ENVIRONMENTAL EFFECTS ON LOCAL ADAPTATION, GENETIC CONNECTIVITY AND MORPHOLOGY OF GENERALIST (*Phaulacridium*) VERSUS SPECIALIST (*Kosciuscola*) AUSTRALIAN GRASSHOPPERS

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DEDICATION

I dedicate this thesis to my beloved grandparents, who taught me the value of hard work, patience, and kindness and to my parents for always having my side.

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Cover images: above- *Phaulacridium vittatum*; below – *Kosciuscola tristis* male.
Photos: Sonu Yadav

SUMMARY

Environmental variables can exert strong effects on a species' morphology, neutral, and adaptive genetic variation. Understanding how environmental variables affect species' genetic connectivity and adaptive genetic responses has implications for conserving species subject to climate change and habitat fragmentation. With field data, statistical modelling and a landscape genomics approach applied to endemic Australian grasshoppers, I compare how patterns of morphology, abundance, gene flow and selection interact in a generalist grasshopper, *Phaulacridium vittatum,* versus three specialist grasshoppers of the genus *Kosciuscola (K. usitatus, K. tristis,* and *K. cognatus),* restricted to alpine regions. Single nucleotide polymorphism (SNP) data from double digest restriction-site associated (ddRAD) DNA sequencing was obtained for *P. vittatum, K. usitatus* and *K. tristis*. For *P. vittatum*, I identified a positive effect of solar radiation on body size and stripe polymorphism, with increasing Foliage Projective Cover (FPC) associated with the presence of winged individuals. Furthermore, latitude, soil moisture, wind speed and FPC were significantly correlated with relative abundance. Mean annual temperature had a stronger positive non-linear effect on genetic connectivity compared to land cover, and despite high gene flow across the 900 km sampling gradient, Environment Association Analysis (EAA) detected signatures of selection in relation to mean annual temperature, latitude and body size. Candidate adaptive SNPs were annotated to gene functions for olfaction, metabolic detoxification and ultra-violet shielding that may be important for environmental adaptation. For the three specialist *Kosciuscola* grasshopper species, the relative abundance of *K. usitatus* and *K. tristis* changed differentially and significantly with elevation, and the relative abundance of *K. cognatus* changed significantly with FPC. Body size changes were observed with increasing elevation that differed within sexes and across species. EAA analysis of SNP data for *K. usitatus* and *K. tristis* indicated that climatic variables including elevation (used as a proxy for temperature due to its high correlation), precipitation seasonality and number of frost days were more strongly associated with selection signatures in both species compared to soil or terrain variables. However, *K. tristis,* with a narrow elevational niche and

lower cold tolerance, showed stronger genetic structure and more pronounced signatures of local adaptation compared to the more widely found *K. usitatus*. Some candidate loci putatively under selection were shared by both species with a greater number associated with elevation, indicating the likelihood of parallel adaptation driven by climatic variables. The highest number of candidate outlier loci were annotated to genes involved in lipid metabolism and development. The study suggests that species with wider environmental niches are likely to maintain higher gene flow and exhibit weaker signatures of selection than species with narrower niche breadths. Furthermore, co-occurring species encountering similar environmental challenges may show parallel adaptation. Taken together, this thesis demonstrates that generalist species are more likely to successfully move and adapt under shifting environments due to land use or climate change than specialist species occupying narrow thermal environments.

CERTIFICATE OF ORIGINALITY

I declare that this thesis entitled “Environmental effects on local adaptation, genetic connectivity and morphology of generalist (*Phaulacridium*) versus specialist (*Kosciuscola*) Australian grasshoppers” is my own work and has not been submitted to any other University or Institute for a higher degree. This thesis is an original piece of research and it has been written by me. Any assistance I received has been appropriately acknowledged in the “Author Contributions” section.

Sonu Yadav
22 August 2019

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CHAPTER 1

General Introduction

"In looking at Nature, it is most necessary to keep the foregoing considerations always in mind- never to forget that every single organic being around us may be said to be striving to the utmost to increase in numbers; that each lives by a struggle at some period of its life; that heavy destruction inevitably falls either on the young or old, during each generation or at recurrent intervals".
-Charles Darwin (Darwin, 1859)

Spatially variable environmental pressures offer challenges that shape species strategies to survive and reproduce in particular environments. From the diverse range of environmental pressures along broad latitudinal gradients to sharp and steep environmental changes along fine-scale altitudinal gradients, species modify their survival strategies to adapt and cope with these contrasting environments. Organisms may show variation in morphological and genetic traits in response to contrasting environmental pressures to develop environmental tolerance, increased survival, and dispersal potential. For instance, in insects distinct colour morphs (relevant for thermoregulation) can be developed according to local environmental conditions (Trullas et al., 2007, Tanaka et al., 2012, reviewed in Simpson et al., 2011), presence or absence of wings (influence dispersal capacity) can be favoured based on habitat stability, temperature and photoperiod (Bai et al., 2016, reviewed in Zhang et al., 2019, Harrison, 1980, McCulloch et al., 2019). Body size variation (relevant for fecundity) can be correlated with latitudinal and altitudinal gradients, which in turn are strongly associated with abiotic and biotic factors (reviewed in Chown and Gaston, 2010, Rosetti and Remis, 2018).

Apart from phenotypic changes, underlying genetic variation (neutral and adaptive) is shaped by the environmental pressures confronted by species (Schoville et al., 2012, Sexton et al., 2014). A large number of studies in insects have shown the influence of abiotic environmental variables, geographic barriers and landscape features on neutral gene flow across space (Lozier et al., 2013, Dreier et al., 2014, Bartlett et al., 2016, Gonzalez-Serna et al., 2018). In addition, recent studies in insects have detected loci under selection in relation to environmental gradients and habitat

variables (Eldon et al., 2019, Feng et al., 2015, Janes et al., 2014). Adaptation to spatially variable environments has been extensively documented in several *Drosophila* species along latitudinal and altitudinal gradients (reviewed in Adrion et al., 2015). Some key examples come from clinal pattern of local adaptation along latitudinal gradients in *Drosophila melanogaster* from Australia (Sezgin et al., 2004, Hoffman and Weeks, 2015) and North America (Berry and Kreitman1993, Fabian et al., 2012). Such clinal allele frequency changes can be significantly associated with environmental conditions such as temperature, UV intensity and seasonality correlated with latitudinal and altitudinal gradient. For example, Schmidt et al., (2008) showed that allele frequency of a SNP associated with adaptation to seasonality in *D. melanogaster* increased clinally with latitude, which in turn is strongly correlated with seasonality.

Among all climatic variables, ambient temperature is shown to have a key impact on genetic connectivity and climatic adaptation in ectothermic insects because of its relation with important life history traits, physiology and reproduction (Waldvogel et al., 2018, Jaffe et al., 2019, Dudaniec et al., 2018). These set of studies provide empirical evidence that the response of individuals to their environment is a complex interaction that involves both phenotypic and/or genotypic changes. Notably, plastic responses such as change in diapause timings, seasonal plasticity and ontogenetic changes in insects may also influence species response to novel environmental conditions (reviewed in Sgro et al., 2016, Gibert et al., 2019). The examination of how environmental variables shape species' morphology, and neutral and adaptive genetic variation is a crucial step towards predicting future distributions and evolutionary changes of species under shifting climatic regimes. Ultimately, information on species' adaptive capacities and dispersal is essential for biodiversity management and conservation as it provides measures of species vulnerability to climate change (Sork and Waits, 2010, Razgour et al., 2019).

Evolutionary responses can be shaped by the relative contribution of neutral gene flow and selection acting in diverse environments. The interaction between gene flow and local adaptation

is widely studied in ecology and evolution (Savolainen et al., 2013). Theory predicts that high gene flow between populations may disrupt local adaptation by bringing locally maladapted alleles and by homogenising locally adapted genotypes (Antonovics, 1976, Yeaman and Whitlock, 2011) whereas restricted gene flow between populations (due to dispersal ability or habitat choice) may lead to greater extent of local adaptation in relation to environmental factors (Tigano and Friesen, 2016). However, local adaptation may still occur if selective pressures are strong enough to overcome the homogenising effects of gene flow (Kawecki and Ebert, 2004). Our knowledge on the interplay between gene flow and local adaptation is therefore inconsistent and varied (Savolainen et al., 2013), suggesting that more studies on different taxa are needed to understand the interaction between these genetic processes. Landscape genomics is a powerful tool to examine such interactions between gene flow and local adaptation (Manel and Holderegger, 2013, Balkenhol et al., 2017). The concurrent study of both neutral connectivity and adaptive divergence in response to environmental variables is crucial to understand the relative contribution of these two processes in shaping adaptive capacity of species.

Species with contrasting geographical distributions and environmental niche tolerances often show different patterns of gene flow and local adaptation and thus are highly suitable to understand the interaction between the two processes. Generalist species with widespread distributions generally occupy large geographic ranges and have a broad ecological niche (Devictor et al., 2008), whereas specialist species with restricted spatial distributions and ecological niches (Clavel et al., 2011) present two such contrasting study systems. Due to their widespread occurrence, generalist species may confront diverse challenging environmental pressures that may promote local adaptation, however greater dispersal and gene flow between locations may disrupt the fixation of locally adapted alleles. On the other hand, specialist species with low dispersal capacity, restricted gene flow, and narrow distribution may be confronted by fine-scale steep environmental pressures along environmental gradients and may exhibit stronger signatures of local adaptation at microgeographic scales. Thus, study systems with striking

differences in their distribution along latitudinal and altitudinal gradients are ideal systems to study evolutionary and genetic processes (Verheyen et al., 2019)

AIMS

The interaction of organisms with their environment is a two-way process where the environment impacts organisms, and organisms shape their environment. In this thesis, I examine how the environment shapes abundance patterns, distribution, morphology, gene flow and local adaptation in two contrasting grasshopper study systems. I study the widely distributed generalist pest grasshopper *Phaulacridium vittatum* (Orthoptera: Acrididae; Key, 1992), and the alpine restricted specialist *Kosciuscola* genus: *K. usitatus, K. tristis,* and *K. cognatus* (Orthoptera: Acrididae; Sjösted, 1933), all endemic to Australia. I examine their relative abundance and morphology changes along latitudinal and altitudinal gradients. Using a landscape genomic approach, in *P. vittatum* I investigate the pattern of genetic structure along latitudinal gradients, examine temperature and landcover effects on genetic connectivity, and common drivers of neutral and adaptive genetic variation. In *Kosciuscola* genus I examine broad and microgeographic scale of genetic structure and isolation by distance, adaptive genetic variation along elevational gradients, parallel signatures of selection in co-occurring species and signals of polygenic adaptation along elevational gradients.

Chapter 2

Widespread distributions and environmental tolerances of a species can be facilitated by morphological variations, for instance different wing morphs (differ in the degree of melanisation) in butterflies can assist with thermoregulation and dispersal across variable thermal conditions (Stoehr and Goux, 2008). Invertebrate pests with widespread distributions (over hundred to thousand kilometres) often exhibit morphological variations likely as a result of differential selection pressures and fitness advantages in diverse habitats. For instance, in the brown planthopper, *Nilaparvata lugens* (a serious pest of rice in Asia), adults possess two wing

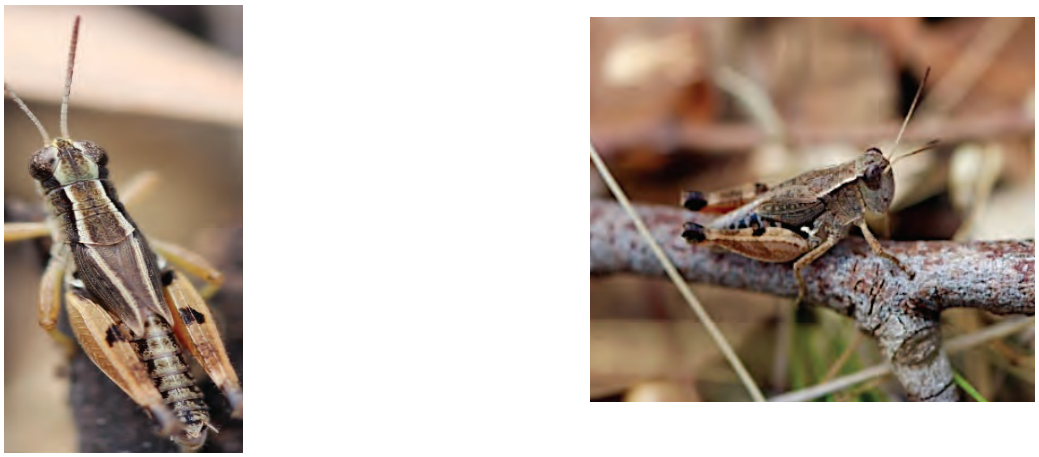
morphs that are linked with migratory responses, whereby long winged adults have high migratory ability and have high heat tolerance than adults with no wings (Lu et al., 2016). These morphological variations may assist widespread species to deal with heterogeneous and challenging environments and can be shaped by environmental conditions (reviewed in Simon and Peccoud, 2018, Hoffmann, 2017). Broad geographical sampling is highly important to study such variations in morphotypes along latitudinal scales because of their power to better account for all sources of variation (reviewed in Shelomi, 2012), however, not many studies have looked at intraspecific variation in shape and size of individuals at broader geographical scales (Bai et al., 2016) except in several *Drosophila* species (Azevedo et al., 1998, Gilchrist et al., 2004, Liefting et al., 2009).

In **chapter 2**, I examine how morphological traits vary along environmental gradients in a widespread agricultural pest, the wingless grasshopper, *Phaulacridium vittatum.* Adults of this species exhibit morphological variation in three key traits (Dearn, 1984, Key, 1992): wing presence/absence, stripe polymorphism (Fig. 1; three distinct morphs: two complete white dorsal stripes, two incomplete white dorsal stripes, and no stripes) and body size variation (both within and between sexes; females are larger than males). *Phaulacridium vittatum* has nonoverlapping generations, with eggs undergoing diapause in winter, and the first hatchlings emerging in spring between October and November (Clark, 1967).

Using a broad-scale sampling design of 900 km, spanning an eight degree latitudinal gradient (from 29.18° S to 36.50° S) along the east coast of Australia, fine scale environmental data and statistical modelling, I examine 1) what is the effect of latitude and environmental variables (i.e., soil moisture, precipitation, wind speed, solar radiation, and foliage projective cover) on site relative abundance and the frequency of morphological traits (body size, wing presence/absence, and stripe polymorphism) and 2) I examine evidence for density-dependent selection on morphology by examining the interaction between site relative abundance and the frequency of

morphological traits. I predict that due to the widespread distribution of this species across heterogeneous landscapes with a striking difference in temperature, precipitation, vegetation cover, and solar radiation, morphological variations are likely to be shaped by environmental variables and thus may show significant correlations. Similarly, I hypothesise that due to thermoregulatory advantages and habitat suitability the relative abundance of *P. vittatum* is likely to be influenced by environmental characteristics (Fig. 2A for an overview of suitable habitat type). An understanding of environmental effects on the spatial distribution of morphological variation and abundance of this pest species is critical to understand its ecology, dispersal potential, and persistence under the climate change context. This chapter has now been published in the Journal of Insect Science (https://doi.org/10.1093/jisesa/iey121).

(A) (B)



(C) (D)

Figure 1: Stripe polymorphism in *Phaulacridium vittatum*: (A) Fully striped; (B) partially striped (C) unstriped morph (photos: Sonu Yadav); (D) *P. vittatum* collection using a plastic jar.

Chapter 3

Landscape genomics approaches are useful to examine the influence of environmental variables and landscape features on genetic connectivity (Manel and Holderegger, 2013), and environmental selection processes (Balkenhol et al., 2017). With the recent advancements in statistical approaches like Environmental Association Analyses (EAA), it is feasible to examine the correlation between allele frequency and environmental variables deemed relevant for the study species (Rellstab et al., 2015, Frichot et al., 2013). A concurrent examination of neutral genetic connectivity and environmental adaptation using the same dataset is crucial for the comprehensive understanding of how neutral genetic connectivity interacts with adaptive genetic divergence and assist in the identification of common drivers of neutral and adaptive genomic variation. Landscape genomics approach also provides the insights on species adaptive potential and vulnerability to climate change (Rellstab et al., 2015) and such knowledge may help informing management interventions and population conservation (Hoffmann et al., 2015).

In **Chapter 3**, with 19 sites across a 900 km latitudinal gradient sampled for *P. vittatum*, and using Single Nucleotide Polymorphism (SNP) data and landscape genomics approach I examine, 1) how temperature and land cover affect neutral genetic connectivity; and 2) using *F*ST outlier tests and Environmental Association Analysis I examine whether patterns of adaptive genetic variation are associated with environmental variables and/or variation in morphological traits. I predict that due to its widespread distribution, a capacity to undertake long distance dispersal and large population size, *P. vittatum* will show a high degree of gene flow across large geographical scales and lower genetic structuring with no isolation by distance. However, as the temperature is a highly crucial climatic factor for ectotherms, influencing movement, and thermoregulation, it may still have subtle effects on genetic connectivity. Furthermore, considering the ecology of the species, I predict that landcover including water bodies and urban areas may provide greater resistance to movement than pastures, agricultural and rural areas. In terms of adaptation to local environmental conditions, I predict that due to the exposure of *P. vittatum* to a diverse range of

environmental pressures along the latitudinal gradients, signatures of local adaptation are anticipated, especially in relation to temperature. Such simultaneous analysis will inform whether similar or different environmental drivers are influencing neutral and adaptive processes. This chapter has now been published in Molecular Ecology Journal (https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.15146).

(A)

(B)

Figure 2: Contrasting study sites: (A) open pasture habitat of *Phaulacridium vittatum* and (B) Mt. Kosciuszko National Park, the habitat of *Kosciuscola* grasshoppers (photos: Sonu Yadav).

Chapter 4

Climate can limit insect distributions by influencing their survival and fecundity (Gaston, 2003). An impact of climatic conditions (such as colder conditions at higher altitudes) on limiting insect distributions has been documented several times (reviewed in Hodkinson, 1999). Due to this dependence of insects on climatic conditions, climate warming is anticipated to shift their ranges and population dynamics. The expansion in distributions and population sizes is particularly true for pest species, which often become favoured with warming conditions (Jepsen et al., 2008). In contrast, changing climatic regimes are negatively influencing specialist insects by decreasing their populations (Piessens et al., 2009, Cerrato et al., 2016), diversity and compositional shifts (Hilt and Fiedler, 2005). Specialist species restricted to alpine habitats are often adapted to narrow thermal ranges and are likely to be adversely affected by climate change (La Sorte and Jetz, 2010). The steep environmental changes along elevation gradients can generate distinct patterns of species abundance and influence morphological traits even at microgeographical scales i.e. over a few metres to kilometres (e.g. Beccacece et al., 2016, Fiedler et al., 2008). Such patterns may further differ in co-occurring species based on their difference in thermal tolerances and niche preferences.

In **Chapter 4**, I study the influence of temperature-elevation gradients on the relative abundance and body size in three co-occurring alpine grasshoppers of the genus *Kosciuscola* (*K. usitatus, K. tristis,* and *K. cognatus*; Fig. 3). All three grasshopper are common in the Kosciuszko region and have been observed to differ in elevational distribution, whereby *K. cognatus* is most widely distributed followed by *K. usitatus*, *K. tristis* (Slatyer et al., 2014). Apart from difference in elevational niche breadth, the three species also show variation in cold tolerance limits (Slatyer et al., 2016), whereby *K. usitatus* has the highest cold tolerance (CTmin= -3 °C), followed by *K. tristis* (CTmin= -2 °C) and *K. cognatus* (CTmin= -1 °C).

Using line transect sampling design within three mountain regions (Thredbo, Guthega and Jagungal) of the Kosciuszko National Park in the Australian Alps (Fig. 2B), I examine, 1) how

species with differences in cold tolerances and elevation niche breadths differ and overlap in relative abundance along temperature-elevation and vegetation gradients, and 2) whether male and female of each species vary in body size along the temperature-elevation gradients. I predict that species relative abundance and body size is likely to differ significantly along elevational gradients (used as a proxy for temperature due to its high correlation), however, the trend of this correlation may differ between species based on their thermal and niche preferences. Furthermore, I predict that differential selection pressures acting on males and females of the same species i.e. natural selection on females (via increased fecundity) and sexual selection on males can generate sex specific variations in body size along elevational gradients. The study will provide information on how populations respond to elevation gradient and associated thermal and vegetation gradients, which is necessary to understand geographically restricted species' vulnerabilities to climate and vegetation change. Baseline studies like this are important to examine environmental influence on alpine species fitness and to predict future population dynamics especially in the Australian alps where knowledge on invertebrate species population dynamics is limited. This chapter has now been published in *Austral Ecology* (https://doi.org/10.1111/aec.12876).

(A) (B)

(C) (D)

Figure 3: Pictures of three study species of the genus *Kosciuscola*. (A) *K. tristis* male, (B) *K. tristis* female, (C) *K. usitatus* male (smaller) and female (larger), (D) *K. cognatus* female (photos: Sonu Yadav).

Chapter 5

Adaptive genetic changes in response to environmental conditions can indicate species potential to respond to existing climate conditions or to exploit new resources. Such changes have been documented in insects in the past few decades (reviewed in Hoffmann, 2010, Hoffmann and Willi, 2008). However, species adaptation to climatic conditions can markedly differ based on their ability to survive and expand or shift ranges under variable thermal conditions. Species inhabiting alpine areas are often specialists with high rates of local endemism (Steinbauer et al., 2016) and have limited opportunities to shift their ranges due to narrow thermal tolerances, local physiological adaptation and restricted areas with suitable habitat (Elsen and Tingley, 2015,

Hodkinson, 2005). The complex topography of alpine areas can isolate populations over microgeographic scales with negative consequence on gene flow (Polato et al., 2018). Furthermore, strong selection pressures along elevation gradients may lead to more pronounced local adaptation (Körner, 2007, Kawecki and Ebert, 2004). The examination of microgeographic patterns of genetic differentiation and local adaptation in specialist species is therefore needed to achieve accuracy in predicting alpine species resilience to climate change.

Alpine ecosystems are characterised by strong and steep shifts in environmental variables and as a result of the steep associations, environmental selection pressures in alpine areas are likely to occur at fine spatial scales (over distances of only a few metres). Species' thermal tolerance and geographic distribution can constrain its potential to adapt to increasing temperatures (For eg., as shown in trees Reich et al., 2015, and dung beetles Birkett et al., 2018) and can influence their patterns of gene flow and local adaptation. A better understanding of adaptive responses can be achieved by simultaneously studying co-occurring species and comparing their adaptive variation (Gugerli et al., 2013, Hand et al., 2015). Lastly, closely related species encountering similar environmental conditions may show common genomic responses leading to parallel adaptation (Yeaman et al., 2016).

In **Chapter 5**, with SNP dataset (two SNP data for individual species and an independent data with markers common in both the species) for *K. usitatus* and *K. tristis* in two mountain regions of Mt Kosciuszko (Thredbo and Guthega) I examine, 1) whether differences in elevational niche preference in two co-occurring species can lead to contrasting patterns of neutral genetic structure and adaptive genetic variation along elevational gradients? 2) whether the two co-occurring species encountering similar environmental challenges show signatures of parallel local environmental adaptation? (examined via multivariate: redundancy and partial redundancy analysis, and univariate EAA approaches, and Fst outlier test) and 3) whether selection signatures show signals of polygenic adaptation along elevational gradients in species (via

additive polygenic score analysis). I predict that the species with narrow elevational distribution and limited thermal tolerance (*K. tristis*) is more likely to show stronger fine and broad scale genetic structuring and prominent signatures of local adaptation compared to the relatively broadly distributed species (*K. usitatus*) and thus is likely to be more vulnerable to environmental change. Furthermore, because similar challenging environmental conditions are encountered by these two closely related specialist species, I predict that they may show signals of parallel local adaptation.

In the **General Discussion** I provide a summary of the main results of my work. I discuss implications of the outcomes of this thesis and provide a snapshot of future directions.

References

ADRION, J. R., HAHN, M. W., & COOPER, B. S. (2015). Revisiting classic clines in Drosophila melanogaster in the age of genomics. *Trends in Genetics,* 31, 434-444.

ANTONOVICS, J. 1976. The nature of limits to natural selection. *Annals of the Missouri Botanical Garden,* 224-247.

AZEVEDO, R. B., JAMES, A. C., MCCABE, J., & PARTRIDGE, L. 1998. Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. *Evolution,* 52, 1353-1362.

BAI, Y., DONG, J. J., GUAN, D. L., XIE, J. Y. & XU, S. Q. 2016. Geographic variation in wing size and shape of the grasshopper *Trilophidia annulata* (Orthoptera: Oedipodidae): morphological trait variations follow an ecogeographical rule. *Sci Rep,* 6**,** 32680.

BALKENHOL, N., DUDANIEC, R. Y., KRUTOVSKY, K. V., JOHNSON, J. S., CAIRNS, D. M., SEGELBACHER, G., SELKOE, K. A., VON DER HEYDEN, S., WANG, I. J. & SELMONI, O. 2017. Landscape Genomics: Understanding Relationships Between Environmental Heterogeneity and Genomic Characteristics of Populations. Springer.

BARTLETT, M., HALE, R. & HALE, M. 2016. Habitat quality limits gene flow between populations of *Bombus ruderatus* in the South Island, New Zealand. *Conservation Genetics,* 17**,** 703-713.

BECCACECE, H. M., ZEBALLOS, S. R. & ZAPATA, A. I. 2016. Changes in Species Richness and Composition of Tiger Moths (Lepidoptera: Erebidae: Arctiinae) among Three Neotropical Ecoregions. *PLoS One,* 11**,** e0162661.

BERRY, A., & KREITMAN, M. 1993. Molecular analysis of an allozyme cline: alcohol dehydrogenase in *Drosophila melanogaster* on the east coast of North America. *Genetics,* 134, 869-893.

BIRKETT, A. J., BLACKBURN, G. A. & MENÉNDEZ, R. 2018. Linking species thermal tolerance to elevational range shifts in upland dung beetles. *Ecography,* 41**,** 1510-1519.

CERRATO, C., LAI, V., BALLETTO, E. & BONELLI, S. 2016. Direct and indirect effects of weather variability in a specialist butterfly. *Ecological Entomology,* 41**,** 263-275.

CHOWN, S. L. & GASTON, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biol Rev Camb Philos Soc,* 85**,** 139-69.

CLARK, D. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.

CLAVEL, J., JULLIARD, R. & DEVICTOR, V. 2011. Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology the Environment,* 9**,** 222-228.

DARWIN, C. 1859. *On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life*, London : John Murray, 1859.

DEARN, J. 1984. Colour Pattern Polymorphism in the Grasshopper *Phaulacridium vittatum*. I. Geographic Variation in Victoria and Evidence of Habitat Association. *Australian journal of zoology,* 32**,** 239-249.

DEVICTOR, V., JULLIARD, R. & JIGUET, F. 2008. Distribution of specialist and generalist species along spatial gradients of habitat disturbance and fragmentation. *Oikos,* 117**,** 507-514.

DREIER, S., REDHEAD, J. W., WARREN, I. A., BOURKE, A. F., HEARD, M. S., JORDAN, W. C., SUMNER, S., WANG, J. & CARVELL, C. 2014. Fine-scale spatial genetic structure of common and declining bumble bees across an agricultural landscape. *Mol Ecol,* 23**,** 3384-95.

DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Mol Ecol,* 27**,** 2576-2593.

ELDON, J., BELLINGER, M. R. & PRICE, D. K. 2019. Hawaiian picture-winged Drosophila exhibit adaptive population divergence along a narrow climatic gradient on Hawaii Island. *Ecol Evol,* 9**,** 2436-2448.

ELSEN, P. R. & TINGLEY, M. W. 2015. Global mountain topography and the fate of montane species under climate change. *Nature Climate Change,* 5**,** 772-U192.
FABIAN, D. K., KAPUN, M., NOLTE, V., KOFLER, R., SCHMIDT, P. S., SCHLÖTTERER, C., & FLATT, T. 2012. Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America. *Molecular ecology,* 21, 4748-4769.
FENG, X. J., JIANG, G. F. & FAN, Z. 2015. Identification of outliers in a genomic scan for selection along environmental gradients in the bamboo locust, *Ceracris kiangsu*. *Sci Rep,* 5**,** 13758.
FIEDLER, K., BREHM, G., HILT, N., SUSSENBACH, D. & HAUSER, C. 2008. Variation of diversity patterns across moth families along a tropical altitudinal gradient. *Ecological Studies,* 198**,** 167.
FRICHOT, E., SCHOVILLE, S. D., BOUCHARD, G. & FRANCOIS, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. *Mol Biol Evol,* 30**,** 1687-99.
GASTON, K. J. 2003. *The structure and dynamics of geographic ranges,* Oxford University Press on Demand.
GIBERT, P., DEBAT, V., & GHALAMBOR, C. K. 2019. Phenotypic plasticity, global change, and the speed of adaptive evolution. *Current opinion in insect science,* 35, 88-95.
GILCHRIST, G. W., HUEY, R. B., BALANYÀ, J., PASCUAL, M., & SERRA, L. 2004. A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution,* 58, 768-780.
GONZALEZ-SERNA, M. J., CORDERO, P. J. & ORTEGO, J. 2018. Using high-throughput sequencing to investigate the factors structuring genomic variation of a Mediterranean grasshopper of great conservation concern. *Scientific Reports,* 8**,** 13436.
GUGERLI, F., BRANDL, R., CASTAGNEYROL, B., FRANC, A., JACTEL, H., KOELEWIJN, H. P., MARTIN, F., PETER, M., PRITSCH, K. & SCHRÖDER, H. 2013. Community genetics in the time of next-generation molecular technologies. *Molecular Ecology,* 22**,** 3198-3207.
HAND, B. K., LOWE, W. H., KOVACH, R. P., MUHLFELD, C. C. & LUIKART, G. 2015. Landscape community genomics: understanding eco-evolutionary processes in complex environments. *Trends in Ecology Evolution,* 30**,** 161-168.
HARRISON, R. G. 1980. Dispersal polymorphisms in insects. *Annual Review of Ecology Systematics,* 11**,** 95-118.
HILT, N. & FIEDLER, K. 2005. Diversity and composition of Arctiidae moth ensembles along a successional gradient in the Ecuadorian Andes. *Diversity and Distributions,* 11**,** 387-398.
HODKINSON, I. D. 1999. Species response to global environmental change or why ecophysiological models are important: a reply to Davis et al. *Journal of Animal Ecology,* 68**,** 1259-1262.
HODKINSON, I. D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol Rev Camb Philos Soc,* 80**,** 489-513.
HOFFMANN, A., GRIFFIN, P., DILLON, S., CATULLO, R., RANE, R., BYRNE, M., JORDAN, R., OAKESHOTT, J., WEEKS, A. & JOSEPH, L. 2015. A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses,* 2**,** 1.
HOFFMANN, A. A. 2010. A genetic perspective on insect climate specialists. *Australian Journal of Entomology,* 49**,** 93-103.
HOFFMANN, A. A. 2017. Rapid adaptation of invertebrate pests to climatic stress? *Curr Opin Insect Sci,* 21**,** 7-13.
HOFFMANN, A. A., & WEEKS, A. R. 2007. Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. *Genetica,* 129, 133.
HOFFMANN, A. A. & WILLI, Y. 2008. Detecting genetic responses to environmental change. *Nat Rev Genet,* 9**,** 421-32.

JAFFE, R., VEIGA, J. C., POPE, N. S., LANES, E. C. M., CARVALHO, C. S., ALVES, R., ANDRADE, S. C. S., ARIAS, M. C., BONATTI, V., CARVALHO, A. T., DE CASTRO, M. S., CONTRERA, F. A. L., FRANCOY, T. M., FREITAS, B. M., GIANNINI, T. C., HRNCIR, M., MARTINS, C. F., OLIVEIRA, G., SARAIVA, A. M., SOUZA, B. A. & IMPERATRIZ-FONSECA, V. L. 2019. Landscape genomics to the rescue of a tropical bee threatened by habitat loss and climate change. *Evolutionary Applications,* 12**,** 1164-1177.

JANES, J. K., LI, Y., KEELING, C. I., YUEN, M. M., BOONE, C. K., COOKE, J. E., BOHLMANN, J., HUBER, D. P., MURRAY, B. W., COLTMAN, D. W. & SPERLING, F. A. 2014. How the mountain pine beetle (*Dendroctonus ponderosae*) breached the Canadian Rocky Mountains. *Mol Biol Evol,* 31**,** 1803-15.

JEPSEN, J. U., HAGEN, S. B., IMS, R. A. & YOCCOZ, N. G. 2008. Climate change and outbreaks of the geometrids *Operophtera brumata* and *Epirrita autumnata* in subarctic birch forest: evidence of a recent outbreak range expansion. *J Anim Ecol,* 77**,** 257-64.

KAWECKI, T. J. & EBERT, D. 2004. Conceptual issues in local adaptation. *Ecology Letters,* 7**,** 1225-1241.

KEY, K. 1992. Taxonomy of the genus *Phaulacridium* and a related new genus (Orthoptera: Acrididae). *Invertebrate taxonomy,* 6**,** 197-243.

KÖRNER, C. 2007. The use of ‘altitude’in ecological research. *Trends in ecology evolution* 22**,** 569-574.

LA SORTE, F. A. & JETZ, W. 2010. Projected range contractions of montane biodiversity under global warming. *Proc Biol Sci,* 277**,** 3401-10.

LIEFTING, M., HOFFMANN, A. A., & ELLERS, J. 2009. Plasticity versus environmental canalization: population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution: International Journal of Organic Evolution*, 63, 1954-1963.

LOZIER, J. D., STRANGE, J. P. & KOCH, J. B. 2013. Landscape heterogeneity predicts gene flow in a widespread polymorphic bumble bee, *Bombus bifarius* (Hymenoptera: Apidae). *Conservation Genetics,* 14**,** 1099-1110.

LU, K., CHEN, X., LIU, W. & ZHOU, Q. 2016. Characterization of heat shock cognate protein 70 gene and its differential expression in response to thermal stress between two wing morphs of *Nilaparvata lugens* (Stål). *Comparative Biochemistry*

Physiology Part A: Molecular Integrative Physiology, 199**,** 47-53.

MANEL, S. & HOLDEREGGER, R. 2013. Ten years of landscape genetics. *Trends Ecol Evol,* 28**,** 614-21.

MCCULLOCH, G. A., FOSTER, B. J., DUTOIT, L., INGRAM, T., HAY, E., VEALE, A. J., DEARDEN, P. K. & WATERS, J. M. 2019. Ecological gradients drive insect wing loss and speciation: The role of the alpine treeline. *Mol Ecol*.

PIESSENS, K., ADRIAENS, D., JACQUEMYN, H. & HONNAY, O. 2009. Synergistic effects of an extreme weather event and habitat fragmentation on a specialised insect herbivore. *Oecologia,* 159**,** 117-26.

POLATO, N. R., GILL, B. A., SHAH, A. A., GRAY, M. M., CASNER, K. L., BARTHELET, A., MESSER, P. W., SIMMONS, M. P., GUAYASAMIN, J. M., ENCALADA, A. C., KONDRATIEFF, B. C., FLECKER, A. S., THOMAS, S. A., GHALAMBOR, C. K., POFF, N. L., FUNK, W. C. & ZAMUDIO, K. R. 2018. Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proc Natl Acad Sci U S A,* 115**,** 12471-12476.

RAZGOUR, O., FORESTER, B., TAGGART, J. B., BEKAERT, M., JUSTE, J., IBÁÑEZ, C., PUECHMAILLE, S. J., NOVELLA-FERNANDEZ, R., ALBERDI, A. & MANEL, S. 2019. Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences,* 116**,** 10418-10423.

REICH, P. B., SENDALL, K. M., RICE, K., RICH, R. L., STEFANSKI, A., HOBBIE, S. E. & MONTGOMERY, R. A. 2015. Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nature Climate Change,* 5**,** 148.

RELLSTAB, C., GUGERLI, F., ECKERT, A. J., HANCOCK, A. M. & HOLDEREGGER, R. 2015. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology,* 24**,** 4348-4370.

ROSETTI, N. & REMIS, M. I. 2018. Spatial Variation in Body Size and Wing Dimorphism Correlates With Environmental Conditions in the Grasshopper *Dichroplus vittatus* (Orthoptera: Acrididae). *Environ Entomol,* 47**,** 519-526.

SAVOLAINEN, O., LASCOUX, M. & MERILA, J. 2013. Ecological genomics of local adaptation. *Nat Rev Genet,* 14**,** 807-20.

SCHOVILLE, S. D., BONIN, A., FRANCOIS, O., LOBREAUX, S., MELODELIMA, C. & MANEL, S. 2012. Adaptive Genetic Variation on the Landscape: Methods and Cases. *Annual Review of Ecology, Evolution, and Systematics, Vol 43,* 43**,** 23-43.

SCHMIDT, P. S., ZHU, C. T., DAS, J., BATAVIA, M., YANG, L., & EANES, W. F. 2008. An amino acid polymorphism in the couch potato gene forms the basis for climatic adaptation in Drosophila melanogaster. *Proceedings of the National Academy of Sciences,* 105, 16207-16211.

SEXTON, J. P., HANGARTNER, S. B. & HOFFMANN, A. A. 2014. Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution,* 68**,** 1-15.

SEZGIN, E., DUVERNELL, D. D., MATZKIN, L. M., DUAN, Y., ZHU, C. T., VERRELLI, B. C., & EANES, W. F. 2004. Single-locus latitudinal clines and their relationship to temperate adaptation in metabolic genes and derived alleles in *Drosophila melanogaster*. *Genetics,* 168, 923-931.

SGRO, C. M., TERBLANCHE, J. S., & HOFFMANN, A. A. 2016. What can plasticity contribute to insect responses to climate change?. *Annual review of Entomology,* 61, 433-451.

SHELOMI, M. 2012. Where are we now? Bergmann's rule sensu lato in insects. *The American Naturalist,* 180**,** 511-519.

SIMON, J. C. & PECCOUD, J. 2018. Rapid evolution of aphid pests in agricultural environments. *Curr Opin Insect Sci,* 26**,** 17-24.

SIMPSON, S. J., SWORD, G. A. & LO, N. 2011. Polyphenism in insects. *Curr Biol,* 21**,** R738-49.

SJÖSTED 1933. Neue australische Acrididen. Ark. Zool.

SLATYER, R. A., NASH, M. A. & HOFFMANN, A. A. 2016. Scale-dependent thermal tolerance variation in Australian mountain grasshoppers. *Ecography,* 39**,** 572-582.

SLATYER, R. A., NASH, M. A., MILLER, A. D., ENDO, Y., UMBERS, K. D. & HOFFMANN, A. A. 2014. Strong genetic structure corresponds to small-scale geographic breaks in the Australian alpine grasshopper *Kosciuscola tristis*. *BMC Evol Biol,* 14**,** 204.

SORK, V. L. & WAITS, L. 2010. Contributions of landscape genetics–approaches, insights, and future potential. *Molecular Ecology,* 19**,** 3489-3495.

STEINBAUER, M. J., FIELD, R., GRYTNES, J. A., TRIGAS, P., AH-PENG, C., ATTORRE, F., BIRKS, H. J. B., BORGES, P. A., CARDOSO, P. & CHOU, C. H. 2016. Topography-driven isolation, speciation and a global increase of endemism with elevation. *Global Ecology Biogeography,* 25**,** 1097-1107.

STOEHR, A. M. & GOUX, H. 2008. Seasonal phenotypic plasticity of wing melanisation in the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera : Pieridae). *Ecological Entomology,* 33**,** 137-143.

TANAKA, S., HARANO, K. & NISHIDE, Y. 2012. Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity. *J Insect Physiol,* 58**,** 89-101.

TIGANO, A. & FRIESEN, V. L. 2016. Genomics of local adaptation with gene flow. *Mol Ecol,* 25**,** 2144-64.
TRULLAS, S. C., VAN WYK, J. H. & SPOTILA, J. R. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology,* 32**,** 235-245.
VERHEYEN, J., TÜZÜN, N. & STOKS, R. 2019. Using natural laboratories to study evolution to global warming: contrasting altitudinal, latitudinal, and urbanization gradients. *Current opinion in insect science*.
WALDVOGEL, A. M., WIESER, A., SCHELL, T., PATEL, S., SCHMIDT, H., HANKELN, T., FELDMEYER, B. & PFENNINGER, M. 2018. The genomic footprint of climate adaptation in *Chironomus riparius*. *Mol Ecol,* 27**,** 1439-1456.
YEAMAN, S., HODGINS, K. A., LOTTERHOS, K. E., SUREN, H., NADEAU, S., DEGNER, J. C., NURKOWSKI, K. A., SMETS, P., WANG, T. & GRAY, L. K. 2016. Convergent local adaptation to climate in distantly related conifers. *Science,* 353**,** 1431-1433.
YEAMAN, S. & WHITLOCK, M. C. 2011. The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution,* 65**,** 1897-1911.
ZHANG, C. X., BRISSON, J. A. & XU, H. J. 2019. Molecular mechanisms of wing polymorphism in insects. *Annu Rev Entomol,* 64**,** 297-314.

CHAPTER 2

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Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae)

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All authors conceptualized the study. I performed fieldwork, collected data, lab work, conducted all statistical analyses and interpreted the results. I wrote the manuscript with editing from Rachael Dudaniec, Adam Stow and Rebecca Harris.

Abstract

Invertebrate pests often show high morphological variation and wide environmental tolerances. Knowledge of how phenotypic variation is associated with environmental heterogeneity can elucidate the processes underpinning these patterns. Here we examine morphological variation and relative abundance along environmental gradients in a widespread agricultural pest, native to Australia, the wingless grasshopper *Phaulacridium vittatum* (Orthoptera: Acrididae). We test for correlations between body size, wing presence, and stripe polymorphism with environmental variables. Using multiple regression and mixed-effects modelling, body size and stripe polymorphism were positively associated with solar radiation, and wing presence was positively associated with foliage projective cover (FPC). There were no associations between body size or morphological traits with relative abundance. However, relative abundance was positively associated with latitude, soil moisture, and wind speed, but was negatively associated with FPC. Therefore, sites with low relative abundance and high forest cover were more likely to contain winged individuals. Overall, our results suggest that environmental and climatic conditions strongly influence the relative abundance and the distribution of morphotypes in *P. vittatum*, which is likely to affect dispersal and fitness in different landscapes. This knowledge is useful for informing how environmental change might influence the future spread and impact of this agricultural pest.

Key Words:

Wingless grasshopper, *Phaulacridium vittatum*, agricultural pest, relative abundance, morphological variation.

Introduction

The distribution, abundance, and phenology of several insect pests have altered in response to changing climatic conditions (Olfert and Weiss 2006, Trnka et al. 2007, Parmesan 2007, Da Silva et al. 2011). Shifts in environmental variables, such as temperature, play a key role in the severity of phytophagous insect pest infestations (e.g. Baker et al. 2015). Major outbreaks of phytophagous insects are exacerbated by ongoing warming conditions, and extreme climatic events such as drought and floods (Jepsen et al. 2008, Zhang et al. 2009). Further, the negative impacts of phytophagous insect pests on crop production (Oerke 2006) and forest harvest (Aukema et al. 2011) are often economically significant (Bradshaw et al. 2016). Therefore, it is important to understand the environmental determinants and interactions that facilitate the occurrence and spread of insect pests under currently shifting climatic regimes.

Some insects show variation in genetic, physiological and morphological traits across broad climatic gradients (Lancaster et al. 2015, 2016, reviewed in Hoffmann et al. 2017, Dudaniec et al. 2018). For insect pests, variations in morphological traits may confer benefits that enable wide environmental tolerances, survival and colonisation across large geographic areas (Gomez and Van 2012, Parsons and Joern 2014, Bai et al. 2016), or increased chances of spread through efficient use of local resources (e.g. as shown in pest aphids; Simon and Peccoud 2018). The relationship between environmental variables and the distribution of morphological variation can therefore be indicative of the capacity of insect pests to persist under shifting environmental conditions and may also provide evidence for local selection processes.

Polymorphism in insects can be maintained by various mechanisms such as balancing selection (Lindtke et al. 2017), a combination of multiple selection pressures (Takahashi et al. 2011) or via adaptive or non-adaptive phenotypic plasticity (Ghalambor et al. 2007, Kelly et al. 2012, Sgrò et al. 2016). Morphological traits may also be regulated via frequency or density dependence. In this case, the fitness of different morphs shifts in response to their prevailing frequencies, or in

response to competitive interactions among conspecifics (Le Rouzic et al. 2015, Lancaster et al. 2017, Nosil et al. 2018). In addition, a clear role of climatic and ecological factors in shaping morphological variations in insects is reported in several studies (Dearn 1990, Zera and Denno 1997, Chown and Gaston 2010). For example, different colour morphs can be selected for according to local environmental variables (Tanaka 2004, Tanaka et al. 2012). Dispersal traits such as wing dimorphism may show variation under temporally and spatially diverse climatic gradients (Harrison 1980, Roff 1986) and in relation to photoperiod, temperature and latitude (Sasaki et al. 2002, Zeng and Zhu 2014, Rosetti and Remis 2018). Variation in climate can also shape the distribution of insect body size along latitudinal gradients (Masaki 1967). A necessary step in understanding these relationships is tearing apart environment effects on morphological traits and their influence on fitness. For example, different morph frequencies may confer differential survival according to local climatic conditions (Forsman 1999). For insect pests, such information offers a starting point for characterizing species resilience under shifting climatic conditions.

Here we examine how morphological traits vary along environmental gradients in a widespread agricultural pest, the wingless grasshopper, *Phaulacridium vittatum*, an endemic species to Australia. Adult grasshoppers exhibit morphological variation in three key traits: wing presence/absence, stripe polymorphism (Fig. 1; three distinct morphs; Key 1992, Dearn 1984), and body size (both within and between sexes; Key 1992). Body size of males (ranging from 6 - 9 mm) is smaller than females (average size of 6 -13 mm) (Key 1992). *Phaulacridium vittatum* has non-overlapping generations, with eggs undergoing diapause in winter, and the first hatchlings emerging in spring between October and November (Clark, 1967).

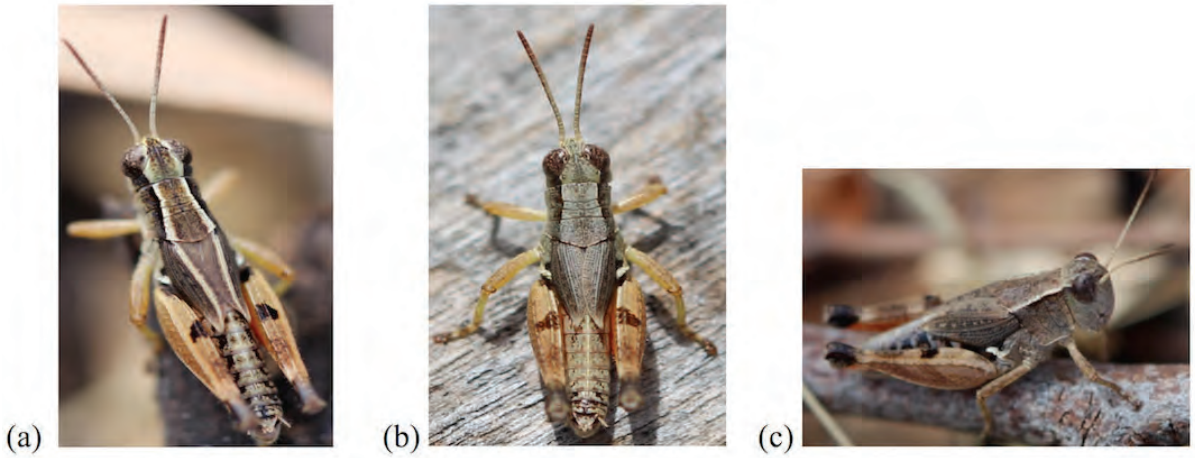

Figure 1: Stripe polymorphism in *Phaulacridium vittatum*: (a) fully striped morph with two full longitudinal white stripes, (b) unstriped morph, (c) partially striped morph with two partial white stripes (photos: Sonu Yadav).

Development involves five instar stages and adults survive until late March and into April. Prior to European colonisation of Australia, *P. vittatum* was restricted to savannah woodlands or grasslands, but with forest clearing for pasture and agriculture, suitable breeding habitat increased dramatically, contributing to the species' current widespread distribution and high abundance that can reach pest proportions (Dearn 1984). Adult grasshoppers cause damage to pasture, gardens, economically important crops (Roberts 1972, Clark 1967), juvenile eucalyptus trees (Loch and Floyd 2001), and can compete with livestock for pasture (Bailey et al. 1994). Severe outbreaks can be cyclic and have been documented every four or five years in areas with suitable habitats and weather conditions (Milner et al. 1994).

Several selective forces may be responsible for maintaining morphological polymorphisms in *P. vittatum*. For example, stripe polymorphism in *P. vittatum* appears to be maintained by disassortative mating (i.e., increased frequency of matings between striped and unstriped individuals) as documented by Dearn (1980 and 1984). Another potential selective force maintaining stripe morph in *P. vittatum* is frequency-dependent selection exerted by predators (Dearn 1984) as reported in other species (Losey 1997, Gray and McKinnon 2007). Evidence

also suggests that morphological variation in *P. vittatum* is influenced by latitude, climatic variables and habitat type. Latitudinal cline in stripe pattern polymorphism has been suggested in previous studies (Dearn 1981). Radiation seasonality and rainfall best explained latitudinal clines in *P. vittatum* body size (Harris et al. 2012). Furthermore, thermoregulatory effects of melanism have been shown in this species, where darker coloured individuals (without stripes) maintained a higher body temperature than lighter colour individuals (Harris et al. 2013a, Harris et al. 2013b). In terms of habitat type, unstriped morphs were present at a higher frequency in more forested habitats, perhaps a result of camouflage advantage (Dearn 1984). Additionally, a higher frequency of winged individuals was observed in shrub-dominated areas, forest margins and gardens than in open pastures (Clark 1967). Despite these early studies, little is known of the broader spatial distribution of morphological variation or abundance of *P. vittatum* in relation to local environmental variables, which is important for understanding the ecology and potential spread and persistence of this agricultural pest.

With morphological, environmental and site relative abundance data collected for *P. vittatum* across 36 sites spanning an eight degree latitudinal gradient (from 29.18°S to 36.50°S) along the east coast of Australia we examine, 1) the effect of latitude and environmental variables (i.e. soil moisture, precipitation, wind speed, solar radiation, and foliage projective cover) on site relative abundance and the frequency of morphological traits (body size, wing presence/absence and stripe polymorphism), and 2) evidence for density-dependent selection on morphology by examining the interaction between site relative abundance and the frequency of morphological traits.

Materials and Methods

Sampling

Phaulacridium vittatum grasshoppers were sampled from late January to early February of 2017, when most individuals had reached maturity. In total, 36 sites were sampled across the east coast

of New South Wales spanning ~900km, covering eight degrees of latitude (from 29.18°S to 36.50°S), a mean annual temperature gradient of 4.6-17.4°C, and an elevation gradient of 133-1966 m asl (Table S1, Fig. 2). Sites were chosen based on previous information on species presence and partly after searching for grasshoppers during the fieldwork. We also followed a paired gradient sampling design as much as possible to capture environmental variation along the sampled latitudinal gradient. Sites were located approximately 50-60 km apart, and sample sizes ranged from 6 to 34 individuals per site (mean = 28.49 ± 0.17). The elevation, latitude, and longitude of each site were recorded using a Garmin e-Trex 20 hand-held GPS. Most sites were situated adjacent to or within open pastures, along roadside edges and some sites were situated inside national parks within eucalyptus woodlands and other native shrubs. A site was declared absent of *P. vittatum* after searching for a minimum of ~15 mins by three people (total search time = 45 mins) without any sightings or captures.

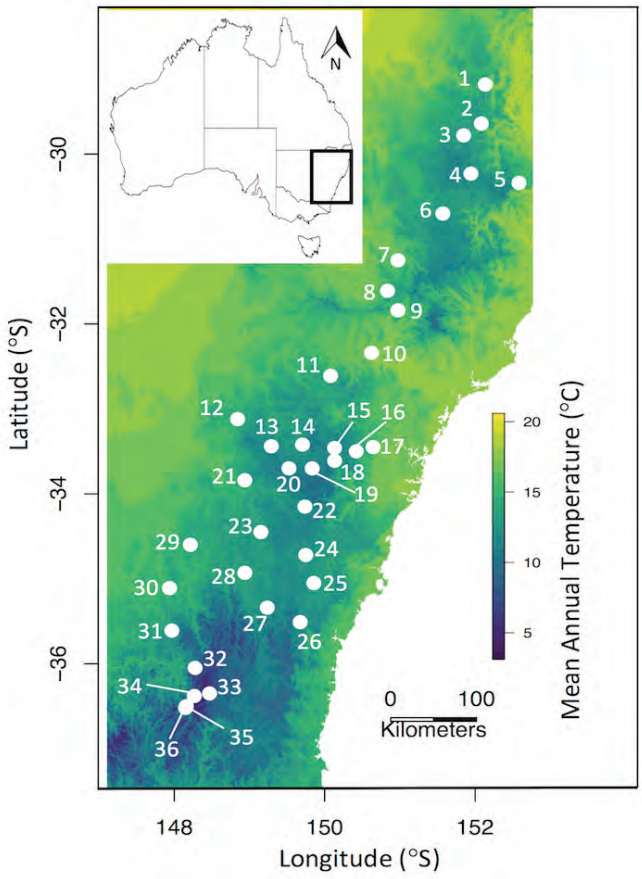

Figure 2: Sample sites (*n*=36) indicated by filled circles over a map of mean annual temperature (°C, BIO1) along the east coast of New South Wales, Australia.

Samples were collected either using a sweep net or by hand using small plastic containers. Habitat type does not have a large effect on spotting and catching grasshoppers as captures are largely cued by the obvious hopping activity of the grasshoppers after disturbing the grasslands and pastures. Similarly, stripe pattern did not introduce sampling bias as stripes are not readily visible from afar and do not influence capture rates. All collected specimens were stored on ice until processed and preserved in 90% ethanol. The following details were recorded on live samples during sample processing: sex (male/female), morphology: (winged/wingless), (striped/partially striped/unstriped) and femur length (mm), the latter being measured with digital callipers to the nearest 0.01 mm as a proxy for body size in grasshoppers (Masaki 1967, Mousseau and Roff, 1989, Davidowitz, 2008). The relative abundance of *P. vittatum* at each site was measured via capture rate per minute, calculated as the number of grasshoppers captured divided by the number of people searching multiplied by the number of minutes spent searching (Lancaster et al. 2015). The same searchers were trained and involved in collection at each site to avoid possible individual based bias. All procedures were performed in accordance with the animal ethics guidelines of Macquarie University, Australia (for compliance purposes), and sampling permissions were obtained from local government authorities and national parks (license number: SL101832).

Environmental data

Environmental variables were selected based on field observations and findings of previous studies on the ecology of *P. vittatum.* Latitude and environmental variables such as, radiation seasonality, and rainfall are known to influence body size variation in *P. vittatum* (Harris et al. 2012). Foliage cover is important for egg laying in this species, as eggs are laid in open areas with bare ground between vegetation patches (Clark 1967). Additionally, soil properties such as soil moisture and soil type have been shown to influence density (Miao et al. 2018), oviposition and therefore further offspring viability in grasshoppers (Herrmann et al. 2010). Wind speed was chosen because wind is beneficial for passive dispersal of insects (Drake 1994, Venette and

Ragsdale 2004) and may aid in the colonisation of new areas by facilitating long-distance dispersal (Anderson et al. 2010, Colombari and Battisti 2016).

Mean annual temperature (BIO1) and mean annual precipitation (BIO12) were extracted per sampling site from WorldClim v1.4 (Hijmans et al., 2005). Mean annual wind speed and mean annual solar radiation were extracted from WorldClim v2.0 (Fick and Hijmans, 2017). All data from WorldClim were used at a spatial resolution of 1 km^2. Foliage Projective Cover (FPC %) is the percentage of ground area occupied by the vertical projection of foliage and was extracted from a buffered area of 100 m surrounding each of the sites from the TERN AusCover database (2011) with a spatial resolution of $5m^2$. A 100m buffer was chosen based on estimates of individual grasshopper movement and range size. Data for each of 36 sites were extracted (Table S1) using the *raster* package (Hijmans and van Etten, 2014) in R v3.3.2 (R Core Team 2016).

Soil type data were downloaded from the New South Wales Office of Environment and Heritage (OEH, 2017) and obtained for each sample site using QGIS v2.18 (QGIS Development Team, 2009). Soil type data were then used to classify soil moisture for each site and was categorised based on the clay content and water holding capacity of the soil, as defined in the Australian Soils Classification (ASC) and Great Soil Group guidelines (GSG) (Raymond 2016). Soils with high to intermediate water holding capacity based on ASC and GSG classifications were scored as 1 (high moisture), whereas soils with low water holding capacity were scored as 0 (low moisture) (Table S4 for site-based information).

A Pearson's correlation matrix was obtained between all environmental variables measured at each site (Table S2, Text S1). After removing variables with very high correlation ($r > 0.80$), a total of six variables were used for further analyses, i.e., i) solar radiation, ii) wind speed, iii) soil moisture, iv) FPC, v) BIO12, and vi) latitude. Despite the high correlation between latitude and

solar radiation (r = 0.81) we retained latitude in order to examine its influence on site relative abundance. Nonetheless, both variables were not used together in the same model.

Statistical approach

All statistical analyses were performed in R v3.3.2. We analysed our data (n = 954 individuals) using multiple regression, and generalized linear mixed-effects models (GLMM) and linear mixed-effects (LMM). The statistical significance of models were examined using likelihood ratio tests within ANOVA by comparing fitted models with the null model including only the response variable, random effects (in case of the mixed model), and intercept. Insignificant fixed effects were removed from the model by applying the "analysis of deviance test" using the *drop1()* command with a Chi-square test. The model was refitted after dropping insignificant fixed effects. We performed a nonlinear transformation on the response variable wherever possible (relative abundance and femur length) by using log-transformation. Site ID was included as a random effect in mixed modelling to account for non-independence between sampling sites. To examine the goodness of fit of the GLMM and LMM, conditional R^2 statistics were calculated using the MuMIn R package (Barton 2018).

Relative abundance

Multiple linear regression modeling was used to examine the influence of latitude, FPC, soil moisture and wind speed on the relative abundance of *P. vittatum* at each site. In addition, the relative importance of each fixed effect and confidence intervals for relative importance were determined using the *boot.relimp()* and *booteval.relimp()* functions of the relaimpo v2.2-2 R package (Grömping 2006) by implementing 1,000 bootstraps.

Wing polymorphism

Wing polymorphism was divided into two categories; winged individuals were categorised as 1 and wingless individuals were categorised as 0. We tested for the association of solar radiation,

wind speed, FPC, soil moisture, relative abundance, body size and stripe pattern on wing presence using GLMM in the lme4 R package (Bates et al. 2015) and the function *glmer()*. The final model was refitted after dropping insignificant fixed effects and using site relative abundance as the random effect to account for its influence on the relationship and to provide additional explanatory power to the model.

Stripe polymorphism

Stripe polymorphism in *P. vittatum* appears to be genetically controlled by three alleles at an autosomal locus (C^S = full stripe, C^I = partial stripe, C^P = plain) following a hierarchical dominance pattern ($C^S > C^I > C^P$) (Dearn 1983). As previously done by Dearn (1978, 1981), and due to a low number of partially striped individuals in our dataset, partially striped morphs were combined with striped morphs into an 'any striped' category, pooling C^S and C^I genotypes. Stripe polymorphism was classified into two categories: any striped individuals were coded as 1 and unstriped individuals were coded as 0. We constructed a GLMM, using stripe polymorphism as a response variable and solar radiation, wind speed, FPC, soil moisture, relative abundance, body size and wing polymorphism as fixed effects. After dropping insignificant fixed effects model was refitted using site relative abundance as the random effect.

Body size

We fitted a LMM to examine the influence of environmental variables on body size of males and females combinedly using the *lmer()* function within the lme4 R package (Bates et al. 2015) with the fixed effects solar radiation, BIO12, FPC, soil moisture, wind speed and relative abundance. Final model was refitted using significant fixed effect. We conducted Type-II Wald Chi-square tests using the "car" R package (Fox and Weisberg 2011) to obtain *P* values for the fitted model. In addition, a linear model was fitted for body size of both males and females individually with significant fixed effect.

Results

Environmental correlations with relative abundance

We found a significant positive association of relative abundance with latitude (P = 0.001), soil moisture (P = <0.001), and wind speed (P = <0.001), whereas FPC showed significant negative association (P = <0.001), with total variance explained by the model = 23% (Fig. 4, Table 1).

Table 1: Summary of multiple linear regression analysis for site relative abundance.

Fixed Effects	Estimate	Standard error	t value	Pr(>|t|)
Latitude	0.03	0.01	3.18	0.001
Soil moisture	0.22	0.03	5.99	<0.001
Wind speed	0.42	0.04	9.88	<0.001
Foliage Projective Cover (%)	-0.02	0.00	-14.78	<0.001

FPC had the highest relative importance amongst other fixed effects followed by wind speed, soil moisture and latitude (Fig. 3, Table S3). Several sites in our study with elevation above 1000 m (n =12 sites) had greater FPC (mean = 23.27 ± 5.92) (Fig. S4), compared to sites below 1000 m elevation (n = 24 sites, mean = 10.01 ± 2.73). In the case of soil moisture, out of 36 sites, 17 were categorized as having intermediate to high soil moisture and had an average relative abundance of 0.36 (± 0.06), compared to 19 sites with low soil moisture that had an average relative abundance of 0.29 (± 0.05).

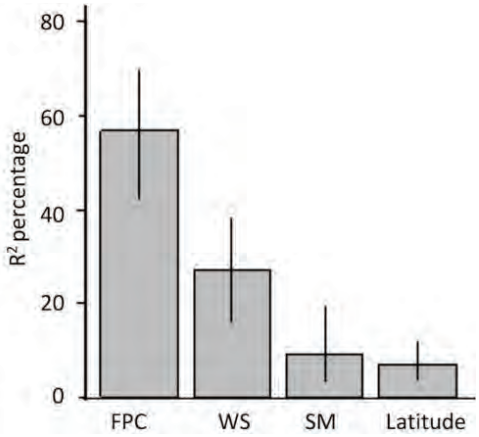

Figure 3: Relative importance of four fixed effects on relative abundance in multiple regression analysis with bootstrapped 95% confidence intervals; FPC= Foliage Projective Cover (%); WS = wind speed (m/s); SM = soil moisture.

Environmental correlations with morphology

In total, 72 out of 954 (7.55%) individuals were winged, and 882 out of 954 (92.45%) were wingless. Within each of the 36 sample sites the proportion of winged individuals ranged from 0-0.73 (mean = 0.10 ± 0.02) and wingless from 0.27-1.00 (mean = 0.90 ± 0.02). A positive correlation was found between FPC and wing presence (GLMM estimate = 0.03, $P = 0.03$ conditional $R^2 = 0.41$) (Fig. S2). However, other variables did not show any significant correlation with wing presence or absence (Table 2). The addition of site relative abundance as a random effect increased the fit of the model (random effect variance: 1.94, SD 1.39).

Table 2: Summary of the generalized linear mixed-effects model (GLMM) for wing polymorphism, and stripe polymorphism. Significant associations are shown in bold.

Fixed effects	Estimate	Pr (>z value)	Estimate	Pr (>z value)
	Wing polymorphism		Stripe polymorphism	
Solar radiation	0.58	0.06	**0.31**	**< 0.001**
Wind speed	-0.23	0.34	-0.08	0.66
Foliage Projective Cover (%)	**0.03**	**0.03**	-0.00	0.87
Soil moisture	-0.08	0.87	0.26	0.18
Relative abundance	-0.56	0.06	0.06	0.40
Body size	-0.00	0.96	-0.04	0.45
Stripe pattern	-0.07	0.82	-	-
Wing pattern	-	-	-0.05	0.85

In total, 15.6% of individuals were striped, 8.1% partially striped and 76.2% individuals were unstriped. Within each of the 36 sample sites the proportion of fully striped individuals ranged from 0-0.36 (mean = 0.15 ± 0.01), partially striped individuals ranged from 0-0.40 (mean = 0.09 ± 0.01) and unstriped individuals ranged from 0.58-0.93 (mean = 0.76 ± 0.01). The proportion of both fully and partially striped morphs in each site was positively associated with solar radiation (GLMM estimate = 0.31, P = < 0.001, conditional R^2 = 0.042). No other significant associations were observed with stripe polymorphism (Table 2). Site relative abundance had no effect on the relationship and did not explain any of the variance.

Body size varied within adult males (range 5.36-10.63 mm; mean = 8.25 ± 0.03 mm) and adult females (range 7.41-12.28 mm; mean = 10.3 ± 0.03 mm) along the sampled gradient. A significant positive correlation was found between body size and solar radiation (LMM: χ^2 = 5.56, df = 1, P = 0.01, conditional R^2 = 0.079; Fig. S1). However, we did not find a significant association with other fixed effects (Table 3). Males were shown to have a stronger relationship (linear model P < 0.001) than females (linear model P = 0.03). Furthermore, site relative abundance had no influence on the relationship (random effect variance= 0.0). The nymphs of the species differ in colour and size from adults and were excluded from body size analysis.

Table 3: Analysis of deviance table summary using Type II Wald Chi-square tests for linear mixed-effects model (LMM) fit for body size. Significant results are indicated in bold.

Fixed effects	Estimate	Df	Chisq	Pr(>Chisq)
Initial LMM				
Solar radiation	**0.03**	**1.00**	**6.66**	**0.01**
Mean annual precipitation	0.03	1.00	2.84	0.09
Foliage Projective Cover (%)	0.00	1.00	1.51	0.21
Soil moisture	0.02	1.00	2.42	0.11

Wind speed	-0.03	1.00	1.67	0.19
Relative abundance	0.02	1.00	0.40	0.52
Final model (after dropping insignificant fixed effects)				
Solar radiation	**0.01**	**1.00**	**5.56**	**0.01**

Discussion

Our study reveals significant relationships between environmental variables, site abundance and the distribution of morphological traits in an agricultural insect pest. We found site relative abundance was positively influenced by latitude, wind speed and soil moisture, and was negatively influenced by foliage cover. Solar radiation was the principal factor influencing stripe polymorphism and body size variation in *P. vittatum*. Furthermore, site relative abundance did not significantly associate with morphological traits. Our study demonstrates how multiple environmental factors can influence the distribution of morphological variation and relative abundance of *P. vittatum,* and contributes knowledge towards our capacity to understand how widespread pests cope with heterogeneous environments via morphological variation.

Environmental variables and relative abundance

Overall, higher numbers of *P. vitattum* were observed at southern latitudinal sites (Fig. 4a). Climatic variables that correlate with latitude can have strong effects on local abundance (Bale et al. 2002). For instance, in grasshoppers, climatic factors such as temperature and precipitation, are critical in controlling population dynamics, both directly by influencing life history traits, and indirectly by controlling resource availability (Fielding and Brusven 1990, Wysiecki et al. 2011, Jonas et al. 2015). However, identifying the influence of individual variables is challenging due to the complex interaction between them. For example, in our study sites, mean annual temperature is strongly correlated with mean annual precipitation (r = -0.83), and wind speed (r = -0.90) (Table S2). Furthermore, some of our sites are located near or within the Australian Alps (latitudinal range of 35-36 °S), which have higher elevation (Table S1) and receive greater

solar radiation (Fig. S3). These strong interactions make it difficult to effectively tease apart which variable is most significant in driving the variation in relative abundance of *P. vittatum*. Given that our model explains a modest 23% of the variance, additional factors such as host plant preferences, and complex interactions between climatic variables may also be involved in regulating the abundances.

A decrease in relative abundance was observed with increased FPC (Fig. 4d). A higher percentage of FPC at high elevation sites in our study (Fig. S4) may hamper dispersal and connectivity among individuals, which in turn may reduce site relative abundances. Furthermore, this observed negative association between relative abundance and FPC is likely to be affected by site preference and resource availability. Open pastures and grazing areas are among the primary habitats of *P. vittatum*, where low FPC provides suitable sites for egg laying (Clark 1967). Furthermore, high vegetation cover negatively affects early instar survival due to a scarcity of preferable food resources that grow in more disturbed areas (i.e., prostrate and rosette-forming plants growing under pasture) (Clark 1967). Lastly, in grasshoppers, basking is a crucial factor to regulate body temperature, facilitated by shuttling between sun and shade (Chappell and Whitman 1990), which is also exhibited by *P. vittatum* (Harris et al. 2015). Compromised thermoregulation due to high vegetation cover and reduction in suitable basking sites, could influence fitness and decrease relative abundance (Kearney et al. 2009), however further study is needed to examine the role of vegetation cover on fitness. A few points towards the end of the distribution indicate variability in the data but given the strong correlation are unlikely to drive the relationship.

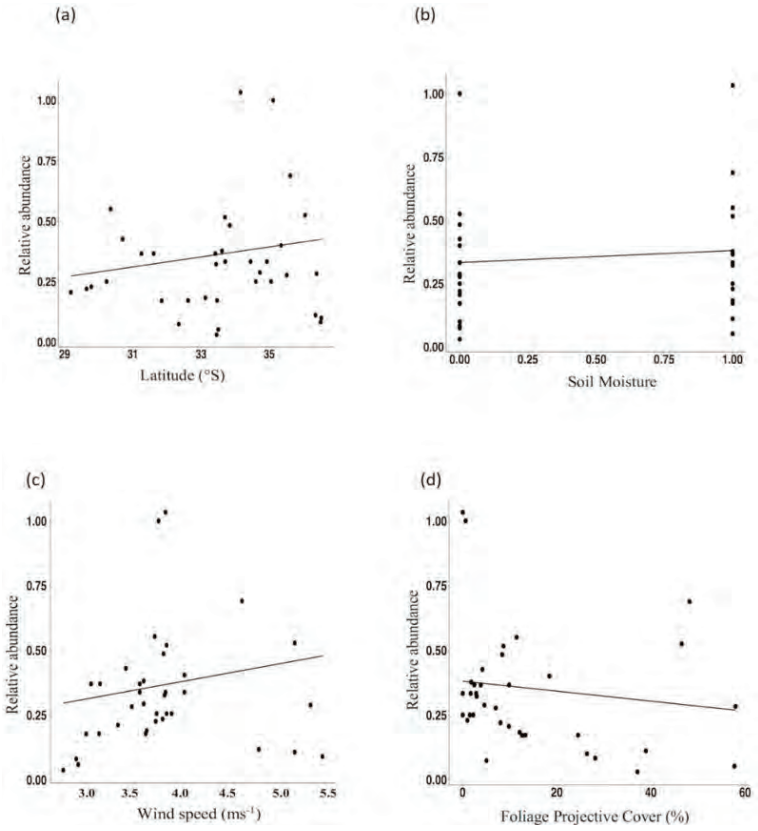

Figure 4: The relationship between four significant fixed effects used in multiple regression analysis and relative abundance; (a) latitude: (P = 0.001); (b) soil moisture (P < 0.001); (c) wind speed (P < 0.0001); and (d) Foliage Projective Cover (%) (P < 0.0001).

Sites with greater wind speed had higher relative abundance (Fig. 4c) suggesting a role of wind-assisted dispersal in the population dynamics of *P. vittatum*. In insects, wind can aid long-distance migration (Drake and Farrow 1988) and drive selection processes (Dudaniec et al. 2018), and for pest species, wind assisted flight can lead to accelerated invasion to new areas, outbreaks and major crop losses (Drake 1994, Liebhold and Tobin 2008, Anderson et al. 2010). Wind speed and the presence of wings in *P. vittatum* are likely to facilitate both passive and active dispersal, respectively, facilitating its broad distribution. However, we did not find a significant relationship between wind speed and presence of wings (P= 0.792) and wind speed was found not to be negatively correlated with FPC. Wind speed can also regulate local ambient temperatures, and indeed, we found that wind speed was highly correlated with mean annual temperature (r = -0.90). Hence, a combined effect of wind speed and temperature is likely to be a key driver of relative abundance.

A significant effect of soil moisture on relative abundance is evident from our study (Fig. 4b). Soil can control insect abundances via influencing life history traits of phytophagous insects, such as egg laying, nymphal development (as shown in the lubber grasshopper, *Romalea microptera*; Herrmann et al. 2010) or via regulation of habitat resources (McColloch and Hayes 1922) and controlling the growth of suitable food resources (Miao et al. 2018). Soil moisture content can support vegetation growth, this may compromise egg laying in *P. vittatum* because females preferentially lay egg pods in patches of bare ground (Clark 1967). However, high soil moisture content may facilitate food resource availability after emergence, supporting higher relative abundances, as observed in our study. Therefore, soil moisture may influence the life stages of *P. vittatum* differentially with greater benefits after emergence than during egg and early instar phases. Furthermore, the local distributions of *P. vittatum* has been found to be influenced by the interaction between soil moisture and the occurrence of parasitic nematodes and entomopathogenic fungi (Clift and Baker 1998, Milner and Prior 1994), indicating evidence for an indirect effect of soil moisture.

Wing polymorphism and foliage projective cover

Wing polymorphism is a common phenomenon in several insects with ecological and evolutionary implications (Harrison 1980, Roff 1986). Winged and wingless morphs may be retained in different frequencies depending on environmental conditions such as temperature, precipitation (Sasaki et al. 2002, Rosetti and Remis 2018) and habitat type (Denno 1994). For instance, planthoppers (Homoptera: Delphacidae), are more likely to have wingless morphs in more stable habitats, and dispersive, winged morphs in less stable habitats (Denno et al. 1991). Our study found a larger proportion of winged individuals at sites with high foliage cover (Fig. S2), which supports the observations of a previous study on *P. vittatum* by Clark (1967), who found that the wingless form was abundant in pastures (>75% wingless adults), and the winged form was abundant in forested areas (60-70% winged individuals). Our concurrent finding that relative abundance also decreases significantly with increased FPC (Fig. 4d) indicates that high

FPC sites are not the preferred habitat of this species. This suggests that high foliage sites are of lower habitat suitability for *P. vittatum* and may select for higher frequencies of traits that facilitate dispersal into new areas, such as wings (Denno et al. 1996).

The development of wings in insects can be a trade-off between dispersal and reproduction (Guerra and Pollack 2007, Guerra 2011, Steenman et al. 2015). For example, an analysis of life history traits of 22 insect species by Roff (1986) showed wingless forms have higher fecundity and reproduce earlier than winged forms. While wingless forms can show high fecundity and reproductive rates in stable, good quality habitats, in unfavorable environments, winged forms may be favoured for their high dispersal ability (Harrison 1980, Denno et al. 1991, Zera and Harshman 2001). The winged form of *P. vittatum* may be advantageous for dispersal during outbreaks to avoid competition with conspecifics and locate new resources. For example, in an outbreak in 1980/1981, the winged form of *P. vittatum* was found to successfully undertake dispersal distances of 100m to >500m from the center of infested areas to favourable peripheral pastures (Farrow et al. 1982). Therefore, the presence of wings can provide additional benefit to this pest species to disperse to new areas, increasing widespread infestation. Lastly, population densities can also influence the frequency of winged and wingless individuals (Poniatowski and Fartmann 2009, Rios Martínez and Costamagna 2018). Our results also indicate a possible influence of relative abundance on the presence of wings in this species, however further study on density-dependent effects of relative abundance on the frequency of wings in *P. vittatum* is needed to clearly disentangle this effect.

Stripe polymorphism and solar radiation

Solar radiation is an important factor for the body temperature regulation in grasshoppers (Pepper and Hastings 1952), while melanism is important for the efficient use of solar radiation (Trullas et al. 2007). However, under high solar radiation, dark colouration might impose negative effects on fitness causing overheating of melanics, whereas lighter colour morphs may

benefit in such conditions (Trullas et al. 2007). We found a positive correlation between solar radiation and individuals with white stripes in *P. vittatum* (Fig. 5, Table 2), which may be involved in reducing heat absorption in sites with higher solar radiation. Likewise, darker individuals without stripes in cooler sites may benefit from increased heat absorption efficiency (True 2003), but this remains to be tested. Effects of melanism on the thermoregulatory behavior of *P. vittatum* have been examined by Harris et al. (2013a, 2013b), who found darker morphs (unstriped morph) warm up rapidly and also have preferred high temperature as compared to lighter morphs. The presence or absence of stripes may therefore be under selection due to its important role in thermoregulation (Harris et al. 2013b).

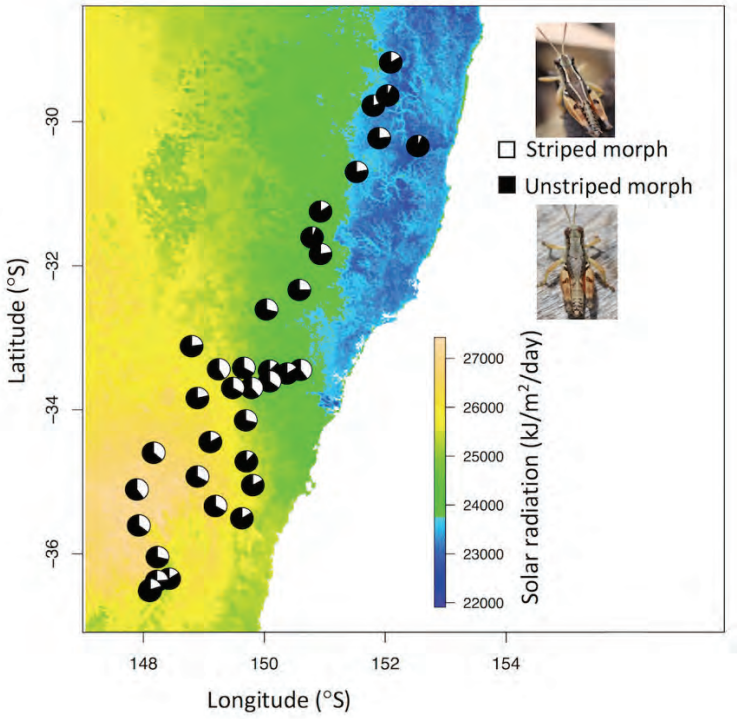

Figure 5: The change in striped morph (striped and partially striped pattern) and unstriped morph frequency is shown along the solar radiation gradient. White corresponds to the proportion of striped morphs within a site, and black corresponds to the proportion of unstriped morph within a site.

Several other factors such as predator avoidance via crypsis, disassortative mating or density-dependent selection exerted by predators (Dearn et al. 1984) may also influence the maintenance and distribution of stripe polymorphism. Disassortative mating is beneficial for maintaining

alleles responsible for a rare phenotypic trait (Follett et al. 2007). In *P. vittatum*, a strong selective constraint on striped individuals with homozygous C^S genotypes has been indicated previously via observations of their reduced viability (Dearn 1984). Strong viability selection on the homozygous genotype indicates a possible role of disassortative mating (observed in *P. vittatum* by Dearn 1980 and 1984) in maintaining heterozygous genotypes for stripe pattern. The co-occurrence of striped and unstriped morphs within sites also suggests that balancing selection may be operating to maintain stripe polymorphism in this species, which may be driven by fitness advantages of different morphs under different environmental conditions (e.g., in relation to solar radiation). Further study is needed to identify the role of disassortative mating and frequency-dependence in maintaining stripe polymorphism, and secondly, whether genes associated with stripes are under environmental selection, which may help to unveil the traits' adaptive significance.

The relatively low frequency of striped and partially striped individuals observed in our study is consistent with observations made in previous studies (Dearn 1981, 1984, Harris et al. 2012). Furthermore, we did not find a significant association between foliage cover and stripe pattern as was observed by Dearn (1984). This is possibly due to our use of foliage cover as a continuous variable (%), as opposed to Dearn (1984), who applied a categorical approach (forest vs pasture) with a much smaller spatial scale with just two 50-100 km transects located approximately 100 km apart. It is also likely that some unmeasured variables influenced the results of Dearn (1984). This inconsistency with the observations made by Dearn (1984) suggests that the relationship between stripe polymorphism and forest habitat does not hold true at broader spatial scales. Furthermore, an increase in the frequency of striped individuals (including striped and partially striped) was found from north to south (ranging from 28°S - 38°S) in Dearn (1981). The significant positive correlation between stripe pattern and solar radiation we find in the current study suggests that solar radiation is likely to be one of the contributing variables shaping this latitudinal increase in stripe pattern morphs.

Body size and solar radiation

Body size variation in insects is a vital life history trait due to its relation with physiology and fitness (Honek 1993, Nylin and Gotthard 1998), and exhibits clinal variation along latitudinal gradients in many ectotherms. Bergmann's rule states that body size increases with latitude, but no single pattern has been found in insects (Blackburn et al. 1999, Chown and Gaston 2010, Cushman et al. 1993, Kaspari and Vargo 1995). Factors such as temperature, seasonality, humidity, diet, and interactions among these factors can cause linear or non-linear clines in ectotherm body size (Roff 1980, Kennington et al. 2003, Ho et al. 2009, Çağlar et al. 2014).

In *P. vittatum*, body size variation was previously explained by rainfall and radiation seasonality (Harris et al. 2012). Our study suggests that solar radiation is significantly associated with increased body size in both males and females (Fig. S1, Table 3). Our finding of an insignificant relationship between annual precipitation and body size (Table 3) contrasts with previous observations made by Harris et al. (2012), which found this association to be significant. The number of study sites analysed (n = 61) in Harris et al. (2012) was larger than in the present study (n = 36) and spans a latitudinal gradient that is approximately 8 degrees wider. However, in the current study we analyse nearly five times the number of individuals as in Harris et al. (2012) (i.e. 198 individuals versus 954 individuals), allowing us to gain meaningful averages per site and higher data resolution.

Furthermore, the strong association between solar radiation and latitude in our study, suggests other factors associated with latitude may also influence body size variation in *P. vittatum.* For example, the availability of a longer growing season at higher latitudes may extend growth and development periods and result in larger adult body size (Blanckenhorn 2000, Blanckenhorn and Demont 2004). Larger body size, in turn, can have further fitness advantage such as increased chances of survival and mating (e.g. Waller and Svensson 2017). Our study suggests that climatic and topographical variables are interacting selection pressures acting on the latitudinal

variation in body size in *P. vittatum*. Further study using genetic analyses that examine signatures of selection along the sampled environmental gradient would be beneficial to elucidate this.

Conclusion

Environmental changes can drive an increased frequency of invertebrate pest outbreaks that bring economic costs and threaten food security (Macfadyen et al. 2018). Knowledge of how environmental conditions and habitat variation are correlated with morphological traits in invertebrate pests is therefore a necessary step for understanding pest responses and persistence under variable climatic conditions. Our study identifies a significant role of environmental and climatic variables on the relative abundance and distribution of morphological variation in *P. vittatum*, indicating that this species exhibits high variability, and potential adaptive responses that assist survival in different environments. We find that variation in body size, and in two discrete morphological traits, wing presence and stripe polymorphism can be maintained at large spatial scales, likely as a result of spatially varying selective pressures that are at least partially explained by environmental gradients. Notably, our study uses a dataset collected in just one season and provides a snapshot of correlations that may or may not be upheld over longer temporal scales, perhaps due to shifts in weather and resources. The results of this study are informative for investigation into the genetic basis of selection on morphology and environment, and ultimately be informative for understanding the future spread and persistence of this agricultural pest.

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References

ANDERSON, K. L., DEVESON, T. E., SALLAM, N. & CONGDON, B. C. 2010. Wind-assisted migration potential of the island sugarcane planthopper *Eumetopina flavipes* (Hemiptera: Delphacidae): implications for managing incursions across an Australian quarantine frontline. *Journal of Applied Ecology,* 47**,** 1310-1319.

AUKEMA, J. E., LEUNG, B., KOVACS, K., CHIVERS, C., BRITTON, K. O., ENGLIN, J., FRANKEL, S. J., HAIGHT, R. G., HOLMES, T. P. & LIEBHOLD, A. M. 2011. Economic impacts of non-native forest insects in the continental United States. *PLoS One,* 6**,** e24587.

BAI, Y., DONG, J.-J., GUAN, D.-L., XIE, J.-Y. & XU, S.-Q. 2016. Geographic variation in wing size and shape of the grasshopper *Trilophidia annulata* (Orthoptera: Oedipodidae): morphological trait variations follow an ecogeographical rule. *Scientific Reports,* 6**,** 32680.

BAILEY, P., FRENSHAM, A., HINCKS, A. & NEWTON, M. 1994. Competition for Herbage by *Phaulacridium vittatum* (Sjöstedt)(Orthoptera: Acrididae) and Sheep During Summer Drought. *Australian Journal of Entomology,* 33**,** 175-179.

BAKER, M. B., VENUGOPAL, P. D. & LAMP, W. O. 2015. Climate change and phenology: *Empoasca fabae* (Hemiptera: Cicadellidae) migration and severity of impact. *PloS one,* 10**,** e0124915.

BALE, J. S., MASTERS, G. J., HODKINSON, I. D., AWMACK, C., BEZEMER, T. M., BROWN, V. K., BUTTERFIELD, J., BUSE, A., COULSON, J. C. & FARRAR, J. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global change biology,* 8**,** 1-16.

BARBERCHECK, M. E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. *Florida Entomologist,* 539-548.

BARTON, K. 2018. Package 'MuMIn'. R package version 1.40. 4.

BATES, D., MÄCHLER, M., BOLKER, B. & WALKER, S. 2014. Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.

BLACKBURN, T. M., GASTON, K. J. & LODER, N. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and distributions,* 5**,** 165-174.

BLANCKENHORN, W. & DEMONT, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integrative and Comparative Biology,* 44**,** 413-424.

BLANCKENHORN, W. U. 2000. The evolution of body size: what keeps organisms small? *The quarterly review of biology,* 75**,** 385-407.

BRADSHAW, C. J., LEROY, B., BELLARD, C., ROIZ, D., ALBERT, C., FOURNIER, A., BARBET-MASSIN, M., SALLES, J.-M., SIMARD, F. & COURCHAMP, F. 2016. Massive yet grossly underestimated global costs of invasive insects. *Nature communications,* 7**,** 12986.

BRAENDLE, C., DAVIS, G. K., BRISSON, J. A. & STERN, D. L. 2006. Wing dimorphism in aphids. *Heredity,* 97**,** 192.

ÇAĞLAR, S. S., KARACAOĞLU, Ç., KUYUCU, A. C. & SAĞLAM, İ. K. 2014. Humidity and seasonality drives body size patterns in males of the bush cricket *Isophya rizeensis Sevgili,* 2003 (Orthoptera: Tettigoniidae: Phaneropterinae). *Insect science,* 21**,** 213-226.

CHAPPELL, M. & WHITMAN, D. 1990. Grasshopper thermoregulation. *Biology of grasshoppers. Wiley, New York,* 143-172.

CHOWN, S. L. & GASTON, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biological Reviews,* 85**,** 139-169.

CLARK, D. 1967. A population study of Phaulacridium vittatum Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.

CLIFT, A. & BAKER, G. 1998. Quantification of the relationship between rainfall and parasitism of *Phaulacridium vittatum* (Sjöstedt) by mermithid nematodes in the Central Tablelands of South-Eastern Australia. *Journal of Orthoptera Research,* 77-82.

COLOMBARI, F. & BATTISTI, A. 2016. Spread of the introduced biocontrol agent *Torymus sinensis* in north-eastern Italy: dispersal through active flight or assisted by wind? *BioControl,* 61**,** 127-139.
DA SILVA, E., KUHN, T. & MONTEIRO, L. 2011. Oviposition behavior of *Grapholita molesta Busck* (Lepidoptera: Tortricidae) at different temperatures. *Neotropical entomology,* 40**,** 415-420.
DAVIDOWITZ, G. 2008. Population and environmental effects on the size-fecundity relationship in a common grasshopper across an aridity gradient. *Journal of Orthoptera Research,* 17, 265-271.
DE WYSIECKI, M. L., ARTURI, M., TORRUSIO, S. & CIGLIANO, M. M. 2011. Influence of weather variables and plant communities on grasshopper density in the Southern Pampas, Argentina. *Journal of Insect Science,* 11.
DEARN, J. 1984. Colour Pattern polymorphism in the grasshopper *Phaulacridium vittatum*. I. Geographic variation in Victoria and evidence of habitat association. *Australian journal of zoology,* 32**,** 239-249.
DEARN, J. 1990. Color pattern polymorphism. *Biology of grasshoppers***,** 517-549.
DEARN, J. M. 1980. Evidence of non-random mating for the colour pattern polymorphism in field populations of the grasshopper *Phaulacridium vittatum*. *Australian Journal of Entomology,* 18**,** 241-243.
DEARN, J. M. 1983. Inheritance of the colour pattern polymorphism in the wingless grasshopper *Phaulacridium vittatum* Sjöstedt (Orthoptera: Acrididae). *Australian Journal of Entomology,* 22**,** 217-218.
DENNO, R. F. 1994. The evolution of dispersal polymorphisms in insects: the influence of habitats, host plants and mates. *Researches on Population Ecology,* 36**,** 127.
DENNO, R. F., RODERICK, G. K., OLMSTEAD, K. L. & DOBEL, H. G. 1991. Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. *The American Naturalist,* 138**,** 1513-1541.
DENNO, R. F., RODERICK, G. K., PETERSON, M. A., HUBERTY, A. F., DOBEL, H. G., EUBANKS, M. D., LOSEY, J. E. & LANGELLOTTO, G. A. 1996. Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. *Ecological Monographs,* 66**,** 389-408.
DRAKE, V. 1994. The influence of weather and climate on agriculturally important insects: an Australian perspective. *Australian Journal of Agricultural Research,* 45**,** 487-509.
DRAKE, V. & FARROW, R. 1988. The influence of atmospheric structure and motions on insect migration. *Annual review of entomology,* 33**,** 183-210.
DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Molecular ecology,* 27**,** 2576-2593.
FARROW, R., NICOLAS, G. & DOWSE, J. 1982. Migration in the macropterous form of the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt) during an outbreak. *Australian Journal of Entomology,* 21**,** 307-308.
FICK, S. E. & HIJMANS, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology,* 37**,** 4302-4315.
FIELDING, D. J. & BRUSVEN, M. 1990. Historical analysis of grasshopper (Orthoptera: Acrididae) population responses to climate in southern Idaho, 1950–1980. *Environmental Entomology,* 19**,** 1786-1791.
FOLLETT, P. A., CALVERT, F. & GOLDEN, M. 2014. Genetic studies using the orange body color type of Nezara viridula (Hemiptera: Pentatomidae): inheritance, sperm precedence, and disassortative mating. *Annals of the Entomological Society of America,* 100**,** 433-438.
FORSMAN, A. 1999. Variation in thermal sensitivity of performance among colour morphs of a pygmy grasshopper. *Journal of Evolutionary Biology,* 12**,** 869-878.
FOX, J. & WEISBERG, S. 2011. Multivariate linear models in R. *An R Companion to Applied Regression. Los Angeles: Thousand Oaks*.

GHALAMBOR, C. K., MCKAY, J. K., CARROLL, S. P. & REZNICK, D. N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional ecology,* 21**,** 394-407.

GRAY, S. M. & MCKINNON, J. S. 2007. Linking color polymorphism maintenance and speciation. *Trends in ecology & evolution,* 22**,** 71-79.

GRÖMPING, U. 2006. Relative importance for linear regression in R: the package relaimpo. *Journal of statistical software,* 17**,** 1-27.

GUERRA, P. A. 2011. Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: a meta-analysis. *Biological Reviews,* 86**,** 813-835.

GUERRA, P. A. & POLLACK, G. S. 2007. A life history trade-off between flight ability and reproductive behavior in male field crickets (*Gryllus texensis*). *Journal of Insect Behavior,* 20**,** 377-387.

HARRIS, R., MCQUILLAN, P. & HUGHES, L. 2012. Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *Journal of Biogeography,* 39**,** 1450-1461.

HARRIS, R. M., MCQUILLAN, P. & HUGHES, L. 2013a. Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). *PloS one,* 8**,** e80243.

HARRIS, R. M., MCQUILLAN, P. & HUGHES, L. 2013b. A test of the thermal melanism hypothesis in the wingless grass-hopper *Phaulacridium vittatum*. *Journal of insect science,* 13.

HARRIS, R. M., MCQUILLAN, P. & HUGHES, L. 2015. The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. *Journal of thermal biology,* 52**,** 75-83.

HARRISON, R. G. 1980. Dispersal polymorphisms in insects. *Annual Review of Ecology and Systematics,* 11**,** 95-118.

HERRMANN, D. L., KO, A. E., BHATT, S., JANNOT, J. E. & JULIANO, S. A. 2010. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Annals of the Entomological Society of America,* 103**,** 227-235.

HIJMANS, R. J., CAMERON, S. E., PARRA, J. L., JONES, P. G. & JARVIS, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International journal of climatology,* 25**,** 1965-1978.

HIJMANS, R. J. & VAN ETTEN, J. 2014. raster: Geographic data analysis and modeling. *R package version,* 2**,** 15.

HO, C.-K., PENNINGS, S. C. & CAREFOOT, T. H. 2009. Is diet quality an overlooked mechanism for Bergmann's rule? *The American Naturalist,* 175**,** 269-276.

HOFFMANN, A. A. 2017. Rapid adaptation of invertebrate pests to climatic stress? *Current opinion in insect science,* 21**,** 7-13.

HONĚK, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos***,** 483-492.

HULTHEN, A. D. & CLARKE, A. R. 2006. The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt)(Diptera: Tephritidae). *Australian Journal of Entomology,* 45**,** 16-19.

JEPSEN, J. U., HAGEN, S. B., IMS, R. A. & YOCCOZ, N. G. 2008. Climate change and outbreaks of the geometrids *Operophtera brumata* and *Epirrita autumnata* in subarctic birch forest: evidence of a recent outbreak range expansion. *Journal of Animal Ecology,* 77**,** 257-264.

JONAS, J. L., WOLESENSKY, W. & JOERN, A. 2015. Weather affects grasshopper population dynamics in continental grassland over annual and decadal periods. *Rangeland ecology & management,* 68**,** 29-39.

KEARNEY, M., SHINE, R. & PORTER, W. P. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences,* 106**,** 3835-3840.
KELLY, S. A., PANHUIS, T. M. & STOEHR, A. M. 2012. Phenotypic plasticity: molecular mechanisms and adaptive significance. *Compr Physiol,* 2**,** 1417-39.
KENNINGTON, W. J., KILLEEN, J. R., GOLDSTEIN, D. B. & PARTRIDGE, L. 2003. Rapid laboratory evolution of adult wing area in Drosophila melanogaster in response to humidity. *Evolution,* 57**,** 932-936.
KEY, K. L. 1992. Taxonomy of the genus Phaulacridium and a related new genus (Orthoptera: Acrididae). *Invertebrate Systematics,* 6**,** 197-243.
LANCASTER, L. T., DUDANIEC, R. Y., CHAUHAN, P., WELLENREUTHER, M., SVENSSON, E. I. & HANSSON, B. 2016. Gene expression under thermal stress varies across a geographical range expansion front. *Molecular ecology,* 25**,** 1141-1156.
LANCASTER, L. T., DUDANIEC, R. Y., HANSSON, B. & SVENSSON, E. I. 2015. Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion. *Journal of Biogeography,* 42**,** 1953-1963.
LANCASTER, L. T., DUDANIEC, R. Y., HANSSON, B. & SVENSSON, E. I. 2017. Do group dynamics affect colour morph clines during a range shift? *Journal of evolutionary biology,* 30**,** 728-737.
LE ROUZIC, A., HANSEN, T. F., GOSDEN, T. P. & SVENSSON, E. I. 2015. Evolutionary time-series analysis reveals the signature of frequency-dependent selection on a female mating polymorphism. *The American Naturalist,* 185**,** E182-E196.
LIEBHOLD, A. M. & TOBIN, P. C. 2008. Population ecology of insect invasions and their management. *Annual review of entomology,* 53.
LINDTKE, D., LUCEK, K., SORIA-CARRASCO, V., VILLOUTREIX, R., FARKAS, T. E., RIESCH, R., DENNIS, S. R., GOMPERT, Z. & NOSIL, P. 2017. Long-term balancing selection on chromosomal variants associated with crypsis in a stick insect. *Molecular ecology,* 26**,** 6189-6205.
LOCH, A. & FLOYD, R. 2001. Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in south-western Australia: History, current perspectives and future prospects. *Austral Ecology,* 26**,** 458-466.
LOSEY, J. E., HARMON, J., BALLANTYNE, F. & BROWN, C. 1997. A polymorphism maintained by opposite patterns of parasitism and predation. *Nature,* 388**,** 269.
MACFADYEN, S., MCDONALD, G. & HILL, M. P. 2018. From species distributions to climate change adaptation: Knowledge gaps in managing invertebrate pests in broad-acre grain crops. *Agriculture, Ecosystems & Environment,* 253**,** 208-219.
MASAKI, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution,* 21**,** 725-741.
MCCOLLOCH, J. & HAYES, W. P. 1922. The reciprocal relation of soil and insects. *Ecology,* 3**,** 288-301.
MIAO, H.-T., LIU, Y., SHAN, L.-Y. & WU, G.-L. 2018. Linkages of plant-soil interface habitat and grasshopper occurrence of typical grassland ecosystem. *Ecological Indicators,* 90**,** 324-333.
MILNER, R., HARTLEY, T., LUTTON, G. & PRIOR, C. 1994. Control of *Phaulacridium vittatum* (Sjöstedt)(Orthoptera: Acrididae) in Field Cages using an Oil-based Spray of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycetina: Hyphomycetes). *Australian Journal of Entomology,* 33**,** 165-167.
MILNER, R. & PRIOR, C. 1994. Susceptibility of the Australian plague locust, *Chortoicetes terminifera*, and the wingless grasshopper, *Phaulacridium vittatum*, to the fungi Metarhizium spp. *Biological control,* 4**,** 132-137.
MOUSSEAU, T. A. & ROFF, D. A. 1989. Adaptation to seasonality in a cricket: Patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution,* 43, 1483-1496.

NOSIL, P., VILLOUTREIX, R., DE CARVALHO, C. F., FARKAS, T. E., SORIA-CARRASCO, V., FEDER, J. L., CRESPI, B. J. & GOMPERT, Z. 2018. Natural selection and the predictability of evolution in Timema stick insects. *Science,* 359**,** 765-770.
NYLIN, S. & GOTTHARD, K. 1998. Plasticity in life-history traits. *Annual review of entomology,* 43**,** 63-83.
OERKE, E.-C. 2006. Crop losses to pests. *The Journal of Agricultural Science,* 144**,** 31-43.
OLFERT, O. & WEISS, R. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus, Meligethes viridescens* and *Ceutorhynchus obstrictus* in Canada. *Agriculture, ecosystems & environment,* 113**,** 295-301.
PARMESAN, C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology,* 13**,** 1860-1872.
PARSONS, S. M. & JOERN, A. 2014. Life history traits associated with body size covary along a latitudinal gradient in a generalist grasshopper. *Oecologia,* 174**,** 379-391.
PEPPER, J. & HASTINGS, E. 1952. The effects of solar radiation on grasshopper temperatures and activities. *Ecology,* 33**,** 96-103.
PONIATOWSKI, D. & FARTMANN, T. 2009. Experimental evidence for density-determined wing dimorphism in two bush-crickets (Ensifera: Tettigoniidae). *European Journal of Entomology,* 106.
QUANTUM, G. Development Team (2014).'QGIS Geographic Information System.'Open Source Geospatial Foundation Project.
RÍOS MARTÍNEZ, A. & COSTAMAGNA, A. 2018. Effects of crowding and host plant quality on morph determination in the soybean aphid, *Aphis glycines*. *Entomologia Experimentalis et Applicata,* 166**,** 53-62.
ROBERTS, R. 1972. A newly recognized from of pasture damage by *Phaulacridium vittatum* (Acrididae) on the northern tablelands of NSW. *Australian Journal of Entomology,* 11**,** 257-258.
ROFF, D. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. *Oecologia,* 45**,** 202-208.
ROFF, D. A. 1986. The evolution of wing dimorphism in insects. *Evolution,* 40**,** 1009-1020.
ROSETTI, N. & REMIS, M. I. 2018. Spatial variation in body size and wing dimorphism correlates with environmental conditions in the grasshopper *Dichroplus vittatus* (Orthoptera: Acrididae). *Environmental entomology,* 47**,** 519-526.
SASAKI, R., NAKASUJI, F. & FUJISAKI, K. 2002. Environmental factors determining wing form in the lygaeid bug, *Dimorphopterus japonicus* (Heteroptera: Lygaeidae). *Applied Entomology and Zoology,* 37**,** 329-333.
SGRO, C. M., TERBLANCHE, J. S. & HOFFMANN, A. A. 2016. What can plasticity contribute to insect responses to climate change? *Annual review of Entomology,* 61**,** 433-451.
SIMON, J.-C. & PECCOUD, J. 2018. Rapid evolution of aphid pests in agricultural environments. *Current opinion in insect science*.
STEENMAN, A., LEHMANN, A. & LEHMANN, G. 2015. Life-history trade-off between macroptery and reproduction in the wing-dimorphic pygmy grasshopper *Tetrix subulata* (Orthoptera Tetrigidae). *Ethology Ecology & Evolution,* 27**,** 93-100.
TAKAHASHI, Y., MORITA, S., YOSHIMURA, J. & WATANABE, M. 2011. A geographic cline induced by negative frequency-dependent selection. *BMC evolutionary biology,* 11**,** 256.
TANAKA, S. 2004. Environmental control of body-color polyphenism in the American grasshopper, *Schistocerca americana*. *Annals of the Entomological Society of America,* 97**,** 293-301.
TANAKA, S., HARANO, K.-I. & NISHIDE, Y. 2012. Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity. *Journal of insect physiology,* 58**,** 89-101.

TEAM, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013.
TRNKA, M., MUŠKA, F., SEMERÁDOVÁ, D., DUBROVSKÝ, M., KOCMÁNKOVÁ, E. & ŽALUD, Z. 2007. European corn borer life stage model: regional estimates of pest development and spatial distribution under present and future climate. *Ecological Modelling,* 207**,** 61-84.
TRUE, J. R. 2003. Insect melanism: the molecules matter. *Trends in ecology & evolution,* 18**,** 640-647.
TRULLAS, S. C., VAN WYK, J. H. & SPOTILA, J. R. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology,* 32**,** 235-245.
VENETTE, R. & RAGSDALE, D. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? *Annals of the Entomological Society of America,* 97**,** 219-226.
WALLER, J. T. & SVENSSON, E. I. 2017. Body size evolution in an old insect order: No evidence for Cope's Rule in spite of fitness benefits of large size. *Evolution,* 71**,** 2178-2193.
WEN, Y., JIN, X., ZHU, C., CHEN, X., MA, T., ZHANG, S., ZHANG, Y., ZENG, S., CHEN, X. & SUN, Z. 2016. Effect of substrate type and moisture on pupation and emergence of *Heortia vitessoides* (Lepidoptera: Crambidae): choice and no-choice studies. *Journal of insect behavior,* 29**,** 473-489.
Y GOMEZ, G. S. M. & VAN DYCK, H. 2012. Ecotypic differentiation between urban and rural populations of the grasshopper *Chorthippus brunneus* relative to climate and habitat fragmentation. *Oecologia,* 169**,** 125-133.
ZENG, Y. & ZHU, D.-H. 2014. Geographical variation in body size, development time, and wing dimorphism in the cricket *Velarifictorus micado* (Orthoptera: Gryllidae). *Annals of the Entomological Society of America,* 107**,** 1066-1071.
ZERA, A. J. & DENNO, R. F. 1997. Physiology and ecology of dispersal polymorphism in insects. *Annual review of entomology,* 42**,** 207-230.
ZERA, A. J. & HARSHMAN, L. G. 2001. The physiology of life history trade-offs in animals. *Annual review of Ecology and Systematics,* 32**,** 95-126.
ZHANG, Z., CAZELLES, B., TIAN, H., STIGE, L. C., BRÄUNING, A. & STENSETH, N. C. 2009. Periodic temperature-associated drought/flood drives locust plagues in China. *Proceedings of the Royal Society of London B: Biological Sciences,* 276**,** 823-831.

(A)

(B)

(A) *Phaulacridium vittatum* female chewing grass; (B) striped and unstriped morph of *Phaulacridium vittatum* at one of the collection sites (photos: Sonu Yadav).

CHAPTER 3

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Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (*Phaulacridium vittatum*)

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Author Contributions

All authors conceptualized the study. SY and RYD designed the study. I conducted fieldwork, collected data, labwork and performed analyses. I wrote the manuscript with editing and comments from Rachael Dudaniec and Adam Stow.

Abstract

Widespread species that exhibit both high gene flow and the capacity to occupy heterogeneous environments make excellent models for examining local selection processes along environmental gradients. Here we evaluate the influence of temperature and landscape variables on genetic connectivity and signatures of local adaptation in *Phaulacridium vittatum,* a widespread agricultural pest grasshopper, endemic to Australia. With sampling across a 900 km latitudinal gradient, we genotyped 185 *P. vittatum* from 19 sites at 11,408 single nucleotide polymorphisms (SNPs) using ddRAD sequencing. Despite high gene flow across sites (pairwise Fst = 0.0003-0.08), landscape genetic resistance modelling identified a positive non-linear effect of mean annual temperature on genetic connectivity. Urban areas and water bodies had a greater influence on genetic distance among sites than pasture, agricultural areas, and forest. Together, Fst outlier tests and Environmental Association Analysis (EAA) detected 242 unique SNPs under putative selection, with the highest numbers associated with latitude, mean annual temperature, and body size. A combination of landscape genetic connectivity analysis together with EAA identified mean annual temperature as a key driver of both neutral gene flow and environmental selection processes. Gene annotation of putatively adaptive SNPs matched with gene functions for olfaction, metabolic detoxification and ultra-violet light shielding. Our results imply that this widespread agricultural pest has the potential to spread and adapt under shifting temperature regimes and land cover change.

Keywords

Landscape genomics, *Phaulacridium vittatum*, grasshopper, landscape resistance, local adaptation, Environmental Association Analysis.

Introduction

Differential local selection pressures can result in spatially structured patterns of adaptive genetic variation (Schoville et al., 2012, Manel and Holderegger, 2013) that are generally considered to arise when gene flow is limited (García-Ramos and Kirkpatrick, 1997). High gene flow between populations may interfere with local adaptation by homogenising locally adapted genotypes and alleles under weak selection (Antonovics, 1976, Lenormand, 2002, Yeaman and Whitlock, 2011). However, the establishment and maintenance of local adaptation also depends on the introduction of novel genetic variation on which selection can act (Kawecki and Ebert, 2004, Tigano and Friesen, 2016, Fitzpatrick et al., 2015), and local adaptation may still occur under the neutralising effects of high gene flow (trees: Gonzalo-Turpin and Hazard, 2009, frogs: Muir et al., 2014, sharks: Momigliano et al., 2017, e.g. birds: Attard et al., 2018). Despite expectations that high genetic connectivity limits selection on adaptive loci within populations (Kawecki and Ebert, 2004, Rasanen and Hendry, 2008, Barton and Partridge, 2000), what we know about the interplay between gene flow and local adaptation remains varied and inconsistent (Savolainen et al., 2013). Therefore, concurrent examination of both neutral genetic connectivity and signatures of selection are needed to understand the relative contribution of these two processes in shaping adaptive genetic processes.

The interaction between gene flow and local adaptation is of particular interest for species that occupy large geographic ranges and a broad ecological niche. Many insect pests fit this description and experience variable environmental conditions across heterogeneous landscapes. Studies have reported the influence of abiotic environmental variables (e.g. Llewellyn et al., 2003, Sandrock et al., 2011, Zhang et al., 2018), geographic barriers (Wang et al., 2017) and landscape features (Keller et al., 2013, Lozier et al., 2013) on neutral gene flow in widespread insects. Regarding local adaptation in insect pests, some studies identify loci under selection in relation to environmental gradients (Janes et al., 2014, Manel et al., 2009, Feng et al., 2015), and insecticide resistance (Crossley et al., 2017, Paris et al., 2010). However, selection processes

relating to climate variables have not been well documented in insect pests (Kirk et al., 2013, Hoffmann, 2017, Pelissie et al., 2018), resulting in limited knowledge about their evolutionary response to shifting climate regimes. Despite their clear interaction, studies that simultaneously examine landscape or climate-mediated gene flow, as well as selection processes in the same study system are scarce.

Polymorphisms in physical characteristics are often observed across the distributions of some invasive or pest insects (e.g. Pener and Simpson, 2009), as well as developmental trait changes in response to temperature gradients, such as changes in hatching time (Falt-Nardmann et al., 2016), diapause (Moraiti et al., 2014), and voltinism (Yamanaka et al., 2008). In insects, morphological traits relevant for dispersal (e.g. wing presence, wing length), thermoregulation (e.g. melanism) and fecundity (e.g. body size) can offer fitness advantages across variable environments (Roff, 1986, Tanaka et al., 2012, Rosetti and Remis, 2018, Trullas et al., 2007), and may lead to selection at the genomic level (Brisson, 2010, Maeno and Tanaka, 2010, Bai et al., 2016, Zhang et al., 2019). The success of widespread generalist insects appears to be largely determined by interactions between morphology, environment, and behavior, which are affected by both neutral and adaptive genetic processes. Teasing apart these processes for economically important insect species is a contemporary challenge under current climate and land use change.

Landscape genomics approaches allow for complementary assessment of how environmental and landscape features influence genetic connectivity (Manel and Holderegger, 2013), and environmental selection processes (Balkenhol et al., 2017, Ahrens et al., 2018). Recent advancements in statistical approaches like Environmental Association Analyses allow for the correlation between allele frequency and environmental variables to be tested (Frichot et al., 2013, Coop et al., 2010, Gunther and Coop, 2013), and may identify selection on many genes of small effect (Rellstab et al., 2015). Identifying key drivers of neutral and adaptive landscape genomic relationships requires concurrent analyses of genetic connectivity and environmental

selection using the same dataset. For widespread species in particular, such a two-tiered analytical approach allows for a more complete understanding of how neutral genetic connectivity interacts with adaptive genetic divergence.

Commonly known as the wingless grasshopper, *Phaulacridium vittatum*, is endemic to Australia and considered a pest of agricultural pasture and crops (Key, 1992). Despite existing historically in low numbers within native forests and grasslands, increasing pastural and agricultural land use is thought to have triggered population expansions and outbreaks (Key, 1992, Dearn, 1984). Adult *P. vittatum* can cause serious damage to pasture and compete with livestock for green clover when green pasture is at a shortage during the summer (Bailey et al., 1994). The species also causes damage to tree nurseries (including native eucalyptus; Loch and Floyd, 2001), and economically important crops such as grapes, vegetables, and fruit trees (Roberts, 1972, Clark, 1967). Cyclic outbreaks of *P. vittatum* have been recorded every four or five years and are often observed during unusually dry weather conditions (Roberts, 1972).

Morphological variation in *P. vittatum* has been described in four distinct morphological traits: wing presence/absence, dorsal stripe polymorphism, color polymorphism, and body size (Yadav et al., 2018, Harris et al., 2012). Previous studies on *P. vittatum* showed significant associations between wing presence, stripe polymorphism and body size with local habitat (i.e. foliage cover) and climatic conditions (i.e. solar radiation, precipitation), and latitude (Dearn, 1984, Harris et al., 2012, Harris et al., 2013a, Harris et al., 2015, Yadav et al., 2018, Clark, 1967). Furthermore, melanism determined by the presence or absence of dorsal stripes provides thermoregulatory advantage such that darker indiviuduals with no stripes maintain higher body temperatures (Harris et al., 2013b, Harris et al., 2013a). These previous findings provide evidence for morphological tracking of environmental gradients and suggest a fitness advantage of different morphotypes in particular environments. However, the genetic basis or selection processes acting on this morphological variation are unknown.

Given that *P. vittatum* is common, with a large population size, broad distribution and a capacity to move long distances (Key, 1992), high levels of gene flow are expected. Nonetheless we predict that landscape and climatic features will affect genetic connectivity and adaptive genetic variation given the diverse environmental conditions the species occupies. With a sampling design spanning a ~900km latitudinal gradient of the *P. vittatum* distribution along the east coast of Australia, we conduct a landscape genomic analysis to examine, 1) how temperature and land cover affect neutral genetic connectivity; and 2) whether patterns of adaptive genetic variation are associated with environmental variables and/or variation in morphological traits (via F_{ST} outlier tests and Environmental Association Analysis; EAA). Our approach demonstrates a simultaneous assessment of landscape and climate effects on neutral gene flow and adaptive genetic variation, with implications for informing spatially-explicit management strategies for pest species across environmental gradients.

Materials and Methods

Study species and sampling

The wingless grasshopper (*P. vittatum*; Acrididae) is endemic to Australia with a widespread distribution in the eastern parts of the continent (Latitude: 23°- 43°S; Elevation: 0-1500m; Key 1992), largely concentrated towards the coastline where agricultural areas and grazed pastures are dominant. This species breeds annually and has non-overlapping generations. Hatching takes place in late spring (October-December) with adults emerging in summer (December-February). Development involves five instar stages and adults survive until late March and sometimes into April or May (Clark, 1967).

Collection of *P. vittatum* (adult and nymph) was undertaken in late January to early February in 2017, for two weeks, from 19 sites that spanned ~900km between -29.18° to -36.52°S (Table S1, Fig. 1A). Spatial coordinates (latitude, longitude) and elevation was recorded for each site using a handheld GPS device (Garmin International Inc., Olathe, KS, USA). We implemented a

paired-gradient sampling design where possible, with at least two sites per latitudinal range (see Dudaniec et al., 2018). This design is recommended to ensure representation of environmental heterogeneity with spatial replication (Rellstab et al., 2015), and is found to be more effective at detecting weak spatial selection in comparison to random or transect designs (Lotterhos and Whitlock, 2015). Grasshoppers were collected from semi-bare grassy patches along roadsides, borders of national parks, dry and green pastures and from shrubland in the Australian Kosciuszko alpine region, with a total elevational range spanning 133-1573 m above sea level (a.s.l.) and mean annual temperature gradient ranging from 6.0 to 17.4°C (Table 1). Insect sweep nets or small plastic jars were used to capture *P. vittatum*, approximately equal proportions of males and females were collected from each site. All samples were stored in 95% ethanol for DNA preservation.

Table 1. Details of total number of samples analysed for each site.

Site ID	No. of samples	Female N	Male N	Elevation (m)	Mean annual temperature (°C)
1	10	5	5	802	15
2	10	5	5	1103	13.2
3	10	5	5	1309	11.6
4	10	5	5	1045	12.8
5	10	5	5	483	16
6	10	5	5	413	15.9
7	8	4	4	133	17.4
8	10	5	5	421	15.5
9	10	5	5	816	12.5
10	10	5	5	984	11.8
11	10	3	7	738	12.5
12	10	5	5	982	11.1
13	10	5	5	400	14.3
14	10	5	5	633	13.1
15	8	4	4	226	15.4
16	10	6	4	665	12.5
17	10	5	5	1004	10.4
18	10	5	5	1573	6.7
19	9	4	5	1548	6

Morphological data

Phaulacridium vittatum exhibits variation in wing presence or absence; the majority of individuals have short non-functional wings whereas others have long functional wings. Studies have demonstrated an increased frequency of winged individuals in areas of high vegetation cover and near forested habitats (Yadav et al., 2018, Clark, 1967). This species also exhibits a stripe pattern polymorphism with three morphotypes (Dearn, 1984): 1) two complete white dorsal longitudinal stripes, 2) partial white stripes from head to pronotum, and 3) complete absence of stripes (Key, 1992). Stripe polymorphism appears to be controlled by a tri-allelic autosomal locus (C^S=full stripe; C^I=partial stripe; C^P=plain) with a dominance hierarchy, represented as, $C^S > C^I > C^P$ (Dearn, 1983). The function of stripes is not fully understood but may have implications for predator avoidance, mate choice or thermoregulation (Dearn, 1984, Harris et al., 2013b, Harris et al., 2013a, Dearn, 1980, Yadav et al., 2018). We examined morphological characters for each captured live individual and recorded data in the following categories 1) stripe polymorphism: fully striped, partially striped and unstriped; 2) wing dimorphism: winged and wingless. We measured femur length (in mm) using vernier calipers (accurate to 0.02 mm), which is a commonly-used proxy for body size in grasshoppers (Masaki, 1967, Mousseau and Roff, 1989, Lehmann and Lehmann, 2008).

DNA extraction, sequencing and filtering

DNA extraction was undertaken using the head tissue of 188 individuals of *P. vittatum* (from 19 sites), performed at the Diversity Arrays Technology sequencing (DArTseq) facility (Canberra, Australia; more detail in Supplementary Information S1). A modified double-digest restriction-site associated DNA (ddRAD) sequencing protocol described in Kilian et al. (2012) was performed using a combination of PstI-HpaII restriction enzymes in library preparation. The PstI enzyme adaptor was modified to contain an Illumina adaptor sequence, primer sequence and a variable-length barcode as described by Elshire et al. (2011), whereas the HpaII adaptor contained an Illumina flow cell attachment and overhang sequence. Digested and ligated

fragments with PstI-HpaII adaptors were amplified and sequenced on an Illumina HiSeq2500 and after trimming, reads of 65bp in length were obtained, each with a unique identifier. DNA sequences were aligned via BLAST using the *Locusta migratoria* reference genome with an E-value: 5e-3 and minimum sequence identity of >70%. To check for contamination, sequences were also aligned to bacterial and fungal genomes (NCBI). Further information on Single Nucleotide Polymorphism (SNP) calling, quality checking and filtering is in the supplementary information (Text S1).

DNA sequences were subject to further filtering using the *filter_dart()* function of the Radiator package (Gosselin, 2017) in R (Team, 2017). SNPs were excluded with a reproducibility of <90%, a call rate of <60% (i.e., the genotype is called in a minimum of 60% of the samples) and a depth of coverage of <8x. A minor allele frequency (MAF) threshold of ≥ 0.02 was applied to filter alleles using a SNP-based approach to exclude alleles potentially resulting from sequencing error. A minimum of two populations (out of 19) was required to pass the MAF threshold in order to retain the SNP marker.

Fst outlier detection

Analyses to detect selection were conducted on the full SNP dataset, which included all SNPs per RAD tag but excluded tightly linked loci identified using PLINK v1.9 (Purcell et al., 2007) (with one marker from each pair excluded with R2 >0.8; following Xuereb et al., 2018a). We used two Fst outlier detection methods to assess for congruence across approaches: 1) OutFLANK (Whitlock and Lotterhos, 2015), and 2) BayeScan 2.1 (Foll and Gaggiotti, 2008). OutFLANK is based on the Lewontin and Krakauer (1973) method, while accounting for sampling error and non-independence between sampled populations. OutFLANK detects outliers under divergent selection by initially inferring the Fst distribution from multiple loci and then fits a χ^2 model to the centre of the distribution, resulting in a null distribution. This null distribution is then used to detect outlier loci. We used a left and right trim value of 0.05 as

suggested by Whitlock and Lotterhos (2015). Two runs of OutFLANK were performed, initially setting the total number of putative genetic clusters (*K*) to 19 (total number of sites) and a second run was performed using *K* based on the outcome of the STRUCTURE analysis (see below). Loci with an expected heterozygosity of <10% were excluded as recommended by Whitlock and Lotterhos (2015), and the false discovery rate (FDR) was set to ≤5%.

BayeScan works on a Dirichlet multinomial distribution and accounts for different rates of immigration across subpopulations. Outlier loci under selection are detected by estimating Bayesian posterior probability using a Reversible Jump Markov Chain Monte Carlo algorithm. BayeScan identifies putative SNPs under positive (i.e. leads to increasing Fst) or negative (i.e. leads to decreasing Fst) selection, the latter having much lower certainty and a higher FDR. In BayeScan, we implemented a 50,000 burn-in, 100,000 total number of iterations, prior odds set to 100 and FDR of ≤5%.

Genetic structure analysis

To obtain a putatively neutral SNP dataset for genetic structure analyses, an 'unlinked' dataset with a single SNP per locus (chosen randomly per RAD tag) was generated to reduce the effects of physical linkage. We tested for loci deviating from Hardy-Weinberg Equilibrium (HWE) using the *pegas* R package (Paradis, 2010) and excluded loci from the 'unlinked' dataset that deviated from HWE at a 0.05 (simulations = 1,000) significance threshold in at least 10 of our 19 sites. Finally, we removed Fst outliers identified above and this filtered dataset was used for genetic structure and connectivity analyses.

We calculated pairwise Fst between 19 sites (Reynolds et al., 1983) in Arlequin 3.5 (Excoffier and Lischer, 2010) with a 0.05 significance level and 1,000 permutations. Isolation by distance (IBD) was assessed using a Mantel test of pairwise Fst and geographic distance (km) between sites (number of permutations=100,000) in VEGAN v2.5-2 package in R (Oksanen et al., 2013).

Genetic structure was further investigated using STRUCTURE 2.3.2 (Pritchard et al., 2000), which uses a Bayesian algorithm to assign individuals with shared ancestry into groups. Genetic structure analysis was carried out without providing prior location information, using the admixture model with correlated allele frequencies. To reduce computational time, we ran STRUCTURE parallelly on multiple cores using StrAuto (Chhatre and Emerson, 2017). We used 50,000 burn-in, 500,000 MCMC repeats with a range of *K* from one to 12 and ten iterations per *K*. The optimal *K* was selected using STRUCTURESELECTOR (Li and Liu, 2018*)*, which assigns a subpopulation to a cluster if the membership coefficient mean MedMeaK (median of means), MaxMeaK (maximum of means) or median MedMedK (median of medians) and 'MaxMedK' (maximum of medians) to a particular cluster is greater than the threshold value of 0.5. In addition, STRUCTURESELECTOR also perform the ΔK method (Evanno et al., 2005, Puechmaille, 2016), which compares the rate of change in log likelihood between each *K* and selects *K* with the maximum value of *ΔK*.

Temperature and landscape data

To test the effects of temperature and landscape variables on genetic connectivity in *P. vittatum*, two geographic datasets were used in raster format: 1) mean annual temperature (BIO1, hereafter referred to as 'Annual Temp') and 2) land cover. Annual Temp data for the study extent was downloaded from WorldClim v1.4 database (Hijmans et al., 2005) at ~1km^2 resolution. Temperature was predicted to be a major factor influencing genetic connectivity due to its important role in dispersal (Whitman, 1987), the population dynamics of grasshoppers (Chapman, 1990) and the distribution of herbivorous ectotherms (Bale et al., 2002, Pepper and Hastings, 1952). The preferred temperature range of *P. vittatum* is 27.5-30°C, and it avoids temperatures below 15°C (Harris et al., 2015). Thus we hypothesized warmer areas provide less resistance to dispersal for *P. vittatum*. The values for Annual Temp were therefore inverted to test for increasing resistance as temperature decreases.

Land cover data were downloaded from the Department of Agriculture and Water Resources, The Australian Bureau of Agricultural and Resource Economics and Sciences, Land use management (ABARES, 2017) and converted to a cell resolution of 150m x 150m. Based on prior ecological knowledge of *P. vittatum's* distribution and abundance in different habitats and locations (Clark, 1967), we collapsed land cover data in to five categories from least to most resistance by recoding the corresponding categories within the raster from 1-5 using the *r.reclass* module in GRASS GIS (Neteler et al., 2012). The categories were as follows: 1) pastures and agricultural areas 2) rural infrastructure, 3) forest and conservation areas, 4) urban area, 5) water bodies (Fig. S1).

The lowest resistance to movement was assigned to pasture and agricultural areas. Our previous study supports this classification because sites with the highest relative abundance of *P. vittatum* had low vegetation cover and occurred in agricultural areas (Yadav et al., 2018), while such areas are also suitable for egg laying in *P. vittatum* (Clark, 1967). The second least-resistance land cover was assigned to rural areas comprised of animal and plant production facilities and rural residential areas with and without agriculture, which are often present close to pastoral areas. The third ranking was assigned to forest and conservation areas. Forested areas were hypothesized to provide more resistance to movement than rural areas because *P. vittatum* is absent from rainforest and dense sclerophyll forested areas (Key, 1992) but can be found near the boundary of pasture and forested areas (Dearn, 1984), and grasslands within forested national parks. Urban areas were assigned with fourth ranking (i.e. manufacturing, urban residential, transport, and other service infrastructure). The final and most resistant land cover was assigned to water bodies (i.e., lake, reservoir, river, marsh and estuary water), which are considered a significant barrier to movement.

Landscape resistance surface generation

To assess the influence of temperature and landscape variables on genetic connectivity, we used an isolation by resistance approach applying circuit theory (McRae and Beier, 2007) and sample size corrected Akaike information criterion (AICc) model selection. This approach has been shown to effectively identify isolation by resistance including linear and non-linear relationships (Dudaniec et al., 2013, Dudaniec et al., 2016, Shirk et al., 2010). We generated multiple resistance surfaces from our parameterised Annual Temp and Land Cover data to test for multiple hypotheses about their effects on genetic distance. To generate alternative resistance surfaces with varying resistance, we apply a similar method as implemented in (Dudaniec et al., 2013, Dudaniec et al., 2016), which is a modified approach of Shirk et al. (2010), whereby different values of intercept (α) and slope (γ) parameters are used to create linear and non-linear resistance surfaces. We applied the following formulas to generate resistance relationships:

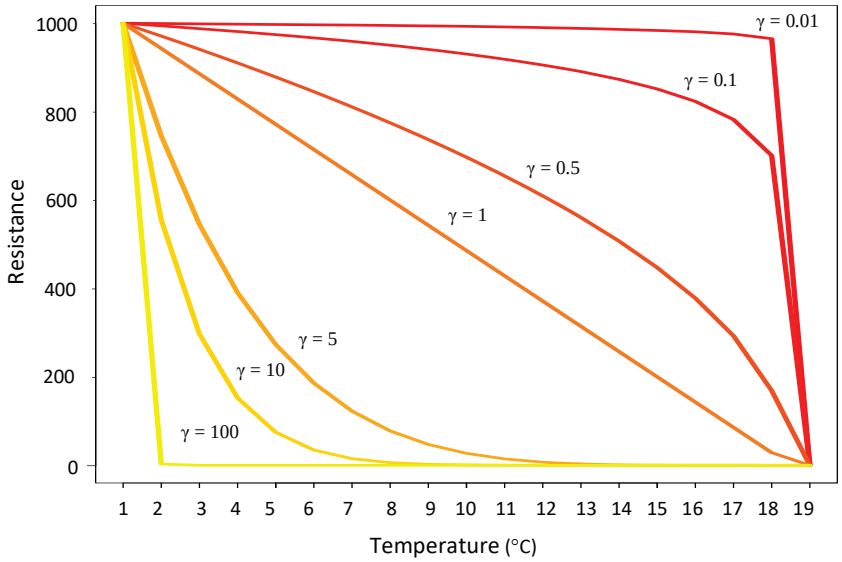
$r_i = 1 + \alpha\,(T_i - 1/max - 1)^{\gamma}$ [1]

$r_i = 1 + \alpha\,(L_i - 1/max - 1)^{\gamma}$ [2]

Here, r_i is the resistance of cell i, T_i is the Annual Temp of cell i [1], L_i is the rank of Land Cover type of cell i [2], and max is the maximum value of the raster surface. Furthermore, α determines the maximum possible resistance value and γ determine the shape of the relationship (slope) between tested variables and resistance. As outlined above, resistance was assumed to be negatively associated with Annual Temp and positively associated with Land Cover rank.

We created resistance surfaces for all combinations by varying the values of parameters α and γ. We used five α values and seven γ values: α=0,5,10,100,1000 and γ=0.01, 0.1,0.5,1,5,10,100 for each of the Annual Temp and Land Cover datasets, resulting in 29 different resistance surfaces for each dataset with subtle differences in the degree of resistance. An α=0 equated to a null IBD model where all raster cells were assigned a value equal to one, indicating no influence of resistance on genetic connectivity. The values of γ <1 are resistance surfaces with increased sensitivity, γ=1 represents a linear resistance relationship and γ >1 are resistance surfaces with reduced resistance sensitivity (Fig. 2).

(A)



(B)

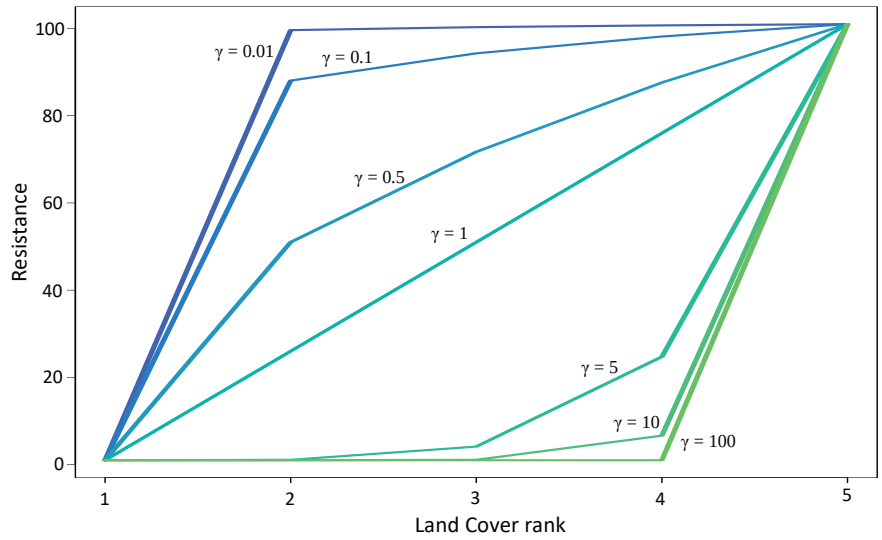


Figure 2. Line plots of landscape resistance models tested for mean annual temperature (BIO1) and Land Cover rank on Fst matrix. The γ values represents the shape of the slope (the linear/non-linear relationship between variables tested and resistance). Values on Y axis represents α with variables degree of resistance. (A) All tested parameters of γ on BIO1 are represented by different lines, line plot with γ=0.5 represents the best selected model. (B) tested values of γ on Land Cover ranking, line plot with γ=10 represents the best selected model. 1=pasture areas and agricultural areas, 2=rural development, 3=forest and conservation areas, 4=urban, 5=water bodies.

Pairwise resistance distance matrices were calculated for all 29 transformed rasters for both the variables (i.e., Annual Temp and Land Cover) using CIRCUITSCAPE v 4.0.5 (Shah and McRae, 2008). CIRCUITSCAPE applies electrical circuit theory to identify all possible pathways of movement between focal points across a given raster dataset and calculates average cumulative resistance between each pair of the sampling site. The pairwise mode was used to obtain pairwise resistance matrices between 19 sample sites with a 4-cell connection scheme such that each sampling site was connected to its neighbouring four cells.

Landscape genetic resistance modelling

Pairwise resistance distances between sites created in CIRCUITSCAPE for both Annual Temp and Land Cover were input as predictors of genetic distance (Fst) between sites using a mixed-effects model with maximum likelihood population-effects (MLPE) (Row et al., 2017) as implemented within the *MLPE_rga()* function of "ResistanceGA" R package (Peterman, 2018). The MLPE approach was found to have the highest accuracy in predicting landscape resistance relationships out of seven regression-based model selection methods assessed in a recent review (Shirk et al., 2018).

To identify which model(s) best described genetic distance between sites, multi-model inference was used to obtain a candidate list of models based on AICc scores for both Annual Temp and Land Cover in the MuMIN v1.40.4 R package (Barton, 2018). As AIC model selection was found to outperform other model selection approaches within MLPE (Row et al., 2017) we used AICc to evaluate the goodness of fit (Burnham and Anderson, 2003). The model with the lowest change in AICc score (ΔAICc=0) and largest AIC weight (wAICc) was considered as most parsimonious. The inference was made from the relative importance of multiple models, which is more informative than a single best-selected model in the case of model uncertainty (Burnham and Anderson, 2003). We followed the ΔAICc criteria classification mentioned in Burnham and Anderson (2003) to obtain the models with the highest support.

Environmental data for EAA

We chose environmental variables deemed relevant for local adaptation in *P. vittatum* based on existing ecological and biological knowledge of the species (Yadav et al., 2018, Harris et al., 2015, Harris et al., 2012). Justification of the environmental variables used in EAA is presented in the supplementary Information (S2). Annual Temp, annual precipitation (BIO12), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), precipitation of the warmest quarter (BIO18), precipitation of the coldest quarter (BIO19) were extracted from WorldClim version 1.4 (Hijmans et al., 2005). Data for annual wind speed and annual solar radiation were extracted from WordClim version 2.0 (Fick and Hijmans, 2017). All data were extracted at a resolution of ~1 km^2 (Table S1 per site information). Foliage projective cover (% FPC) was extracted from Terrestrial Ecosystems Research Network AusCover database (TERNAusCover, 2011) at a resolution of 5x5m. To extract FPC values, a buffer of 100m was used around each sampling site from which cell values were averaged using the *raster* R package (Hijmans and van Etten, 2014).

To determine soil moisture and soil acidity per site, soil type data for each site were obtained from the Office of Environment and Heritage, New South Wales (OEH, 2017) and raster values were extracted for each site using QGIS v2.18 (QGIS, 2016). Soil moisture per site was classified based on clay content and water holding capacity of the soil as described by The Australian Soils Classification guidelines (ASC) and 'Great Soil Group' (GSG) (Raymond, 2016). Likewise, pH was used to assess soil acidity, soils with pH <5.5 were classified as 'acidic' and coded as 1, whereas soils >5.5 pH were classified as 'non-acidic' and were coded as 0 (Table S1), which is consistent with the soil acidity classification criteria of ASC and GSG.

To assess collinearity between the 11 environmental variables, latitude, longitude and elevation for use in EAA, we performed pairwise Pearson's correlation analyses of the site-based environmental data in R (Table S2) and discarded highly correlated variables ($|r| > 0.80$). The

removal of highly correlated environmental variables is recommended for EAA as high collinearity may limit the ability to tease apart effects of individual variables and may lead to inconclusive results (Rellstab et al., 2015). After removing highly correlated variables, latitude plus six environmental variables were used in LFMM: (i) Annual Temp, (ii) BIO18, (iii) Wind Speed, (iv) % FPC, (v) soil moisture and (vi) soil acidity. We chose to retain Wind Speed despite having a high correlation with Annual Temp (r=-0.86) due to its significant role in determining relative abundance of *P. vittatum* (Yadav et al., 2018) and potential role in selection in insects (e.g. Dudaniec et al. 2018).

Morphological association analysis

Three discrete morphological traits were examined for significant SNP x morphology associations: wing dimorphism, stripe polymorphism and body size. Collinearity among the three morphological predictors and between the morphological and environmental predictors was assessed (Pearson $r| > 0.80$) before including them in the analysis (Table S2). Winged individuals were coded as 1, and wingless individuals as 0. Stripe pattern was divided into four categories:1) fully striped 2) partially striped 3) both fully and partially striped ("Striped"), and 4) unstriped (described in Yadav et al., 2018). Finally, body size (based on femur length) was tested for SNP associations as a continuous variable. Body size is sex biased in this species, whereby females have larger body size than males, therefore to confirm outliers obtained from EAA are associated with body size and not biased by sex, we performed a t-test on allele frequencies of each outlier SNP and sex (coded as a binary variable).

Detecting environmental and morphological signatures of selection - EAA

Using the full dataset, we conducted SNP-association analyses for both environmental variables and morphological traits using the Latent Factor Mixed Model (LFMM) program (Frichot et al., 2013) within the LEA R package (Frichot and Francois, 2015). LFMM examines the association between environmental factors and allele frequencies using Markov Chain Monte Carlo

algorithm while accounting for neutral genetic structure by introducing it as a latent factor and can effectively detect loci under weak selection (Frichot et al., 2013). We performed LFMM with 100,000 iterations after a 10,000 burn-in, five repetitions for each variable and the number of latent factors was based on STRUCTURE analysis outcome. Median z scores were combined across five runs, and adjusted p values were calculated as recommended by Frichot and Francois (2015). The Benjamini-Hochberg procedure was applied to obtain candidate loci with an expected FDR of 5%. To examine inflation in the test statistics Genomic Inflation Factor (GIF) was calculated using the formula λ=median (z^2)/0·456 as described by (Devlin and Roeder, 1999) for each variable individually. GIF scores of each variable were then assessed for its closeness to 1.0 as suggested by Frichot and Francois (2015). When LFMM detected more than one SNP marker on the same RAD tag, we randomly retained one of the markers for analyses of common and unique associations among variables.

Gene annotation

Gene annotations were sought for all unique RAD tags that contained SNPs under putative selection (identified via Fst outlier tests and EAA) using the NCBI BLAST platform (Johnson et al., 2008). Due to the unavailability of a *P. vittatum* genome, RAD tags were aligned to the *Locusta migratoria* genome (Wang et al., 2014) using the BLASTN mode, with an E-value threshold of 0.0001. Sequences were also annotated to genes classified as 'insects' (Taxid:6960), using BLAST with an E-value threshold of 0.0001.

Results

Data filtering

The sample size of sequenced individuals ranged from 8-10 individuals per site (mean=9.72 ± 0.16; Table 1). A total of 37,644 SNP markers (including polymorphic and monomorphic) were initially obtained from DArTseq with an average of 0.11 polymorphic information content (PIC; an index ranging from zero to one that indicates allelic variation per SNP marker). After filtering

SNPs using *filter_dart()*, we identified 56 tightly linked SNPs (with LD correlation R^2 >0.8) using PLINKv1.9 that were then excluded, resulting in a final full dataset of 11,408 SNPs. For genetic structure analysis, a total of 3,257 SNPs were retained, which included only one SNP per RAD tag (i.e. 'unlinked') and excluded Fst outliers (see below), and loci deviating from HWE (*n*= 203). Three samples from three different sites were excluded from the dataset due to poor sample quality or a high proportion of missing data, resulting in a final sample size of 185 individuals.

Fst outlier detection

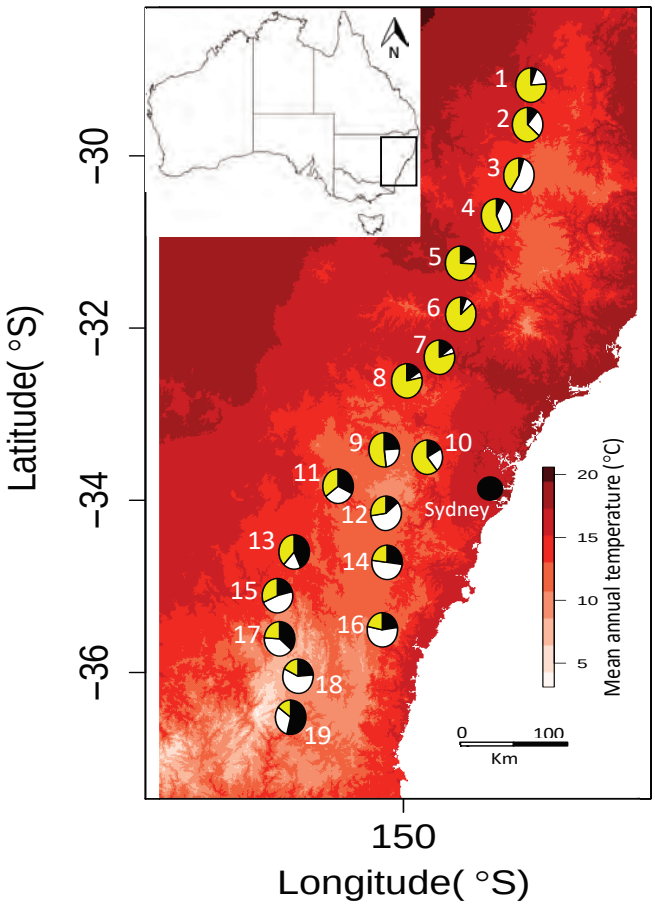
Out of 11,408 total SNPs, OutFLANK identified three outlier SNPs under divergent selection that showed no evidence of linkage as they were located on separate DNA fragments and were not identified to be in linkage disequilibrium using PLINKv1.9 (Table S3, Fig. S2A). Both runs of OutFlank (i.e. with *K*=19 and *K*=3; identified by STRUCTURE, below) identified the same three outlier SNPs. BayeScan identified 16 outlier SNPs under divergent selection, also located on distinct DNA fragments (Table S4, Fig. S2B) and a single outlier under balancing selection. All three outliers detected by OutFLANK were in common with the outliers detected by BayeScan.

Genetic structure

Pairwise Fst analysis using 3,257 SNPs (filtered as described above) varied between 0.0003-0.08 across sites with mean Fst=0.030 (Table S5). A Mantel test indicated no IBD (*P*=0.79, Mantel statistic *r*=-0.080; Fig. S3). STRUCTURE (Pritchard et al., 2000) analysis indicated the most likely number of genetic clusters to be three (*K*=3), based on the agreement between ΔK method and estimators of STRUCTURESELECTOR (Fig. S4). Out of the four estimators of STRUCTURESELECTOR i.e. MedMed, MedMean, MaxMed, and MaxMean, all except MaxMed indicated optimal *K* to be 3 (Fig. S4). Based on these results we used *K*=3 as the optimal K for further analyses. However, individuals were weakly assigned to each cluster with

high genetic admixture (Fig. 1B). Based on a ≥ 0.50 assignment probability cut-off, mean (±s.e.) assignment probabilities to each cluster was as follows: cluster 1=0.70 (±0.01), cluster 2=0.68 (±0.05) and cluster 3=0.67 (±0.02). Individuals from more northern and mid latitudinal ranges (sites 1-11) were predominantly assigned to cluster 1 (Fig. 1B), whereas individuals from southern latitudes (sites 12-19) were chiefly assigned to cluster 2. Some north latitude sites (sites 1-4) also showed a greater assignment to cluster 2, than mid latitudinal sites (sites 5-8). Cluster 3 has a low frequency overall but was represented more in southern sites (sites 15-19).

(A)



(B)

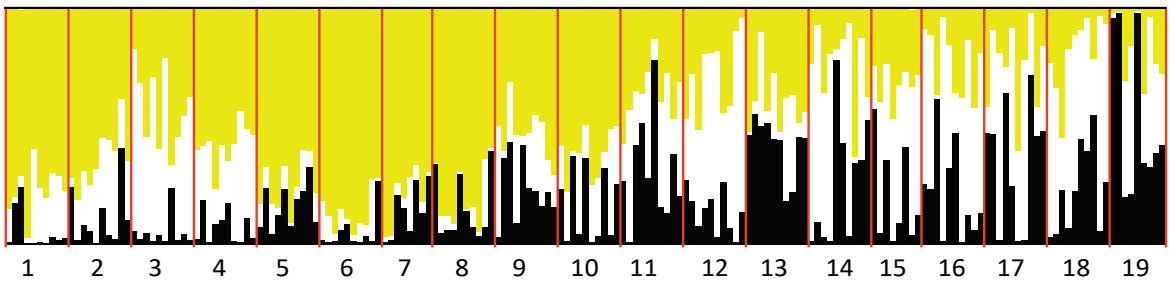


Figure 1. A) Map of sample sites (*n*=19) displayed against the mean annual temperature map (BIO1). STRUCTURE analysis indicated three genetic clusters (*K*=3) shown in three different colors in the pie chart (Yellow=Cluster 1; White=Cluster 2; Black=Cluster 3). The proportion of the pie chart indicates the assignment probability of individuals to the respective clusters based on the population Q-matrix obtained from STRUCTURE. B) Bar plot of assignments of individuals to each cluster, the proportion of colour represents the proportion of individual assignment to each of the three genetic clusters. Clusters are grouped together based on sampled sites divided by a red line.

Landscape genetic resistance

For Annual Temp, models with ΔAICc >1 were excluded from the confidence set of models (Table 2), which resulted in a candidate set of eight resistance surfaces (out of 29). The best supported resistances surface for Annual Temp was α=1000 and γ=0.5 (ΔAICc=0.00, w_i=0.07), which indicates that maximum resistance is 1000 times greater than zero (Fig. 3A). The value of γ=0.5 indicates temperature influenced genetic connectivity non-linearly, with upper mean annual temperatures of around 19°C providing close to zero resistance to genetic connectivity (Fig. 2A). Together the eight-confidence set of models covered a weight of 0.46 (Table 2). All confidence sets of resistance surfaces had γ values <1, indicating resistance surfaces with increased resistance sensitivity were most supported.

For Land Cover, models with ΔAICc >2 were excluded from the candidate set of models, which provided a set of 10 candidate resistance surfaces (Table 2). The most supported resistance surface had α=100 and γ=10 (ΔAICc=0.00, w_i=0.18) (Table 2, Fig. 3B). This suggests that the landscape resistance to gene flow is 100 times greater than zero and shows a non-linear relationship across sites. Resistance due to land cover rank was close to zero until rank three (i.e. pasture/agricultural areas, rural infrastructure, and forest/conservation areas), but started to increase up to rank five (i.e. water bodies; Fig. 2B). Together a confidence set of 10 models

covered a total weight of 1.00 (Table 2). The confidence set of models had γ values ranging from 5,10 to 100 (i.e. above γ=1), indicating resistance surfaces with lower land cover resistance are favoured in the model selection. Furthermore, our best-selected resistance surface for Land Cover was ten times less resistant to genetic connectivity (α=100) compared to the best-selected Annual Temp resistance surface (α=1000) (Fig. 3B), indicating a higher impact of temperature on genetic connectivity than land cover.

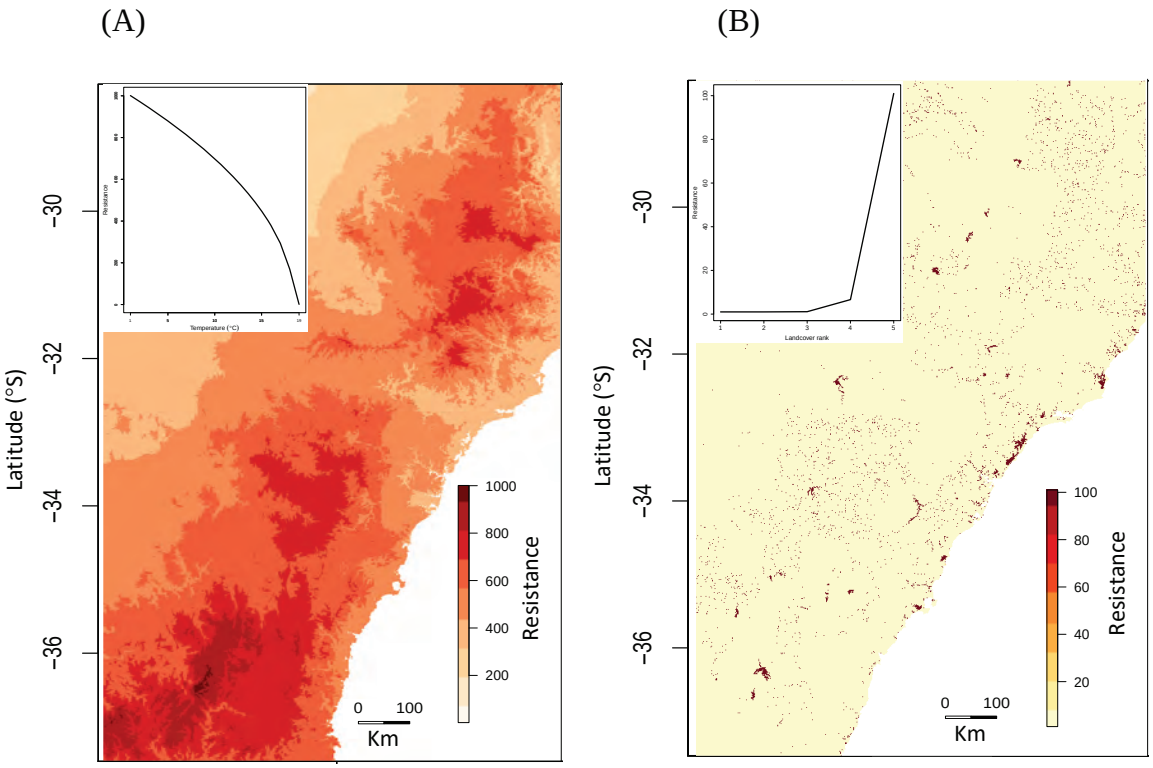


Figure 3. Landscape surfaces identified as the best-selected surfaces in landscape genetic modelling analysis; A) Mean annual temperature (BIO1); B) Land Cover. The darker regions represent highest resistance. Land Cover provides 10 times less resistance than BIO1. (Inset=Line plot of best selected model). Land cover ranking: 1) Pastures and agricultural areas 2) Rural infrastructure, 3) Forest and conservation areas, 4) Urban area, 5) Water bodies.

Table 2. List of the candidate set of models and null model (maximum possible resistance $\alpha = 0$; represents no resistance by landscape) for mean annual temperature (BIO1) and Land Cover. α=degree of resistance; γ=shape of the correlation.

Alpha (α)	Gamma (γ)	logLik	AICc	Delta	Weight (*wi*)
Mean annual temperature					
1000	0.5	473.58	-938.92	0.00	0.07
100	0.5	473.57	-938.91	0.01	0.07
10	0.5	473.52	-938.79	0.13	0.07
1000	0.1	473.23	-938.21	0.71	0.05
5	0.5	473.23	-938.21	0.71	0.05
100	0.1	473.22	-938.21	0.71	0.05
10	0.1	473.21	-938.17	0.75	0.05
5	0.1	473.19	-938.14	0.78	0.05
Null model	-	473.02	-937.81	1.12	0.04
Land Cover					
100	10	473.73	-939.22	0.00	0.18
10	10	473.25	-938.25	0.98	0.11
10	5	473.17	-938.09	1.13	0.10
5	5	473.16	-938.08	1.15	0.10
1000	100	473.12	-938.00	1.23	0.10
5	10	473.09	-937.94	1.28	0.09
100	100	473.06	-937.89	1.34	0.09
10	100	472.97	-937.69	1.53	0.08
5	100	472.94	-937.64	1.59	0.08
Null model	-	472.87	-937.50	1.72	0.07

Environmental and Morphological Association Analysis

LFMM analysis identified a total of 262 SNPs under putative selection out of 11,408 total SNPs (2.3%) of which 231 (mean Fst=0.17±0.01; Fig. S5) were located on unique DNA fragments and these were used in further analysis. Out of 231 SNPs, 177 SNPs were associated with environmental variables (unique and overlapping) and 54 SNPs were associated with morphological variables (Table 3). For geographic and environmental variables, the highest number of SNPs detected as being under putative selection were associated with latitude (*n*=100, with *n*=84 uniquely associated) and Annual Temp (*n*=37, with *n*=22 uniquely associated) (Table 3). Fourteen SNPs associated with Wind Speed (Fig. S6), but these also associated with other variables, including Annual Temperature (*n*=7 in common), providing limited evidence for an independent effect of Wind Speed on local adaptation.

Table 3. Details of outliers detected by Fst outlier tests (BayeScan and OutFlank) and EAA approach. A total of 251 SNPs (unique and overlapping) were identified as outliers by all the methods. Numbers of common outliers (i.e. those overlapping among tested variables) are shown here for each variable used in the analysis. GIF=Genomic Inflation Factor; BIO1=Annual mean temperature (°C); BIO18=Precipitation of warmest quarter (mm); FPC=Foliage Projective Cover (%), Striped includes fully striped and the partial striped pattern.

Approach	Variables	Total outliers	Unique outlier	GIF	Latitude	BIO1	BIO18	Wind speed	FPC	Soil moisture	Soil acidity	Fully striped	Partial striped	Striped	Unstriped	Body size	BayeScan
LFMM	Latitude	100	84	1.01	-												
Environment	BIO1	37	22	1.02	10	-											
	BIO18	6	4	0.79	1	1	-										
	Wind speed	14	4	1.06	5	7	1	-									
	FPC	6	4	0.78	2	2	0	0	-								
	Soil moisture	10	10	0.76	0	0	0	0	0	-							
	Soil acidity	4	4	0.80	0	0	0	0	0	0	-						
LFMM	Fully striped	1	0	0.77	0	0	0	0	0	0	0	-					
Morphology	Partial striped	12	12	0.63	0	0	0	0	0	0	0	0	-				
	Striped	1	0	0.76	0	0	0	0	0	0	0	1	0	-			
	Unstriped	1	0	0.76	0	0	0	0	0	0	0	1	0	1	-		
	Body size	39	36	0.97	2	0	0	0	0	0	0	1	0	1	1	-	
Fst outlier	BayeScan	17		-	1	0	0	0	0	0	1	0	0	0	0	1	-
	OutFlank	3		-	1	0	0	0	0	0	0	0	0	0	0	1	3
All		251															

For morphological associations, the highest number of SNPs (*n*=39) were associated with body size (Table 3, Fig. S7A). Of these associations, 36 were unique to body size. All SNPs with possibly high linkage were excluded from the analysis during filtering thus it would be difficult to assess whether these SNPs unique to body size are part of a gene block or are in linkage. Only three outliers that associated with body size were also significantly associated with sex (*P* value <0.05), indicating that body size outliers were not biased by differences between the sexes. For signatures of selection in relation to stripe polymorphism, the highest number of SNP-associations were with the partially striped morph (*n*=12, all unique SNP associations). Only a few associations were found with the fully striped morph (*n*=1) and unstriped morph (*n*=1; Table 3, Fig. S7B). No associations were found in relation to wing dimorphism. GIF values (mean= 0.84 ±0.03) ranged from a minimum of 0.63 for partially striped to a maximum of 1.06 for Wind Speed, while other variables had GIF scores close to 1.0. GIF scores and p-value distributions for each tested variable indicated that confounding genetic structure was well-accounted for in the model and our chosen FDR threshold was appropriate (Fig. S8).

Gene annotation

Gene annotation of DNA fragments that contained SNPs identified via Fst outlier tests or EAA resulted in successful annotation of four SNPs, using the *L. migratoria* genome on the BLAST database (Table S6). All four annotated SNPs were identified only using EAA and showed significant associations with latitude (*n*=1), Annual Temp (*n*=1), and body size (*n*=2) in LFMM. The SNPs associated with latitude and body size were annotated to cell membrane receptors (glutamate receptor 3 and ionotropic glutamate receptor gene), which are involved in neurotransmission, chemical signaling, and olfaction (Dingledine et al., 1999, Usherwood, 1994). The SNP associating with Annual Temp was annotated to the gene glucosyl glucuronosyl transferase (mRNA gene), which transcribes an enzyme involved in the transfer of a glucuronoyl group to other molecules and has a role in metabolism (Ahn et al., 2012). Furthermore, annotation to ‘insects’ as an organism using BLAST did not result in any additional annotations.

Discussion

Using complementary analyses of neutral landscape genetic connectivity and adaptive genetic variation, we find evidence for local adaptation in the presence of high gene flow along an environmental gradient for the grasshopper, *P. vittatum*. Using a landscape genetic resistance approach, we were able to detect subtle barriers to genetic connectivity, mainly due to low mean annual temperatures. Cleared, agricultural habitat was associated with increased genetic connectivity, however resistance of this land cover was 10 times less than for temperature. Further, mean annual temperature was associated with the greatest number of SNPs under putative selection compared with other variables. Our independent analyses of landscape resistance to gene flow and detection of selection signatures show that temperature is a dominant variable influencing evolutionary and dispersal processes in this agricultural pest. With respect to morphological variables, selection on body size was most evident based on the number of positive SNP associations. SNP annotations indicated the involvement of genes that may be relevant for local environmental adaptation across latitudinal gradients. By applying this two-tiered approach to a widespread pest species, we provide insights into the spatial drivers of genetic connectivity and the capacity of this agricultural pest to adapt to diverse local environments.

Temperature and land cover effects on genetic connectivity

Widespread species with high dispersal capacity often show less pronounced spatial genetic structure with lower Fst distributions (e.g. Llewellyn et al., 2003, Yin et al., 2017, Vorburger et al., 2003). Minimal genetic structuring in *P. vittatum* was anticipated due to the species' high abundance and mobility across pastures and agricultural areas of Australia (Yadav et al., 2018, Farrow et al., 1982). The lower genetic differentiation in wide-ranging species is often coupled with an absence of IBD (e.g. in cotton aphids: Gholamian et al., 2018) as found in the current study for *P. vittatum.* However, in species with broad tolerances in terms of habitat and climatic

conditions, environmental factors may still facilitate or restrict gene flow in more subtle ways that are not detectable using standard, non-spatially explicit analyses of genetic structure.

In ectotherms, temperature predominanty controls locomotion (Whitman, 1987), developmental rates (Clissold and Simpson, 2015) and habitat selection (Gillis and Possai, 1983) with important consequences for population size and dispersal (Fielding and Brusven, 1990, Cannon, 1998). Our analysis found that resistance due to mean annual temperature had an effect on genetic connectivity that was ten times greater than land cover, providing evidence for the crucial role of temperature in regulating neutral genetic connectivity in *P. vittatum*. Studies have found that *P. vittatum* maintains its body temperature via behavioral thermoregulation, and this is facilitated by body melanism (Harris et al., 2013b, Harris et al., 2013a). Lower temperatures may therefore prevent preferred body temperatures from being maintained, which in turn may significantly restrict the dispersal and distribution of individuals with consequences for genetic connectivity.

The best resistance model for land cover indicated that pasture, agricultural areas, rural areas and forested areas facilitate movement and do not limit genetic connectivity of *P. vittatum* (Fig. 2B). These land cover types provide preferred temperatures and offer food resources such as pasture and clover (Roberts, 1972). Further, previous findings of high abundance of the winged form (i.e. morph relevant for increased dispersal) within shrub dominated areas and forest margins (Yadav et al., 2018, Clark, 1967) is consistent with our finding of low resistance to gene flow across partially forested areas. Though less of an effect than temperature, water bodies and urban areas were found to be significant barriers to gene flow and may restrict the movement of *P. vittatum*. This is possibly due to the difficulty in crossing large water bodies, and a limited availability of food resources in urban areas. Hence, our landscape genetic connectivity analysis suggests that the future spread of *P. vittatum* will be facilitated by the expansion of pastoral and agricultural land use, in combination with increasing temperatures under climate change. In Australia, a 0.4-2.0°C increase in annual average temperature is predicted by 2030 (Hughes,

2003). Additionally, Australia is undergoing rapid land clearing for pasture and agricultural purposes (Bradshaw, 2012, Evans, 2016). These rapid changes in landscape and temperature may therefore facilitate more frequent outbreaks of this species by supporting high population sizes and increasing economic damage.

Evidence for local environmental adaptation

From an evolutionary perspective, gene flow can either facilitate or impede local adaptation, depending partially on the strength of local selection pressures (Garant et al., 2007). We find evidence for selection along our sampled environmental gradient, indicating local adaptive processes in *P. vittatum.* Similar to our finding of a dominating effect of mean annual temperature on landscape genetic connectivity, temperature was associated with the highest number of SNPs under putative selection in *P. vittatum.* Our results are consistent with other studies that have reported (via EAA and outlier tests) a key role of temperature in driving adaptive genetic differentiation in insects (e.g. Dudaniec et al., 2018, Feng et al., 2015, Waldvogel et al., 2018), and in other systems (e.g. trees: Jordan et al., 2017, marine invertebrates: Xuereb et al., 2018b, vertebrates: Manthey and Moyle, 2015).

Although there was a small number of SNPs associated with soil moisture, all were unique to this variable, indicating that soil moisture may be a selection pressure for *P. vittatum.* This possibility is supported by our recent study on *P. vittatum* that reported a positive correlation between soil moisture and relative abundance along the same gradient (Yadav et al., 2018). Soil moisture significantly influences the viability of offspring in grasshoppers (Herrmann et al., 2010) and may affect local population distributions via the presence of parasitic nematodes and entomopathogenic fungi of *P. vittatum* (Clift and Baker, 1998, Milner and Prior, 1994) and in other Orthoptera (Barbercheck, 1992). Notably, of all tested variables, latitude had the highest number of SNP associations (*n*=100, unique= 84), which likely reflects a combination of environmental changes along the sampled latitudinal gradient. Given the latitudinal pattern of

genetic admixture we found (Fig. 1B), the unique SNPs associating with latitude may be false positive signatures of selection affected by neutral genetic structure or may be related to untested environmental variables.

Morphological variations and signature of selection

Out of all morphological variables tested, body size had the highest number of SNP associations in *P. vittatum.* Notably, the latitudinal cline in body size and the correlation of latitude with other environmental variables makes it difficult to tease apart selection signatures on body size alone. However, 92% (36 out of 39) of the SNPs under putative selection for body size were not significantly associated with any other environmental variable, and all except three outlier SNPs (92%) were unbiased by sex, indicating that these associations are not likely to be confounded by the effects of latitudinal genetic structure and sex. Notably, one of the SNP outliers relating to body size only was detected by both the Fst outlier approaches as well as EAA and exhibits a striking change in allele frequency along the sampled gradient (Fig. 4). Previously we found larger body size in higher solar radiation sites towards southern latitudes suggesting a possible underlying thermoregulatory advantage (Yadav et al., 2018). Our current results indicate that the latitudinal cline in body size of *P. vittatum* may have an adaptive genetic basis.

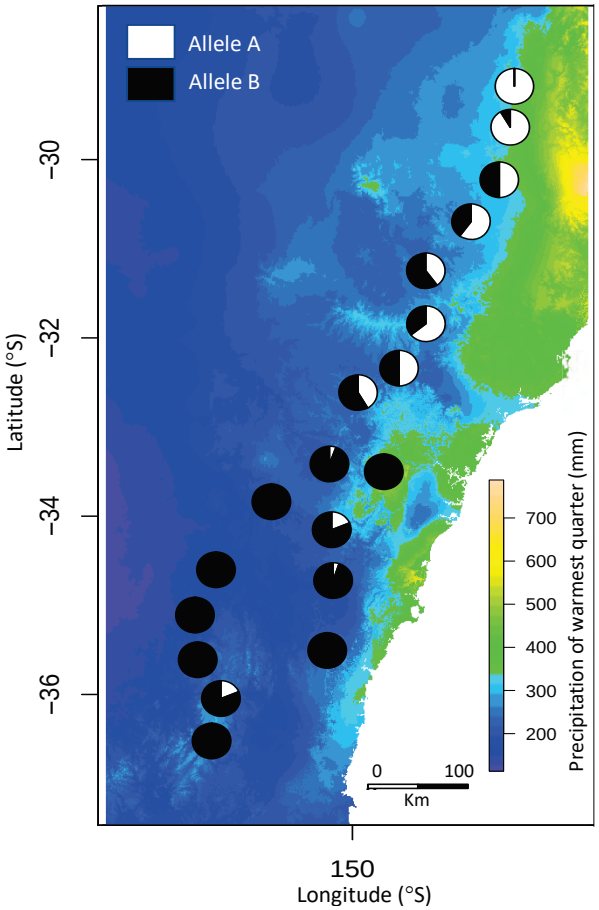


Figure 4. Allele frequency change across sites is shown for one SNP that was correlated with body size using all outlier detection methods (i.e., two Fst outlier approaches and LFMM). This SNP shows a distinct north-south divide in allele frequency. The frequency of each SNP allele is displayed as the proportion of individuals with the allele per site (white – 'Allele A'; black – 'Allele B'), shown over BIO18=Precipitation of the warmest quarter (mm).

The lack of selection signatures in relation to wing dimorphism may suggest more complex or subtle genetic processes governing this trait, such as polygenic and additive gene interactions (e.g. Lotterhos et al., 2018, Babin et al., 2017) or polyphenism (phenotypic plasticity where a single genotype produces different phenotypes) as reported in several insect groups including Orthoptera (Zera, 2003, Simpson et al., 2011, Zhang et al., 2019). The small number of SNP associations with stripe polymorphism indicated some evidence for selection on this trait, and associations were largely detected for the partially striped morph. Notably, the sensitivity of the EAA analysis as indicated by the GIF value within the LFMM (Table 3) and conservative *P*

values for this variable (Figure S8) is indicating higher confidence in these outliers (Frichot and Francois, 2015).

Gene annotation of candidate SNPs

Our annotation of candidate SNPs provided some evidence that functional genes (i.e. glutamate and ionotropic receptors) may be under selection and thus, involved in local adaptation of *P. vittatum* across environmental gradients. SNPs associated with these gene functions were also associated with body size and latitude (Table S6). Glutamate receptors (GluRs) mediate neurotransmission in the brain and are expressed largely in the central nervous system (Dingledine et al., 1999). Notably, the ionotropic receptors (IRs), a subfamily of ionotropic GluRs have been shown to be involved in olfaction in migratory locusts (Wang et al., 2015) as well as chemical signalling and environmental cue detection in other insects (Benton et al., 2009, Croset et al., 2010, Rytz et al., 2013). This involvement of GluRs and IRs in key pathways of detecting environmental cues, suggests that genes coding for these membrane proteins are putatively under differential selective pressures across variable environments and may provide a fitness advantage in *P. vittatum* via improved perception of the immediate environment.

Another annotation identified glucosyl glucuronosyltransferases among our candidate SNPs. Uridine diphosphate-glucuronosyl transferase (UGT) is an important enzyme of the detoxification pathway and catalyses the addition of sugars to lipophilic compounds (Ahn et al., 2012). The detoxification role of UGT has also been well demonstrated in herbivorous insects (Ahn et al., 2011, Heidel-Fischer and Vogel, 2015) and pest insects (e.g. Krempl et al., 2016). Additionally, UGT is involved in UV-shielding (Daimon et al., 2010), and pigmentation (Bock, 2016, Krempl et al., 2016). Along our sampled gradient, the annotated SNP relating to UGT was uniquely associated with mean annual temperature, a variable where selection pressures relating to ultra-violet light and the development of melanism are relevant. However, further experiments

are required to establish this relationship between temperature and expression of UGT and to examine the underlying genetic architecture of the genomic regions under selection.

Conclusion

Using a landscape genomic approach, we identify the relative contribution of common environmental drivers of both neutral and adaptive genetic processes, which are otherwise challenging to detect under conditions of high gene flow. In the widely distributed pest, *P. vittatum* we identify subtle landscape and climatic effects on genetic connectivity and environmental selection processes, despite weak genetic structure. Regarding the future impacts of *P. vittatum* as an agricultural pest, increased future temperatures under climate warming and land clearing are likely to facilitate a wider distribution and spread of this species to other areas. Increased availability of suitable habitat and temperatures may support higher population sizes leading to greater chances of outbreak and higher economic costs due to reduced agricultural production. Our study highlights the value of considering neutral and adaptive genetic processes together to understand the roles of gene flow and local adaptation in driving evolutionary responses in widely distributed species.

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References

ABARES. 2017. *Catchment scale land use of Australia* [Online]. Available: http://www.agriculture.gov.au/abares/aclump/ [Accessed 2018].

AHN, S. J., BADENES-PEREZ, F. R., REICHELT, M., SVATOS, A., SCHNEIDER, B., GERSHENZON, J. & HECKEL, D. G. 2011. Metabolic detoxification of capsaicin by UDP-glycosyltransferase in three *Helicoverpa* species. *Arch Insect Biochem Physiol,* 78**,** 104-18.

AHN, S. J., VOGEL, H. & HECKEL, D. G. 2012. Comparative analysis of the UDP-glycosyltransferase multigene family in insects. *Insect Biochem Mol Biol,* 42**,** 133-47.

AHRENS, C. W., RYMER, P. D., STOW, A., BRAGG, J., DILLON, S., UMBERS, K. D. L. & DUDANIEC, R. Y. 2018. The search for loci under selection: trends, biases and progress. *Mol Ecol,* 27**,** 1342-1356.

ANTONOVICS, J. 1976. The nature of limits to natural selection. *Annals of the Missouri Botanical Garden***,** 224-247.

ATTARD, C. R. M., BRAUER, C. J., SANDOVAL-CASTILLO, J., FAULKS, L. K., UNMACK, P. J., GILLIGAN, D. M. & BEHEREGARAY, L. B. 2018. Ecological disturbance influences adaptive divergence despite high gene flow in golden perch (*Macquaria ambigua*): Implications for management and resilience to climate change. *Mol Ecol,* 27**,** 196-215.

BABIN, C., GAGNAIRE, P. A., PAVEY, S. A. & BERNATCHEZ, L. 2017. RAD-Seq Reveals Patterns of Additive Polygenic Variation Caused by Spatially-Varying Selection in the American Eel (*Anguilla rostrata*). *Genome Biol Evol,* 9**,** 2974-2986.

BAI, Y., DONG, J. J., GUAN, D. L., XIE, J. Y. & XU, S. Q. 2016. Geographic variation in wing size and shape of the grasshopper *Trilophidia annulata* (Orthoptera: Oedipodidae): morphological trait variations follow an ecogeographical rule. *Sci Rep,* 6**,** 32680.

BAILEY, P., FRENSHAM, A., HINCKS, A. & NEWTON, M. 1994. Competition for herbage by *Phaulacridium vittatum* (Sjöstedt)(Orthoptera: Acrididae) and sheep during summer drought. *Australian Journal of Entomology,* 33**,** 175-179.

BALE, J. S., MASTERS, G. J., HODKINSON, I. D., AWMACK, C., BEZEMER, T. M., BROWN, V. K., BUTTERFIELD, J., BUSE, A., COULSON, J. C., FARRAR, J., GOOD, J. E. G., HARRINGTON, R., HARTLEY, S., JONES, T. H., LINDROTH, R. L., PRESS, M. C., SYMRNIOUDIS, I., WATT, A. D. & WHITTAKER, J. B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology,* 8**,** 1-16.

BALKENHOL, N., DUDANIEC, R. Y., KRUTOVSKY, K. V., JOHNSON, J. S., CAIRNS, D. M., SEGELBACHER, G., SELKOE, K. A., VON DER HEYDEN, S., WANG, I. J. & SELMONI, O. 2017. *Landscape Genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations,* Springer.

BARBERCHECK, M. E. 1992. Effect of soil physical factors on biological-control agents of soil insect pests. *Florida Entomologist,* 75**,** 539-548.

BARTON, K. 2018. Package 'MuMIn'. R package version 1.40. 4.

BARTON, N. & PARTRIDGE, L. 2000. Limits to natural selection. *Bioessays,* 22**,** 1075-84.

BENTON, R., VANNICE, K. S., GOMEZ-DIAZ, C. & VOSSHALL, L. B. 2009. Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. *Cell,* 136**,** 149-62.

BOCK, K. W. 2016. The UDP-glycosyltransferase (UGT) superfamily expressed in humans, insects and plants: Animal-plant arms-race and co-evolution. *Biochem Pharmacol,* 99**,** 11-7.

BRADSHAW, C. J. A. 2012. Little left to lose: deforestation and forest degradation in Australia since European colonization. *Journal of Plant Ecology,* 5**,** 109-120.

BRISSON, J. A. 2010. Aphid wing dimorphisms: linking environmental and genetic control of trait variation. *Philos Trans R Soc Lond B Biol Sci,* 365**,** 605-16.

BURNHAM, K. P. & ANDERSON, D. R. 2003. *Model selection and multimodel inference: a practical information-theoretic approach*, Springer Science & Business Media.
CANNON, R. J. C. 1998. The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology,* 4**,** 785-796.
CHAPMAN, A. J. 1990. *Biology of grasshoppers,* John Wiley & Sons.
CHHATRE, V. E. & EMERSON, K. J. 2017. StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics,* 18**,** 192.
CLARK, D. P. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.
CLIFT, A. & BAKER, G. L. 1998. Quantification of the relationship between rainfall and parasitism of *Phaulacridium vittatum* (Sjöstedt) by mermithid nematodes in the Central Tablelands of South-Eastern Australia. *Journal of Orthoptera Research,* 77-82.
CLISSOLD, F. J. & SIMPSON, S. J. 2015. Temperature, food quality and life history traits of herbivorous insects. *Curr Opin Insect Sci,* 11**,** 63-70.
COOP, G., WITONSKY, D., DI RIENZO, A. & PRITCHARD, J. K. 2010. Using environmental correlations to identify loci underlying local adaptation. *Genetics,* 185**,** 1411-23.
CROSET, V., RYTZ, R., CUMMINS, S. F., BUDD, A., BRAWAND, D., KAESSMANN, H., GIBSON, T. J. & BENTON, R. 2010. Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLoS Genet,* 6**,** e1001064.
CROSSLEY, M. S., CHEN, Y. H., GROVES, R. L. & SCHOVILLE, S. D. 2017. Landscape genomics of Colorado potato beetle provides evidence of polygenic adaptation to insecticides. *Mol Ecol,* 26**,** 6284-6300.
DAIMON, T., HIRAYAMA, C., KANAI, M., RUIKE, Y., MENG, Y., KOSEGAWA, E., NAKAMURA, M., TSUJIMOTO, G., KATSUMA, S. & SHIMADA, T. 2010. The silkworm Green b locus encodes a quercetin 5-O-glucosyltransferase that produces green cocoons with UV-shielding properties. *Proc Natl Acad Sci U S A,* 107**,** 11471-6.
DEARN, J. 1983. Inheritance of the colour pattern polymorphism in the wingless grasshopper *Phaulacridium vittatum* Sjöstedt (Orthoptera: Acrididae). *Australian Journal of Entomology,* 22**,** 217-218.
DEARN, J. M. 1980. Evidence of non-random mating for the colour pattern polymorphism in field populations of the grasshopper *Phaulacridium vittatum*. *Australian Journal of Entomology,* 18**,** 241-243.
DEARN, J. M. 1984. Colour pattern polymorphism in the grasshopper *Phaulacridium Vittatum*. I. Geographic variation in Victoria and evidence of habitat association. *Australian journal of zoology,* 32**,** 239-249.
DEVLIN, B. & ROEDER, K. 1999. Genomic control for association studies. *Biometrics,* 55**,** 997-1004.
DINGLEDINE, R., BORGES, K., BOWIE, D. & TRAYNELIS, S. F. 1999. The glutamate receptor ion channels. *Pharmacol Rev,* 51**,** 7-61.
DUDANIEC, R. Y., RHODES, J. R., WORTHINGTON WILMER, J., LYONS, M., LEE, K. E., MCALPINE, C. A. & CARRICK, F. N. 2013. Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology,* 22**,** 3752-3765.
DUDANIEC, R. Y., WORTHINGTON WILMER, J., HANSON, J. O., WARREN, M., BELL, S. & RHODES, J. R. 2016. Dealing with uncertainty in landscape genetic resistance models: a case of three co-occurring marsupials. *Mol Ecol,* 25**,** 470-86.
DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Mol Ecol,* 27**,** 2576-2593.
ELSHIRE, R. J., GLAUBITZ, J. C., SUN, Q., POLAND, J. A., KAWAMOTO, K., BUCKLER, E. S. & MITCHELL, S. E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One,* 6**,** e19379.

EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol,* 14**,** 2611-20.
EVANS, M. C. 2016. Deforestation in Australia: drivers, trends and policy responses. *Pacific Conservation Biology,* 22**,** 130-150.
EXCOFFIER, L. & LISCHER, H. E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour,* 10**,** 564-7.
FALT-NARDMANN, J., KLEMOLA, T., ROTH, M., RUOHOMAKI, K. & SAIKKONEN, K. 2016. Northern geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures than their southern conspecifics: Implications of climate change. *European Journal of Entomology,* 113**,** 337-343.
FARROW, R., NICOLAS, G. & DOWSE, J. 1982. Migration in the macropterous form of the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt) during an outbreak. *Australian Journal of Entomology,* 21**,** 307-308.
FENG, X. J., JIANG, G. F. & FAN, Z. 2015. Identification of outliers in a genomic scan for selection along environmental gradients in the bamboo locust, *Ceracris kiangsu*. *Sci Rep,* 5**,** 13758.
FICK, S. E. & HIJMANS, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology,* 37**,** 4302-4315.
FIELDING, D. J. & BRUSVEN, M. 1990. Historical analysis of grasshopper (Orthoptera: Acrididae) population responses to climate in southern Idaho, 1950–1980. *Environmental Entomology,* 19**,** 1786-1791.
FITZPATRICK, S., GERBERICH, J., KRONENBERGER, J., ANGELONI, L. & FUNK, W. 2015. Locally adapted traits maintained in the face of high gene flow. *Ecology Letters,* 18**,** 37-47.
FOLL, M. & GAGGIOTTI, O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics,* 180**,** 977-993.
FRICHOT, E. & FRANCOIS, O. 2015. LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution,* 6**,** 925-929.
FRICHOT, E., SCHOVILLE, S. D., BOUCHARD, G. & FRANCOIS, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. *Mol Biol Evol,* 30**,** 1687-99.
GARANT, D., FORDE, S. E. & HENDRY, A. P. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology,* 21**,** 434-443.
GARCÍA-RAMOS, G. & KIRKPATRICK, M. 1997. Genetic models of adaptation and gene flow in peripheral populations. *Evolution,* 51**,** 21-28.
GHOLAMIAN, E., RAZMJOU, J., BANI HASHEMIAN, S. M. & SABOURI, A. 2018. Genetic structure of populations of *Aphis gossypii* (Hemiptera: Aphididae) on citrus trees in Northern Iran. *European Journal of Entomology,* 115**,** 7-14.
GILLIS, J. E. & POSSAI, K. W. 1983. Thermal niche partitioning in the grasshoppers *Arphia conspersa* and *Trimerotropis suffusa* from a montane habitat in Central Colorado. *Ecological Entomology,* 8**,** 155-161.
GONZALO-TURPIN, H. & HAZARD, L. 2009. Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology,* 97**,** 742-751.
GOSSELIN, T. 2017. radiator: RADseq data exploration, manipulation and visualization using R. R package version 0.0.5
GUNTHER, T. & COOP, G. 2013. Robust identification of local adaptation from allele frequencies. *Genetics,* 195**,** 205-+.
HARRIS, R., MCQUILLAN, P. & HUGHES, L. 2012. Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *Journal of Biogeography,* 39**,** 1450-1461.

HARRIS, R. M., MCQUILLAN, P. & HUGHES, L. 2013a. Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). *PLoS One,* 8**,** e80243.

HARRIS, R. M. B., MCQUILLAN, P. & HUGHES, L. 2013b. A test of the thermal melanism hypothesis in the wingless grass-hopper *Phaulacridium vittatum*. *Journal of insect science,* 13.

HARRIS, R. M. B., MCQUILLAN, P. & HUGHES, L. 2015. The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. *Journal of thermal biology,* 52**,** 75-83.

HEIDEL-FISCHER, H. M. & VOGEL, H. 2015. Molecular mechanisms of insect adaptation to plant secondary compounds. *Current Opinion in Insect Science,* 8**,** 8-14.

HERRMANN, D. L., KO, A. E., BHATT, S., JANNOT, J. E. & JULIANO, S. A. 2010. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Annals of the Entomological Society of America,* 103**,** 227-235.

HIJMANS, R. J., CAMERON, S. E., PARRA, J. L., JONES, P. G. & JARVIS, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology,* 25**,** 1965-1978.

HIJMANS, R. J. & VAN ETTEN, J. 2014. raster: Geographic data analysis and modeling. *R package version,* 2.

HOFFMANN, A. A. 2017. Rapid adaptation of invertebrate pests to climatic stress? *Curr Opin Insect Sci,* 21**,** 7-13.

HUGHES, L. 2003. Climate change and Australia: trends, projections and impacts. *Austral Ecology,* 28**,** 423-443.

JANES, J. K., LI, Y. S., KEELING, C. I., YUEN, M. M. S., BOONE, C. K., COOKE, J. E. K., BOHLMANN, J., HUBER, D. P. W., MURRAY, B. W., COLTMAN, D. W. & SPERLING, F. A. H. 2014. How the mountain pine beetle (*Dendroctonus ponderosae*) breached the Canadian rocky mountains. *Molecular Biology and Evolution,* 31**,** 1803-1815.

JOHNSON, M., ZARETSKAYA, I., RAYTSELIS, Y., MEREZHUK, Y., MCGINNIS, S. & MADDEN, T. L. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res,* 36**,** W5-9.

JORDAN, R., HOFFMANN, A. A., DILLON, S. K. & PROBER, S. M. 2017. Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: Implications for adaptive potential to projected climate change. *Mol Ecol,* 26**,** 6002-6020.

KAWECKI, T. J. & EBERT, D. 2004. Conceptual issues in local adaptation. *Ecology Letters,* 7**,** 1225-1241.

KELLER, D., VAN STRIEN, M. J., HERRMANN, M., BOLLIGER, J., EDWARDS, P. J., GHAZOUL, J. & HOLDEREGGER, R. 2013. Is functional connectivity in common grasshopper species affected by fragmentation in an agricultural landscape? *Agriculture, Ecosystems & Environment,* 175**,** 39-46.

KEY, K. 1992. Taxonomy of the genus *Phaulacridium* and a related new genus (Orthoptera: Acrididae). *Invertebrate taxonomy,* 6**,** 197-243.

KILIAN, A., WENZL, P., HUTTNER, E., CARLING, J., XIA, L., BLOIS, H., CAIG, V., HELLER-USZYNSKA, K., JACCOUD, D. & HOPPER, C. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. *Data production and analysis in population genomics.* Springer.

KIRK, H., DORN, S. & MAZZI, D. 2013. Molecular genetics and genomics generate new insights into invertebrate pest invasions. *Evolutionary Applications,* 6**,** 842-856.

KREMPL, C., SPORER, T., REICHELT, M., AHN, S. J., HEIDEL-FISCHER, H., VOGEL, H., HECKEL, D. G. & JOUSSEN, N. 2016. Potential detoxification of gossypol by UDP-glycosyltransferases in the two Heliothine moth species *Helicoverpa armigera* and *Heliothis virescens*. *Insect Biochem Mol Biol,* 71**,** 49-57.

LEHMANN, G. U. & LEHMANN, A. W. 2008. Variation in body size among populations of the bushcricket *Poecilimon thessalicus* (Orthoptera: Phaneropteridae): an ecological adaptation. *Journal of Orthoptera Research,* 17**,** 165-170.
LENORMAND, T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology & Evolution,* 17**,** 183-189.
LEWONTIN, R. & KRAKAUER, J. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics,* 74**,** 175-195.
LI, Y. L. & LIU, J. X. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular ecology resources,* 18**,** 176-177.
LLEWELLYN, K. S., LOXDALE, H. D., HARRINGTON, R., BROOKES, C. P., CLARK, S. J. & SUNNUCKS, P. 2003. Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology,* 12**,** 21-34.
LOCH, A. & FLOYD, R. 2001. Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus,* in south-western Australia: History, current perspectives and future prospects. *Austral Ecology,* 26**,** 458-466.
LOTTERHOS, K. E. & WHITLOCK, M. C. 2015. The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Mol Ecol,* 24**,** 1031-46.
LOTTERHOS, K. E., YEAMAN, S., DEGNER, J., AITKEN, S. & HODGINS, K. A. 2018. Modularity of genes involved in local adaptation to climate despite physical linkage. *Genome Biol,* 19**,** 157.
LOZIER, J. D., STRANGE, J. P. & KOCH, J. B. 2013. Landscape heterogeneity predicts gene flow in a widespread polymorphic bumble bee, *Bombus bifarius* (Hymenoptera: Apidae). *Conservation Genetics,* 14**,** 1099-1110.
MAENO, K. & TANAKA, S. 2010. Genetic and hormonal control of melanization in reddish–brown and albino mutants in the desert locust *Schistocerca gregaria*. *Physiological entomology,* 35**,** 2-8.
MANEL, S., CONORD, C. & DESPRÉS, L. 2009. Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread insect. *BMC Evolutionary Biology,* 9**,** 288.
MANEL, S. & HOLDEREGGER, R. 2013. Ten years of landscape genetics. *Trends Ecol Evol,* 28**,** 614-21.
MANTHEY, J. D. & MOYLE, R. G. 2015. Isolation by environment in White-breasted Nuthatches (Sitta carolinensis) of the Madrean Archipelago sky islands: a landscape genomics approach. *Mol Ecol,* 24**,** 3628-38.
MASAKI, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution,* 21**,** 725-741.
MCRAE, B. H. & BEIER, P. 2007. Circuit theory predicts gene flow in plant and animal populations. *Proc Natl Acad Sci U S A,* 104**,** 19885-90.
MILNER, R. J. & PRIOR, C. 1994. Susceptibility of the Australian plague locust, *Chortoicetes terminifera,* and the wingless grasshopper, *Phaulacridium vittatum,* to the Fungi Metarhizium Spp. *Biological Control,* 4**,** 132-137.
MOMIGLIANO, P., HARCOURT, R., ROBBINS, W. D., JAITEH, V., MAHARDIKA, G. N., SEMBIRING, A. & STOW, A. 2017. Genetic structure and signatures of selection in grey reef sharks (*Carcharhinus amblyrhynchos*). *Heredity,* 119**,** 142-153.
MORAITI, C., NAKAS, C. & PAPADOPOULOS, N. 2014. Diapause termination of *Rhagoletis cerasi* pupae is regulated by local adaptation and phenotypic plasticity: escape in time through bet-hedging strategies. *Journal of Evolutionary Biology,* 27**,** 43-54.
MOUSSEAU, T. A. & ROFF, D. A. 1989. Adaptation to Seasonality in a Cricket: Patterns of Phenotypic and Genotypic Variation in Body Size and Diapause Expression Along a Cline in Season Length. *Evolution,* 43**,** 1483-1496.

MUIR, A. P., BIEK, R., THOMAS, R. & MABLE, B. K. 2014. Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). *Molecular ecology,* 23**,** 561-574.
NETELER, M., BOWMAN, M. H., LANDA, M. & METZ, M. 2012. GRASS GIS: A multi-purpose open source GIS. *Environmental Modelling & Software,* 31**,** 124-130.
OEH. 2017. *Great Soil Group (GSG) Soil Type map of NSW, NSW Office of Environment and Heritage, Sydney* [Online]. Available: https://datasets.seed.nsw.gov.au/dataset/great-soil-group-gsgsoil-type-map-of-nsw1cf19 [Accessed].
OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R., SIMPSON, G. L., SOLYMOS, P., STEVENS, M. H. H. & WAGNER, H. 2013. Package 'vegan'. Community ecology package, version, 2(9).
PARADIS, E. 2010. pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics,* 26**,** 419-420.
PARIS, M., BOYER, S., BONIN, A., COLLADO, A., DAVID, J. P. & DESPRES, L. 2010. Genome scan in the mosquito *Aedes rusticus*: population structure and detection of positive selection after insecticide treatment. *Mol Ecol,* 19**,** 325-37.
PELISSIE, B., CROSSLEY, M. S., COHEN, Z. P. & SCHOVILLE, S. D. 2018. Rapid evolution in insect pests: the importance of space and time in population genomics studies. *Current Opinion in Insect Science,* 26**,** 8-16.
PENER, M. P. & SIMPSON, S. J. 2009. Locust phase polyphenism: An update. *Advances in Insect Physiology, Vol 36,* 36**,** 1-272.
PEPPER, J. & HASTINGS, E. 1952. The effects of solar radiation on grasshopper temperatures and activities. *Ecology,* 33**,** 96-103.
PETERMAN, W. E. 2018. ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *Methods in Ecology and Evolution,* 9**,** 1638-1647.
PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* 155**,** 945-959.
PUECHMAILLE, S. J. J. M. E. R. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. 16**,** 608-627.
PURCELL, S., NEALE, B., TODD-BROWN, K., THOMAS, L., FERREIRA, M. A., BENDER, D., MALLER, J., SKLAR, P., DE BAKKER, P. I. & DALY, M. J. J. T. A. J. O. H. G. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. 81**,** 559-575.
QGIS, D. T. 2016. QGIS geographic information system V. 2.18. 10.
RASANEN, K. & HENDRY, A. P. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters,* 11**,** 624-636.
RAYMOND, I. 2016. *The Australian soil classification,* CSIRO publishing.
RELLSTAB, C., GUGERLI, F., ECKERT, A. J., HANCOCK, A. M. & HOLDEREGGER, R. 2015. A practical guide to environmental association analysis in landscape genomics. *Mol Ecol,* 24**,** 4348-70.
REYNOLDS, J., WEIR, B. S. & COCKERHAM, C. C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics,* 105**,** 767-779.
ROBERTS, R. J. 1972. A newly recognized from of pasture damage by *Phaulacridium vittatum* (Acididae) on the northern tablelands of NSW. *Australian Journal of Entomology,* 11**,** 257-258.
ROFF, D. A. 1986. The evolution of wing dimorphism in insects. *Evolution,* 40**,** 1009-1020.
ROSETTI, N. & REMIS, M. I. 2018. Spatial variation in body size and wing dimorphism correlates with environmental conditions in the grasshopper *Dichroplus vittatus* (Orthoptera: Acrididae). *Environ Entomol,* 47**,** 519-526.

ROW, J. R., KNICK, S. T., OYLER-MCCANCE, S. J., LOUGHEED, S. C. & FEDY, B. C. 2017. Developing approaches for linear mixed modeling in landscape genetics through landscape-directed dispersal simulations. *Ecology evolution,* 7**,** 3751-3761.

RYTZ, R., CROSET, V. & BENTON, R. 2013. Ionotropic receptors (IRs): chemosensory ionotropic glutamate receptors in Drosophila and beyond. *Insect Biochem Mol Biol,* 43**,** 888-97.

SANDROCK, C., RAZMJOU, J. & VORBURGER, C. 2011. Climate effects on life cycle variation and population genetic architecture of the black bean aphid, *Aphis fabae*. *Mol Ecol,* 20**,** 4165-81.

SAVOLAINEN, O., LASCOUX, M. & MERILA, J. 2013. Ecological genomics of local adaptation. *Nat Rev Genet,* 14**,** 807-20.

SCHOVILLE, S. D., BONIN, A., FRANCOIS, O., LOBREAUX, S., MELODELIMA, C. & MANEL, S. 2012. Adaptive Genetic Variation on the Landscape: Methods and Cases. *Annual Review of Ecology, Evolution, and Systematics, Vol 43,* 43**,** 23-43.

SHAH, V. B. & MCRAE, B. Circuitscape: a tool for landscape ecology. Proceedings of the 7th Python in Science Conference, 2008. SciPy 2008, Pasadena, California, USA, 62-66.

SHIRK, A. J., LANDGUTH, E. L. & CUSHMAN, S. A. 2018. A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Mol Ecol Resour,* 18**,** 55-67.

SHIRK, A. J., WALLIN, D. O., CUSHMAN, S. A., RICE, C. G. & WARHEIT, K. I. 2010. Inferring landscape effects on gene flow: a new model selection framework. *Mol Ecol,* 19**,** 3603-19.

SIMPSON, S. J., SWORD, G. A. & LO, N. 2011. Polyphenism in insects. *Curr Biol,* 21**,** R738-49.

TANAKA, S., HARANO, K. & NISHIDE, Y. 2012. Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity. *J Insect Physiol,* 58**,** 89-101.

TEAM, R. C. 2017. *R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.* [Online]. Available: https://www.R-project.org/ [Accessed].

TERNAUSCOVER. 2011. *Woody extent and Foliage Projective Cover - SPOT, OEH algorithm, NSW coverage* [Online]. Available: http://www.auscover.org.au/purl/spot-woody-extent-fpc-nsw-2011 [Accessed].

TIGANO, A. & FRIESEN, V. L. 2016. Genomics of local adaptation with gene flow. *Mol Ecol,* 25**,** 2144-64.

TRULLAS, S. C., VAN WYK, J. H. & SPOTILA, J. R. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology,* 32**,** 235-245.

USHERWOOD, P. 1994. Insect glutamate receptors. *Advances in Insect Physiology.* Elsevier.

VORBURGER, C., LANCASTER, M. & SUNNUCKS, P. 2003. Environmentally related patterns of reproductive modes in the aphid *Myzus persicae* and the predominance of two 'superclones' in Victoria, Australia. *Molecular Ecology,* 12**,** 3493-3504.

WALDVOGEL, A. M., WIESER, A., SCHELL, T., PATEL, S., SCHMIDT, H., HANKELN, T., FELDMEYER, B. & PFENNINGER, M. 2018. The genomic footprint of climate adaptation in *Chironomus riparius*. *Mol Ecol,* 27**,** 1439-1456.

WANG, X. H., FANG, X. D., YANG, P. C., JIANG, X. T., JIANG, F., ZHAO, D. J., LI, B. L., CUI, F., WEI, J. N., MA, C. A., WANG, Y. D., HE, J., LUO, Y., WANG, Z. F., GUO, X. J., GUO, W., WANG, X. S., ZHANG, Y., YANG, M. L., HAO, S. G., CHEN, B., MA, Z. Y., YU, D., XIONG, Z. Q., ZHU, Y. B., FAN, D. D., HAN, L. J., WANG, B., CHEN, Y. X., WANG, J. W., YANG, L., ZHAO, W., FENG, Y., CHEN, G. X., LIAN, J. M., LI, Q. Y., HUANG, Z. Y., YAO, X. M., LV, N., ZHANG, G. J., LI, Y. R., WANG, J., WANG, J., ZHU, B. L. & KANG, L. 2014. The locust genome provides insight into swarm formation and long-distance flight. *Nature Communications,* 5**,** 1-9.

WANG, Y. Z., LI, B. Y., HOFFMANN, A. A., CAO, L. J., GONG, Y. J., SONG, W., ZHU, J. Y. & WEI, S. J. 2017. Patterns of genetic variation among geographic and host-plant associated populations of the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae). *BMC Evol Biol,* 17**,** 265.

WANG, Z., YANG, P., CHEN, D., JIANG, F., LI, Y., WANG, X. & KANG, L. 2015. Identification and functional analysis of olfactory receptor family reveal unusual characteristics of the olfactory system in the migratory locust. *Cell Mol Life Sci,* 72**,** 4429-43.

WHITLOCK, M. C. & LOTTERHOS, K. E. 2015. Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of FST. *American Naturalist,* 186**,** S24-S36.

WHITMAN, D. W. 1987. Thermoregulation and daily activity patterns in a black desert rrasshopper, *Taeniopoda eques*. *Animal Behaviour,* 35**,** 1814-1826.

XUEREB, A., BENESTAN, L., NORMANDEAU, E., DAIGLE, R. M., CURTIS, J. M., BERNATCHEZ, L. & FORTIN, M. J. 2018a. Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RAD seq, in a highly dispersive marine invertebrate (*Parastichopus californicus*). *Molecular ecology,* 27**,** 2347-2364.

XUEREB, A., KIMBER, C. M., CURTIS, J. M., BERNATCHEZ, L. & FORTIN, M. J. 2018b. Putatively adaptive genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as revealed by environmental association analysis of restriction-site associated DNA sequencing data. *Molecular ecology*.

YADAV, S., STOW, A. J., HARRIS, R. M. B. & DUDANIEC, R. Y. 2018. Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae). *J Insect Sci,* 18**,** 13.

YAMANAKA, T., TATSUKI, S. & SHIMADA, M. 2008. Adaptation to the new land or effect of global warming? An age-structured model for rapid voltinism change in an alien lepidopteran pest. *J Anim Ecol,* 77**,** 585-96.

YEAMAN, S. & WHITLOCK, M. C. 2011. The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution,* 65**,** 1897-1911.

YIN, Y. Q., LI, X. Y., CHU, D., ZHAO, X. Q., SATHYA, K., DOUANGBOUPHA, B., KYAW, M. M., KONGCHUENSIN, M., SOMRITH, A., NGO, V. V., NGUYEN, H. C., SHEN, S. C., LIU, S. F. & CHEN, A. D. 2017. Extensive gene flow of white-backed planthopper in the Greater Mekong Subregion as revealed by microsatellite markers. *Scientific Reports,* 7**,** 15905.

ZERA, A. J. 2003. The endocrine regulation of wing polymorphism in insects: state of the art, recent surprises, and future directions. *Integr Comp Biol,* 43**,** 607-16.

ZHANG, C. X., BRISSON, J. A. & XU, H. J. 2019. Molecular mechanisms of wing polymorphism in insects. *Annu Rev Entomol,* 64**,** 297-314.

ZHANG, L. J., CAI, W. Z., LUO, J. Y., ZHANG, S., LI, W. D., WANG, C. Y., LV, L. M. & CUI, J. J. 2018. Population genetic structure and expansion patterns of the cotton pest *Adelphocoris fasciaticollis*. *Journal of Pest Science,* 91**,** 539-550.

(A)

(B)

(A) *Kosciuscola tristis* pair (smaller male and larger female); (B) *Kosciuscola tristis* male (photos: Sonu Yadav)

CHAPTER 4

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Elevational partitioning in species distribution, abundance and body size in Australian alpine grasshoppers (*Kosciuscola*)

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Author contributions

This chapter was conceived by Sonu Yadav and Rachael Dudaniec. I carried out the fieldwork with the help of Kate Umbers and Justin McNab. I collected datasets, carried out species identification, lab work, data analysis and interpretation of results. I discussed my findings with Rachael Dudaniec and Adam Stow. I wrote the chapter, which was greatly improved by comments and edits from Rachael Dudaniec and Adam Stow.

Abstract

Elevation gradients are strongly associated with abiotic factors that can influence the abundance and morphological traits of species. Population size and morphological variation have consequences for species persistence during the current, and unprecedented rates of anthropogenic climate change. In mountainous regions, temperature gradients can structure communities and partition intraspecific variation, and this may reflect susceptibility to climate change. Across three regions (Thredbo, Guthega and Jagungal) within Kosciuszko National Park, Australia, we examine elevational variation in species relative abundance and intraspecific body size among three co-occurring grasshopper species of the genus *Kosciuscola (K. usitatus, K. tristis,* and *K. cognatus)*, which are endemic to the Australian Alpine region. Species-specific variation in relative abundances was observed, whereby the relative abundance of *K. usitatus* decreased with increasing elevation. On the contrary, the relative abundance of *K. tristis* increased with elevation. No significant change in the relative abundance of *K. cognatus* with elevation was observed, but it was negatively correlated with foliage cover. Body size changes were observed with increasing elevation according to sex and species. Body size decreased with elevation in both male and female *K. usitatus*, and this pattern was similarly observed in female *K. tristis* and male *K. cognatus* only. Our results demonstrate spatial partitioning of species and body size in relation to elevational gradients within an alpine habitat. Changes in relative abundance, and morphology within this assemblage might be indicative of sensitivity to climate change and may be useful for predicting the future distribution of this grasshopper assemblage.

Keywords

Australian Alps, *Kosciuscola*, grasshopper, species composition, Orthoptera, elevation.

Introduction

Alpine species are often adapted to narrow thermal ranges, making them sensitive to increasing temperatures (Williams et al., 2008, Laurance et al., 2011) and are likely to be more adversely influenced by climate change than widespread species (Dirnböck et al., 2011, La Sorte and Jetz, 2010). Elevational gradients in alpine ecosystems are characterised by steep changes in climatic and vegetation variables. Such sharp environmental clines may impose differential selection pressures on species that may lead to changes in their composition and diversity (Hilt and Fiedler, 2005, Zenker et al., 2015, Beccacece et al., 2016, Sundqvist et al., 2013). Changes to species composition and diversity along elevation gradients have been reported in several systems (plants: Mota et al., 2018; moths: Brehm and Fiedler, 2003, Zenker et al., 2015; dung beetles: Herzog et al., 2013; birds: Rahbek, 1997), indicating that many species are sensitive to these gradients across spatial scales. For specialist species with narrow thermal and spatial niches, increased sensitivity to changing thermal regimes is expected, so understanding how populations respond to elevation gradients, which in turn are strongly associated with environmental variables, is necessary to understand species vulnerabilities to climate change.

Alpine ecosystems provide an ideal system to investigate processes that determine species assemblages along environmental gradients. Rapid changes in temperature and vegetation patterns along elevation gradients may occur over short distances (Körner, 2007) and correspond with shifts in species assemblages (Bateman et al., 2010, Ramirez-Bautista and Williams, 2019, Martin-Regalado et al., 2019). Studies of assemblages along elevational gradients have identified high species richness at mid altitudes, a phenomenon consistent across many regions and taxa (reviewed in Colwell et al., 2004). However, studies that have looked at changes in species assemblages with elevation in ectotherms (invertebrates) have reported diverse results that span negative (Lee et al., 2011), positive (Mccoy, 1990) and hump-shaped relationships (Werenkraut and Ruggiero, 2014). Environmental conditions such as climate (i.e. temperature, radiation, precipitation), topography and productivity are amongst the major drivers of such changes in

species assemblages (Lomolino, 2001, Currie and Kerr, 2008). In addition, elevational segregation in species distributions are also influenced by physiological tolerances, climatic preferences, and microhabitat use (Wachter et al., 1998, Werenkraut and Ruggiero, 2014, Röder et al., 2017, Birkett et al., 2018). Thus, understanding changes in species abundance in response to temperature-elevation gradients may provide insight into the drivers of species assemblage structure across fine spatial scales.

Body size is an important functional trait related to fitness, survival, growth, and physiology (reviewed in Chown and Gaston, 2010, Kleiber, 1947) and in insects, body size is largely influenced by temperature (Whitman, 2008, Sheridan and Bickford, 2011). Bergmann's rule states that body size will increase with an increase in latitude, and this rule was later extended to elevational gradients (Brehm and Fiedler, 2004, Shelomi, 2012, Bergmann, 1847). While no single pattern has been applied in ectotherms, this rule is largely applied to mammals and birds (reviewed in Shelomi, 2012, Vinarski, 2014, Cardilini et al., 2016). In contrast to endotherms, body size in ectotherms (i.e. including insects), is generally positively correlated with temperature with smaller individuals in cooler climates (Mousseau, 1997, Berner and Blanckenhorn, 2006, Buckley et al., 2014, Telfer and Hassall, 1999), however there is no single consistent pattern across the insect taxa (Brehm et al., 2018, Maveety and Browne, 2014). This inconsistency indicates that the underlying mechanisms controlling body size may vary across species and locations, and more studies on different taxa may offer possible explanations for these inconsistent patterns in ectotherms as well sex-specific body size changes.

Insects frequently differ in body size between sexes with females being the larger sex (Chown and Gaston, 2010), which is also observed in Orthoptera (Bidau et al., 2016, Hochkirch and Gröning, 2008). In species with sexual size dimorphism, males and females may show distinct variation in body size along environmental gradients, likely due to different selection pressures acting on males and females of the same species (Stamps, 1993, Fairbairn, 1997, Teder and

Tammaru, 2005). For instance, a study by Blanckenhorn et al. (2006b) showed 66 out of 98 species of vertebrates and invertebrates showed steeper latitudinal cline in male body size than females. One key mechanism driving such pattern is possibly the presence of greater selection pressures on female body size compared to male body size (Fairbairn, 2005). However, other studies have shown females may show variable body size as a consequence of variable environmental conditions and ecological pressures such as habitat, substrate use, and developmental period (García-Navas et al., 2017, Fairbairn et al., 2007, Laiolo et al., 2013). Understanding sex-specific variation in body size along elevation gradients could provide insight into the environmental mechanisms generating such clines.

Alpine ecosystems are particularly susceptible to climate change (Gobiet et al., 2014, IPCC, 2014) and the Australian Alps are no exception. Since 1979 the Australian Alps have experienced a decline in precipitation by 6%, an increase in temperature at an average of 0.4°C and a substantial decline in snow depth since 1954 (Nicholls, 2005, Hennessy et al., 2003, Sánchez-Bayo and Green, 2018, Wahren et al., 2013). The Australian alpine bioregion is further threatened by fire, sub-alpine vegetation encroachment and increased impact of invasive species (Hoffmann et al., 2018). Such impacts have been linked to alterations to the survival rates, distribution and population dynamics of endemic alpine species (Green, 2010, Nicholls, 2005). For instance, the overwintering survival rates of invertebrates subject to reduced snow cover may be reduced because snow acts as a buffer from fluctuating temperatures and insulates eggs during cold winter months (Bale and Hayward, 2010). However, important baseline studies examining environmental impacts on alpine communities' fitness with subsequent effects on their population demography and persistence are limited in the Australian alps, particularly in case of invertebrates.

Here we examine the effect of temperature-elevation gradients on species relative abundances and body size in three co-occurring alpine grasshoppers of the genus *Kosciuscola* (*K. usitatus, K.*

tristis, and *K. cognatus*), all endemic to Australia. The three *Kosciuscola* species (Orthoptera: Acrididae) occur commonly in the Kosciuszko region but are thought to differ in elevational distribution, with *K. cognatus* being the most widely distributed species (Tatarnic et al., 2013) followed by *K. usitatus*. *K. tristis* has the narrowest elevational distribution among all the species in the *Kosciuscola* genus (Slatyer et al., 2014). However, the distributions are anecdotal observations and are not empirically examined in the three species. The three grasshopper species show variation in cold tolerance limits (Slatyer et al., 2016), whereby *K. usitatus* has the highest cold tolerance (CTmin= −3 °C), followed by *K. tristis* (CTmin= −2 °C) and *K. cognatus* (CTmin= −1 °C). Further, populations at higher elevation showed a higher CTmax compared to populations at lower elevation for *K. usitatus* and *K. tristis*, but not for *K. cognatus* (Slatyer et al., 2016). Despite differences in elevational niche, the three species have been shown to overlap in their elevational distributions. *K. cognatus* can occupy low to middle elevation areas (900m - 1700m), whereas *K. tristis* (1500-2000m) and *K. usitatus* (1400-2200) are found more commonly at middle to high elevations (Slatyer et al., 2016, Campbell and Dearn, 1980).

We examine a grasshopper assemblage (three species) of the genus *Kosciuscola* along elevational transects within three mountain regions of the Kosciusko National Park in the Australian Alps: Thredbo, Guthega and Jagungal. Given differences in species' cold tolerances and elevational niche breadths, we test, (1) how species differ and overlap in relative abundances along temperature, elevation and vegetation gradients, and (2) whether temperature-elevation gradients are associated with variation in body size among males and females of each species.

Materials and methods

Study species

The genus *Kosciuscola* is comprised of six known species that are all are endemic and spread throughout the alpine, sub-alpine region and lowlands of Australia (i.e. in New South Wales and Victoria) (Rehn, 1957, Tatarnic et al., 2013). The three focal species of this genus: *K. usitatus, K.*

tristis, and K. cognatus are flightless and are commonly distributed in the Kosciuszko alpine region ranging from montane woodlands to alpine areas (Slatyer et al., 2016). All three-grasshopper species have non-overlapping generations (Campbell and Dearn, 1980, Umbers et al., 2013). Adults lay eggs during autumn and summer and hatching takes place in spring (Campbell and Dearn, 1980, Umbers et al., 2013). Sexual dimorphism in body size is known in all three species whereby females have a larger body size than males (Umbers et al., 2013, Slatyer et al., 2016).

Study sites and collection

Samples were collected in the Kosciuszko region within the south-east of New South Wales of Australia, which is a large alpine and sub-alpine region on the Australian mainland. Sampling was undertaken for two weeks in late February to early March 2017. Using a line transect approach we collected samples starting from elevational range of 1400 m to 2,000 m at every 50m increment in elevation. However, at some sites sampling with an increment of 50m was not possible due to topological challenges. In such cases samples were collected from points with an approximate 50m increment, which lead to collection from two sites at 1550 m and three sites at 1600 m in Thredbo and one 1730 m site in Guthega. Furthermore, species were absent from some of the points on the transect, resulting in no capture. Samples were collected from a total of 48 sites (where a minimum one of the species was present) across three locations: 1) Thredbo (-36.49°S), 2) Guthega (-36.37°S) and 3) Mt. Jagungal (-36.03°S; Table 1).

Table 1: Sample site information (sites where a minimum of one of the species is present are shown here) of the three regions, with environmental variables extracted per site. FPC= Foliage Projective Cover, Elevation (metre above sea level).

Latitude (°S)	Longitude (°S)	Region	Transect	Elevation (m a.s.l.)	Annual precipitation (mm)	Temperature (°C)	FPC (%)
-36.52	148.26	Thredbo	T1	1549	1872.34	18.78	26.14
-36.52	148.26	Thredbo	T1	1551	1895.21	18.64	0
-36.52	148.26	Thredbo	T1	1596	1892.91	18.67	40.31
-36.53	148.27	Thredbo	T1	1600	1898.01	18.59	48.7
-36.52	148.26	Thredbo	T1	1600	1898.18	18.63	18.72
-36.52	148.26	Thredbo	T1	1636	1951	18.35	39.58
-36.52	148.26	Thredbo	T1	1650	1974.07	18.23	30.84
-36.52	148.26	Thredbo	T1	1697	2103.15	17.64	53.63
-36.51	148.27	Thredbo	T1	1741	2161.72	17.38	1.97
-36.51	148.27	Thredbo	T1	1797	2257.41	16.9	0
-36.51	148.27	Thredbo	T1	1849	2328.05	16.53	24.84
-36.5	148.27	Thredbo	T1	1896	2356.2	16.37	0.03
-36.5	148.27	Thredbo	T1	1937	2402.31	16.12	10.33
-36.5	148.3	Thredbo	T2	1414	1701.74	19.86	62.32
-36.49	148.3	Thredbo	T2	1672	2064.72	17.72	64.57
-36.49	148.29	Thredbo	T2	1802	2298.34	16.61	60.32
-36.49	148.29	Thredbo	T2	1869	2363.26	16.26	47.33
-36.52	148.26	Thredbo	T3	1746	2183.8	17.29	11.4
-36.51	148.25	Thredbo	T3	1796	2265.53	16.92	9.76
-36.51	148.25	Thredbo	T3	1845	2335.62	16.57	56.38
-36.51	148.25	Thredbo	T3	1890	2387.43	16.29	50.37
-36.51	148.25	Thredbo	T3	1940	2437.5	16.01	20.76
-36.51	148.25	Thredbo	T3	1990	2496.12	15.65	21.34
-36.38	148.37	Guthega	T1	1600	1747.55	18.52	18.53
-36.38	148.37	Guthega	T1	1636	1840.26	18.12	62.58
-36.37	148.37	Guthega	T1	1648	1899.22	17.9	39.7
-36.37	148.36	Guthega	T1	1688	1976.37	17.61	32.55
-36.37	148.36	Guthega	T1	1728	2048.6	17.34	40.98
-36.37	148.36	Guthega	T1	1734	2046.47	17.33	12.03
-36.37	148.36	Guthega	T1	1767	2079.3	17.2	49.64
-36.38	148.37	Guthega	T2	1669	1841.22	18.08	31.88
-36.38	148.37	Guthega	T2	1694	1874.66	17.93	40.65
-36.38	148.38	Guthega	T2	1732	1932.15	17.69	35.75
-36.38	148.38	Guthega	T2	1788	2049.22	17.21	2.5
-36.38	148.38	Guthega	T2	1847	2141.49	16.69	0
-36.37	148.37	Guthega	T3	1591	1788.16	18.37	40.46
-36.38	148.38	Guthega	T3	1642	1878.29	17.87	5.22

-36.38	148.38	Guthega	T3	1699	1941.28	17.61	0
-36.38	148.38	Guthega	T3	1744	2013.68	17.32	0
-36.38	148.38	Guthega	T3	1797	2096.15	16.94	0
-36.38	148.38	Guthega	T3	1846	2173.81	16.52	9.22
-36.05	148.38	Jagungal	T1	1548	1713.95	19.47	37.08
-36.03	148.37	Jagungal	T1	1589	1780.02	18.97	2.76
-36.15	148.37	Jagungal	T1	1648	1868.58	18.18	2.27
-36.15	148.37	Jagungal	T1	1682	1854.51	18.24	1.72
-36.14	148.38	Jagungal	T1	1749	1954.5	17.6	3.97
-36.15	148.38	Jagungal	T1	1837	2043.11	17.13	0
-36.14	148.39	Jagungal	T1	1891	2164.22	16.49	0

In Thredbo, we collected grasshoppers from three transects covering a total of 20 points (T1= 10 points, T2= 4 points, and T3= 6 points) with an elevational gradient ranging from ~1400-2,000 m a.s.l. A total of three transects were laid in Guthega with 17 points (T1= 6 points, T2= 5 points, and T3= 6 points) with an elevational range of ~1600-1850 m a.s.l. and in Jagungal one transect was covered with seven sampling sites and an elevation range of ~1550-1900 m a.s.l. (Fig. 1). Only one transect was covered in Jagungal due to poor accessibility in the area and absence of well-defined trails.

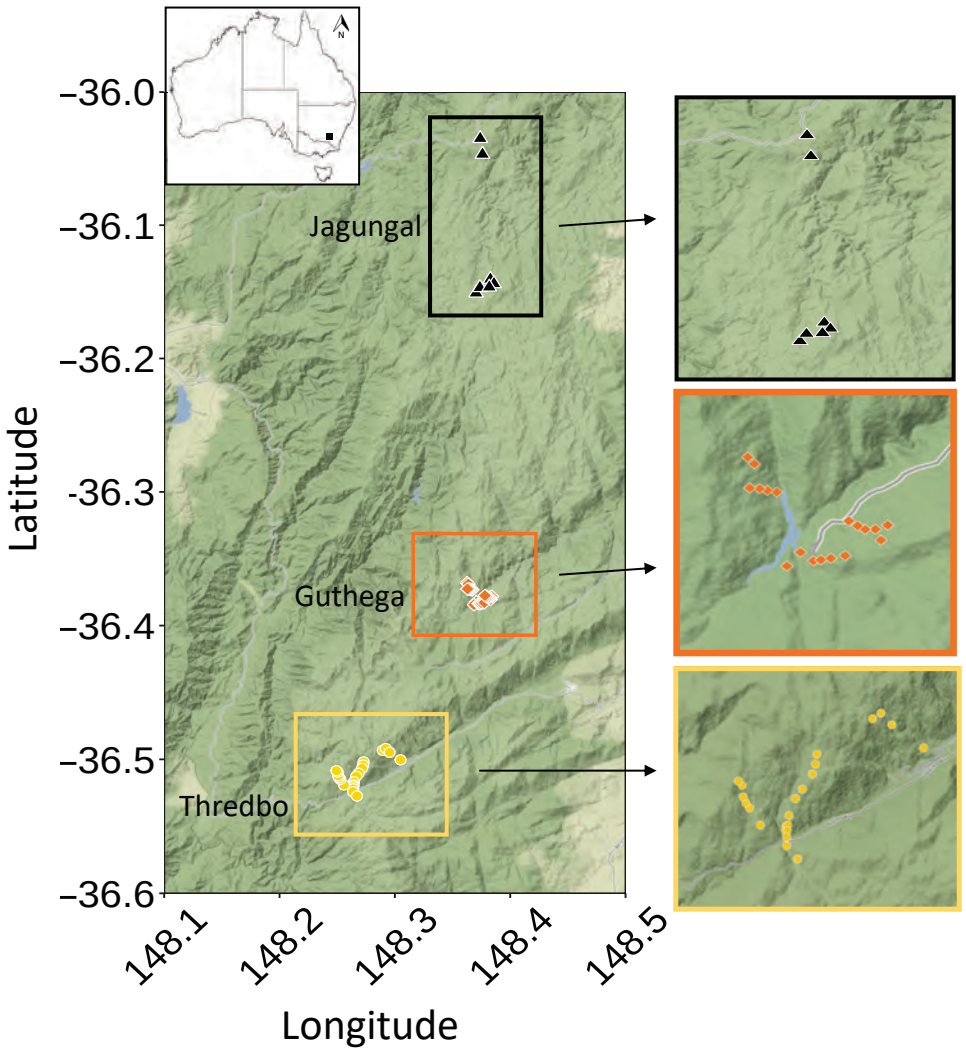

Figure 1: Collection sites for all three species across three mountain regions (Thredbo, Guthega and Jagungal) shown on a topographic map. Only presence sites are shown here (sites where at least one of the species was present).

Spatial coordinates (latitude, longitude) per site were recorded using a handheld GPS device (Garmin Etrex10). All three species were searched for within a ~30m^2 plot at each 50 m interval sampling point for 20 mins by two people (40 minutes in total; except in 11 sites where the number of people varied from 3-5; see Tables S1, S2, S3 for species-specific sampling information). Samples were captured using small plastic jars and the time of the day, species name and sex were recorded from live specimens upon processing. To further confirm species identification, features such as eye colour, cheek pattern, pronotum shape, body shape and size were compared against type specimens stored in the Australian National Insect Collection,

CSIRO, Canberra. Hind leg femur length was measured on the left side for each specimen using vernier calipers (accurate to 0.02 mm), which is a commonly-used proxy for body size in grasshoppers (Masaki, 1967, Mousseau and Roff, 1989, Lehmann and Lehmann, 2008). All samples were stored in 95% ethanol for DNA preservation.

Environmental data

Environmental variables were identified based on field observations, expert opinion and knowledge of the species ecology and thermal tolerance (Slatyer et al., 2016, Slatyer et al., 2014, Umbers et al., 2013). Temperature, precipitation (including rainfall or snow cover) and topography are known to affect reproduction, growth, and the distribution of alpine insects including grasshoppers (Roland and Matter, 2016, Joern and Gaines, 1990, Buckley et al., 2013, Hodkinson, 2005). For example, temperature clines along elevational gradients have been found to affect the hopping performance, feeding behaviour and movement of ectothermic grasshoppers (Buckley and Nufio, 2014). Host plants may largely impact distribution and abundance of specialist species, however, due to the difficulty in measuring host plant with a limited field season foliage cover was used as a measure of vegetation. Foliage cover is an important factor in regulating movement, egg laying and distribution of wing/wingless morphs in grasshoppers via controlling the presence of preferred sites and food resource availability (Yadav et al., 2018, Clark, 1967). Foliage cover have also been shown to be associated with adaptive genetic variations in Australian grasshoppers of the genus *Phaulacridium*, which overlap in distribution with *Kosciuscola* (Yadav et al., 2019).

Maximum summer temperature ("Temperature" now onwards) and annual rainfall ("Precipitation" now onwards) were obtained from ANUCLIM 6.1 (Xu and Hutchinson, 2013) and resampled to an original 30m resolution to achieve fine scale measurements of the variables in Kosciuszko national park region (Lyons et al., 2017). A 30m resolution was sufficient to test fine scale correlations between morphological and environment variables as it covers site-based

variation and is relevant based on species ecology. Elevation data were derived from a Digital Elevation Model (DEM) obtained from Geoscience Australia at a 30m resolution. Foliage Projective Cover (FPC) refers to the percentage of land obscured by woody vegetation and trees, thus FPC provides a fine-scale measurement of vegetation cover. FPC was obtained from the Terrestrial Ecosystems Research Network AusCover database (TERNAusCover, 2011) at a resolution of 5 metres. To extract FPC values, a buffer of 30m was used around each sampling site from which cell values were averaged using the raster (Hijmans and van Etten, 2014) package in R (Team 2017).

Temperature, precipitation and FPC association with elevation

We conducted all analyses in R (Team 2017). We examined whether temperature, precipitation and FPC changed significantly with elevation within our three focal study regions by fitting a linear mixed effect model (LMM) with elevation as a fixed effect and region (Thredbo, Guthega and Jagungal) as a random intercept. The LMMs were fitted using the *lmer* function of the lme4 package (Bates et al., 2015) and goodness of fit was examined by calculating conditional R^2 using the *r.squaredGLMM* function in MUMIn (Barton, 2018). Borderline p values were dropped using "Drop1" function. The statistical significance of the fixed effect in LMM was examined using ANOVA with a Chi-square test in the R package car (Fox and Weisberg, 2011). Diagnostic plots for each LMM were examined to ensure model assumptions were met.

We also examined the correlation between temperature, precipitation, FPC and elevation using a Pearson correlation analysis. A high correlation was obtained between elevation and temperature (R = -0.98, Table S4) and elevation and precipitation (R = 0.91; Table S4), therefore, all further models were fitted just using elevation, which serves as a proxy for temperature and precipitation.

Relative abundance along elevation

The relative abundance of each species at each site was calculated via capture rate per minute, calculated as the number of grasshoppers caught divided by the number of searchers, multiplied by the total number of minutes spent collecting (number of grasshoppers/ (no of searchers x time spent collecting; Yadav et al., 2018). The influence of elevation on relative abundance in the three sampled mountain regions was evaluated by fitting a LMM. Models were fitted individually for each species with elevation as a fixed effect and region and time of the day as random effects. To maintain consistency between captures most sampling was carried out in the morning and in the afternoon at the same time each day. Days with higher cloud cover and lower temperatures were avoided due to no activity of grasshoppers on such days. Time of the day was used as a random effect to account for possible bias in the number of samples collected due to variation in temperature and weather differences during the day. Model fit and significance were assessed using ANOVA with a Chi-square test as described above.

Body size correlation with elevation

Body size differs markedly between the sexes in the genus *Kosciuscola* (Campbell and Dearn, 1980). To test if body size of each species changed significantly with elevation and to account for sex-specific differences, we first fitted the model with body size (measured from femur length) as a response variable, elevation as a predictor, sex as an interaction term with elevation and region and site ID as random effect. We then examined the change in body size along elevation gradients across all transects for male and females separately for each of the three species. LMM was fitted using femur length body size as a response variable and elevation as a fixed effect and region and site ID as random effect. In addition to region, site ID was also used as a random effect to account for potential correlation between individuals from the same site.

Results

Temperature, precipitation and FPC association with elevation

Precipitation was significantly positively associated with elevation (P= <0.0001), indicating higher precipitation levels at higher elevations. Temperature (P= <0.001) and FPC (P=0.03) both showed significant negative association with elevation indicating a significant decrease in temperature and vegetation at higher elevations (Table 2, Fig. S1).

Table 2: Association of temperature (Temp) and precipitation (Prec), and Foliage Projective Cover (FPC) with elevation.

Model	Estimate ± SE	Chisq	DF	Pr (>Chisq)	R^2
Prec ~Elevation	179.36 ± 5.27	1154.5	1	<0.0001	0.96
Temp ~ Elevation	-0.920 ± 0.02	1749.5	1	<0.0001	0.97
FPC ~ Elevation	-6.057 ± 2.849	4.519	1	0.03	0.20

Relative abundance along elevation

Correlations between the relative abundance of each species and elevation were variable. For *K. usitatus*, relative abundance significantly decreased linearly with elevation (χ^2 = 6.72, DF=1, P= 0.009, R^2 = 0.26, Fig. 2A) indicating lower relative abundance of this species at higher elevational zones. The relative abundance of *K. tristis* significantly increased linearly with elevation (χ^2 =17.61, DF=1, P = <0.0001, R^2 = 0.44; Fig. 2B). No significant correlation was evident between the relative abundance of *K. cognatus* and elevation (χ^2= 0.006, DF=1, P= 0.93; Fig. 2C).

Similarly, the correlation between the relative abundance of each species and FPC was inconsistent. *K. cognatus* showed a significant negative correlation (χ^2= 4.7, DF=1, P=0.03, R^2 = 0.36; Fig. 2D), indicating higher abundances at sites with low vegetation cover, however relative abundance was not significantly correlated with FPC for *K. usitatus* (P= 0.10; Table S5) or *K. tristis* (P= 0.80; Table S5).

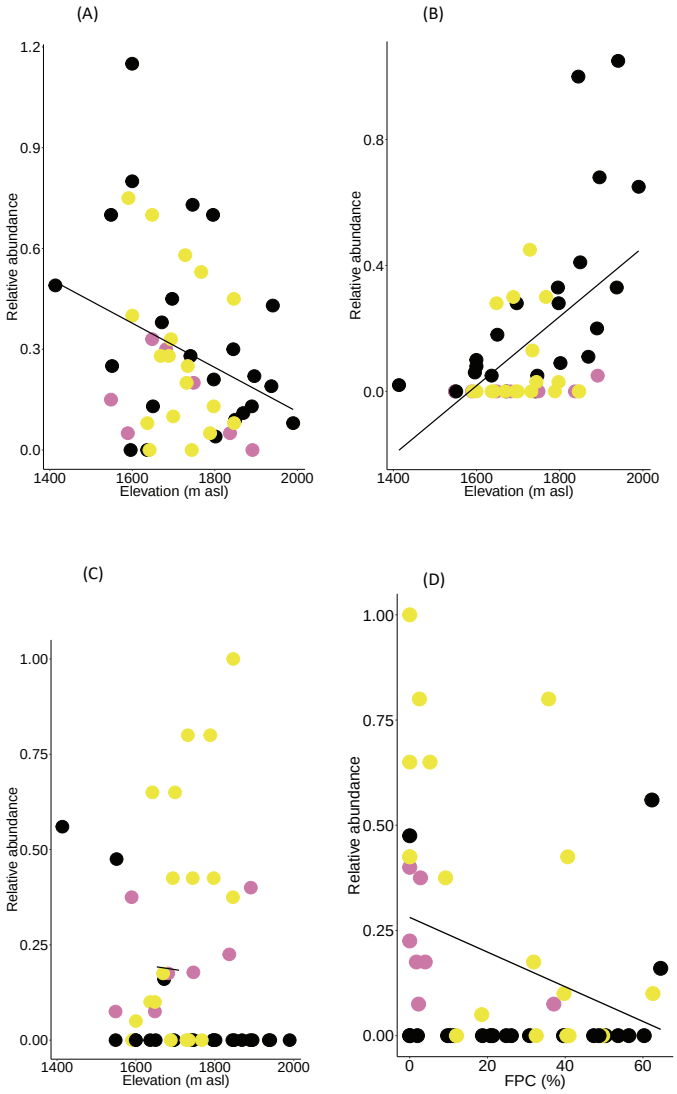

Figure 2: Scatter plots of the relative abundance (Capture rate) of *Kosciuscola* species along the elevational gradient and Foliage Projective Cover. (A) *K. usitatus*; (B) *K. tristis*; (C) *K. cognatus;* (D) *K. cognatus* vs FPC. Colours represent different mountains; Black= Thredbo, Yellow= Guthega and Pink= Jagungal.

Body size change with elevation and sex

The relation between body size and elevation varied between species and sexes. The interaction term was insignificant (P = 0.14) for *K. usitatus* indicating that the change in body size with elevation is not influenced by sex differences, with each sex showing a decline in body size with elevation (male = P= 0.0001, R^2 = 0.34 and female P=0.026, R^2 = 0.04, Table 3, Fig. 3A,3B). However, the interaction effect of sex and elevation was significant for *K. tristis* (P=0.009, Table

3) showing that the observed change in body size is explained by sex, and only females significantly declined in body size with elevation (females: P= 0.03, R^2 = 0.21; males: P= 0.99; Table 3, Fig. 3C). For *K. cognatus* the interaction effect of sex and elevation was also significant (P=0.045) indicating that body size change with elevation is influenced by sex, but in this case, male body size significantly decreased with increasing elevation (P= 0.019, R^2 =0.70, Table 3, Fig. 3D), but this was not upheld in females (P= 0.92, Table 3).

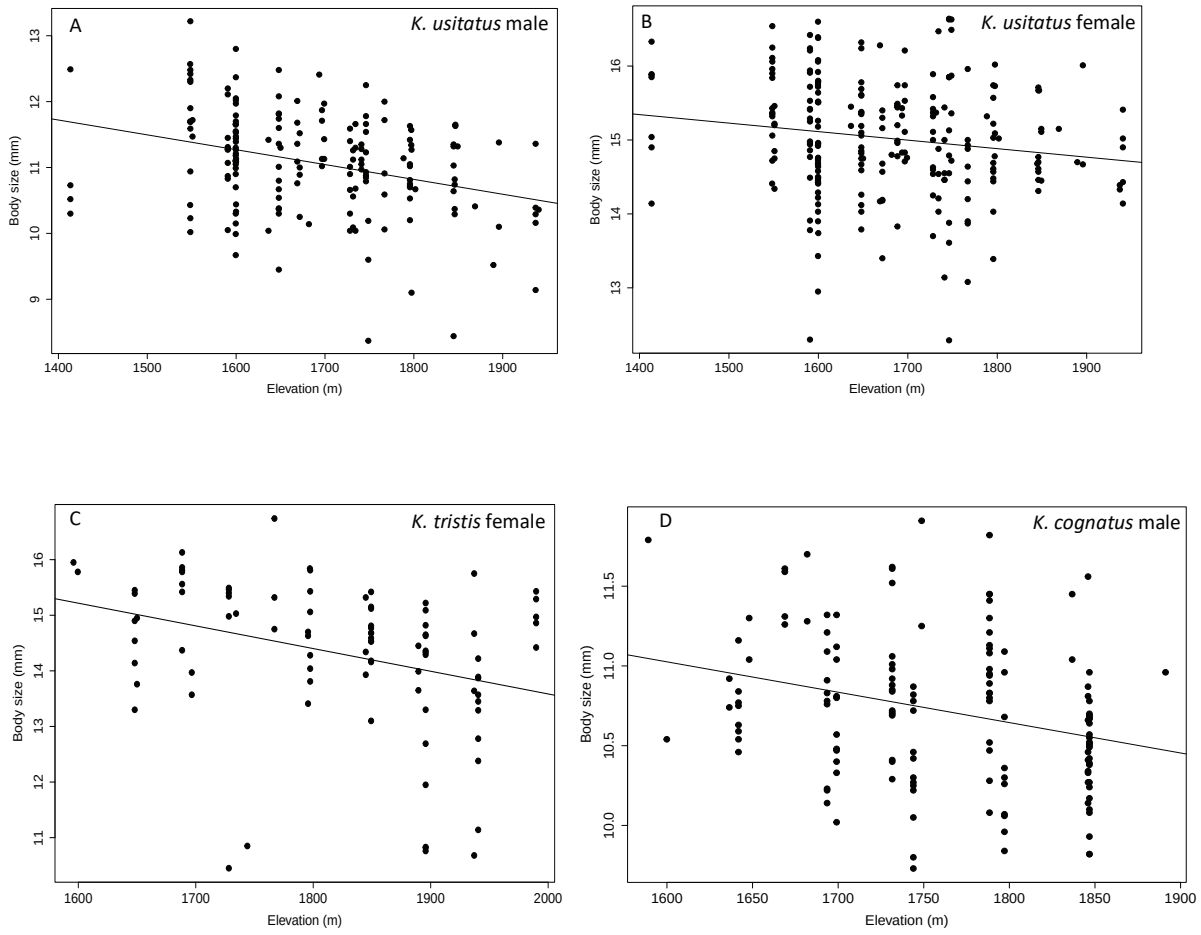

Figure 3: Change in body size with elevation in all three studied species of *Kosciuscola* genus. *K. usitatus* A) male and B) female. Both male (P= 0.0001, R^2 = 0.34 and female (P= 0.02, R^2=0.04) *K. usitatus* showed significant decrease in body size with elevation. C) Negative significant correlation between *K. tristis* female body size (P= 0.03, R^2 = 0.21l) with elevation. D) Significant negative correlation of *K cognatus* male body size (P= 0.01 R^2 =0.70) with elevation.

Table 3: Correlations between body size and elevation in three *Kosciuscola* species, shown for males and females individually and for the interaction between sex and elevation (indicated by * mark).

Sex	Observations	Region	Estimate ± SE	Chisq	DF	P
			K. usitatus			
Male	174	3	-0.002 ± 0.00	14.295	1	0.0001
Female	237	3	-0.001 ± 0.00	4.93	1	0.02
Elevation*Sex	411	3	-	2.17	1	0.14
			K. tristis			
Male	188	2	-	0	1	0.99
Female	91	2	-0.003 ± 0.001	4.27	1	0.03
Elevation*Sex	279	2	-	6.7162	1	0.009
			K. cognatus			
Male	143	2	-0.012 ± 0.00	5.473	1	0.01
Female	128	2	-	0.008	1	0.92
Elevation*Sex	271	2	-	4.016	1	0.045

Discussion

We show variation in relative abundance and body size in three grasshopper species of the genus *Kosciuscola* across elevational gradients and three mountain regions of the Australian Alps. Elevation was strongly associated with temperature and precipitation and was found to be a strong driver of changes in the relative abundances of *K. usitatus* and *K. tristis*. The relative abundance of *K. cognatus* was not significantly influenced by elevation, but significantly decreased with increasing foliage cover suggesting an important role of vegetation in regulating abundance patterns. Body size decreased with increasing elevation in all three species, however, the change in body size was dependent on sex for *K. tristis* and *K. cognatus*. Our study highlights

that local environmental variables (temperature and precipitation) and vegetation cover significantly influence differences in relative abundances and body size of alpine specialist species that differ in their physiological tolerances. A further change in environmental conditions as a consequence of anthropogenic climate change and habitat encroachment will thus largely affect specialist insect species distributions and morphological traits, which are crucial to determine species persistence and survival. In the Australian alpine bioregion, reduction in snow cover, increase in temperature, sub-alpine vegetation encroachment and depletion in food sources is already threatening the abundance and survival of alpine specialist species such as mountain pygmy-possums (Greenville et al., 2018). An altitudinal shift in the distribution of avian and mammalian biodiversity in the Australian alpine bioregion has been visible, as examined over 30 years prior to 1999 (Green and Pickering, 2002). These observations suggest that Australian alpine species are already responding to climate change and further change in environmental conditions may significantly influence their persistence.

Abundance change with elevation

Different trends in relative abundance change along elevation gradient have been observed in the three species (positive: *K. tristis*, negative: *K. usitatus* and insignificant: *K. cognatus*). In ectotherms including insects, thermal preferences, interspecific and intraspecific physiological adaptations and the presence of optimal environments along elevation gradients may lead to difference in species' compositions (Birkett et al., 2018, Röder et al., 2017) and distributions, possibly by influencing their thermoregulatory capacity (Monasterio et al., 2009). Significant variation in the abundance patterns of *K. usitatus* and *K. tristis* is likely due to differences in their thermal tolerances, and elevational preference, whereby *K. usitatus* has been shown to occupy broader elevational gradients and maintain higher cold tolerance as compared to *K. tristis* (Slatyer et al., 2016). In addition, interspecific differences in heat tolerance have been observed in a previous study on *K. usitatus* and *K. tristis*, whereby populations at higher alpine habitats (i.e. treeless) are more tolerant to heat compared to lower-elevation populations (Slatyer et al.,

2016). Furthermore, temperature extremes and an increase in open bare habitats at higher elevations can impose higher selection pressures via decreased thermoregulatory capacity, and fitness (for e.g. reduced fecundity). The decrease in abundance at higher elevations is likely to be related with reduced fecundity due to reduced food quality or a shortened time to oviposit (Hodkinson, 2005). For instance, a previous study on *K. cognatus*, *K. usitatus* and *Praxibulus* sp. by Dearn (1977), showed a decline in egg production per female with increase in elevation. This decline in egg production may negatively influence relative abundance as indicated in *K. usitatus*.

For ectothermic insects to cope with variable thermal regimes in alpine ecosystems it is beneficial to regulate body temperature via colour polymorphism in order to exploit particular elevation zones (Trullas et al., 2007). The increase in relative abundance of *K. tristis* at higher elevations is likely to be assisted by its rapid colour changing behaviour that occurs primarily in response to ambient temperature, as found by Umbers (2011), whereby males change colour from turquoise blue to black at lower temperatures. *Kosciuscola tristis* grasshoppers are black below an ambient temperature of 15°C and the head, pronotum and abdomen turn turquoise when the ambient temperature reaches 25°C (Umbers, 2011, Umbers et al., 2013). Such colour changing behaviour of *K. tristis* males might have an additional thermoregulatory advantage (Umbers, 2011), and may contribute towards maintaining higher densities of individuals at higher elevations with lower temperatures and lower vegetation cover. Several insect species have been shown to maintain different colour morphs along elevational gradients (examples in Hodkinson, 2005, Dearn, 1990, Valverde and Schielzeth, 2015). For instance, in the grasshopper, *Pseudochorthippus parallelus*, lowland populations have a dominance of green coloured individuals, whereas high-altitude populations have a higher dominance of brown coloured individuals (Köhler et al., 2017), which assist in efficient thermoregulation at higher altitudes. Therefore, the maintenance of different colour morphs can thus be advantageous when dealing with harsh climatic changes at higher elevations and may help to increase population densities.

Vegetation cover along the sampled transects was not a significant predictor of abundance in *K. usitatus* and *K. tristis* but was significant for *K. cognatus*. A large number of individuals were observed at sites with lower vegetation cover (Fig. 2D). In grasshoppers, vegetation cover is highly important for egg laying, early instar survival, food resources (Clark, 1967) and basking which in turn is very important for thermoregulation (Kearney et al., 2009, Chappell and Whitman, 1990). A previous study by us on a widespread grasshopper *Phaulacridium vittatum* detected associations of candidate loci putatively under selection with foliage cover, highlighting its potential role in shaping adaptive genetic variations.

Grasshoppers regulate body temperature by shuttling between sun and shade, therefore vegetation cover can affect thermoregulatory behaviour (principal means of controlling body temperature with respect to ambient temperature) by altering the availability of basking sites. The degree to which ectotherms can withstand changes in their ambient thermal environment is crucial in regulating their abundance (Willott and Hassall, 1998). Similar effects of vegetation on abundance, relative abundance and population dynamics of grasshoppers have been observed by other studies (Kemp et al., 1990, Anderson, 1964, Yadav et al., 2018), indicating a key role of vegetation cover in driving insect species distribution and abundance change (Oliver et al., 2016, Andrew et al., 2019). The absence of a significant relationship in *K. usitatus* and *K. tristis* with FPC is likely due to lower variability in the foliage cover in the species preferred elevation zones. A further examination of the correlation in other areas might shed light on the interspecific differences in the relative abundance change with FPC. Notably, our study was carried out for one season and further sampling efforts in other seasons will help to provide more accuracy and a better understanding of spatial and temporal changes in species abundance. A further spread of sampling efforts to other areas in the Australian Alps will be useful to understand population dynamics of these alpine restricted species.

Body size variation along the elevation gradient

A negative association between elevation and body size observed within and across all three species is converse to Bergmann's rule, a pattern been reported in a number of ectothermic species (Mousseau, 1997, Blanckenhorn and Demont, 2004, Baroni et al., 2018) including a large number of Orthoptera (Bidau and Martí, 2007, Ciplak et al., 2008, Laiolo et al., 2013, Eweleit and Reinhold, 2014) and grasshoppers (Berner and Blanckenhorn, 2006, Telfer and Hassall, 1999, Buckley et al., 2014). Temperature and precipitation gradients associated with elevation are important climatic variables associated with thermoregulation and resource availability, which in turn exert selective constraints on body size along spatially variable environments in alpine grasshoppers (Laiolo et al., 2013, Whitman, 2008). At higher elevations with lower temperature, heat gain in insects may dominate over heat conservation and insects with smaller body sizes may rapidly gain and lose heat (Kubota et al., 2007). A quick heat gain and loss in small ectothermic species suggests that they undergo a rapid change in body temperature in relation to variable environmental temperature and thus are likely to be more thermally tolerant (Peters and Peters, 1986), which may allow them to occupy higher elevations. The observed decline in body size along elevation gradients in our study may be related to efficient thermoregulation of ectothermic grasshoppers at lower temperatures. Yet our study does not provide information on what extent variation in body size affects the basal metabolism of *Kosciuscola* species, and further studies on thermoregulation will help to provide further explanation.

Constraints on resources and season length at higher elevations may lead to variation in egg hatching timing, and/or alter developmental periods in grasshoppers (Berner and Blanckenhorn, 2006, Berner et al., 2004) and in other insects (Hodkinson, 2005). Season length and development time are both important for determining body size. Short season length may reduce a species growth period, and since this is positively correlated with body size (Roff, 1980), short seasons may lead to smaller body size (Blanckenhorn and Demont, 2004). This is particularly important for univoltine species (sensitive to season length) than multivoltine species because of

the limited time available to grow and reproduce, which in turn may impose limitations on adult body size and fecundity (Chown and Gaston, 1999, Kozłowski et al., 2004, Horne et al., 2018, Honěk, 1993). As univoltine species, the three studied *Kosciuscola* grasshopper species may experience growth limitation at higher elevations due to shorter growing season length. In addition, grasshoppers at higher elevations may experience later hatching compared to those at lower elevations (Mousseau, 1997), leading to further shortening of the season length for higher elevation populations. This might lead to an overall smaller body size in individuals at a higher elevation than those at lower elevations. An extension of growing season length as a result of climate change induced warming (Robeson, 2002, Linderholm, 2006) may lead to insects at higher elevations showing an increase in body size.

Geographic trends in body size change often differ between males and females, and among species (Stillwell et al., 2007). Sex-specific differences in body size variation within *K. tristis* and *K. cognatus* indicate differential selection and fitness pressures acting on the sexes. Our results are consistent with observations made in a previous study using femur length of only female *K. cognatus* and *K. usitatus* in the same study area (Dearn, 1977). Similar to the current study, Dearn (1977) failed to observe any significant change in body size of female *K. cognatus* with altitude, however, identified significant negative correlation between female femur length and altitude for *K. usitatus.* Although, sex specific clines along geographical scales are common, the underlying causes leading to such sex-specific difference in body size clines are not clear (Blanckenhorn et al., 2006b). One explanation states that the environmental variables associated with geographical gradients may influence males and females differently and can create sex specific clines (e.g. Teder and Tammaru, 2005, Laiolo et al., 2013). Elevational gradients in our study area are strongly correlated with precipitation and temperature, and these associations are likely to influence one sex more than other as shown in *K. tristis* and *K. cognatus*. The absence of a significant correlation of female *K. cognatus* body size with elevation may also highlight

that females in this species have a more variable body size than males in relation to environmental pressures (as shown in nine grasshopper species: Laiolo et al., 2013).

Another explanation behind interspecific body size difference is that the extent of natural selection on females (via increased fecundity) may lead to a difference in body size clines (Blanckenhorn et al., 2006a, Teder, 2014). Predominance of female biased sexual size dimorphism in Orthoptera (Hochkirch and Gröning, 2008) and in our study system suggests that fecundity selection is an important selective force in this Order, which often results in a proportional change in body size in females compared to males (Laiolo et al., 2013) as observed in *K. tristis*. Taken together, the evolution of male and female body size may follow a divergent evolutionary trajectory as a consequence of sexual selection on male or natural selection on female, or sex-specific plasticity due to ecological pressures such as habitat and environment (Fairbairn et al., 2007, Fairbairn, 2013, García-Navas et al., 2017), which could lead to decoupling in body size evolution between sexes.

Conclusion

Our study suggests that the fine-scale elevation gradients and habitat variables can significantly influence relative abundance and body size of alpine insect assemblages, which implies that further change in climatic and vegetation conditions may lead to changes in species distributions and fitness related traits, such as body size. While these patterns were observed only in one season, the strong statistical support and transect-based study design allowed for strong conclusions to be drawn within the sampled time period. An understanding of species distributions and abundances in alpine regions is necessary to monitor future abundance changes, however, not much is known about invertebrate species responses to such variables and our study is an important contribution to fill this gap. This is particularly important as the Australian Alps have relatively flat and rounded mountains with shallow elevational gradients, (1600-2228m on the mainland; Tatarnic, Umbers, & Song, 2013) and the opportunity in alpine species

for migration towards higher elevations is restricted in the Australian Alps (Tatarnic et al., 2013). Furthermore, the results of our study are useful to investigate selection signatures on body size and local adaptation at fine spatial scales, which will further help in understanding adaptive capacity and future persistence of alpine restricted specialist species.

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References

ANDERSON, N. L. 1964. Some relationships between grasshoppers and vegetation. *Annals of the Entomological Society of America,* 57**,** 736-742.

ANDREW, N. R., MILLER, C., HALL, G., HEMMINGS, Z., & OLIVER, I. 2019. Aridity and land use negatively influence a dominant species' upper critical thermal limits. *PeerJ, 6,* e6252.

BALE, J. S. & HAYWARD, S. A. 2010. Insect overwintering in a changing climate. *J Exp Biol,* 213**,** 980-94.

BARONI, D., MASOERO, G., STEWART, A. & HASSALL, C. 2018. Complex influence of climate on the distribution and body size of an Alpine species. *Insect Conservation and Diversity,* 11**,** 435-448.

BARTON, K. 2018. Package 'MuMIn'. R package version 1.40. 4.

BATEMAN, B. L., KUTT, A. S., VANDERDUYS, E. P. & KEMP, J. E. 2010. Small-mammal species richness and abundance along a tropical altitudinal gradient: an Australian example. *Journal of Tropical Ecology,* 26**,** 139-149.

BATES, D., MACHLER, M., BOLKER, B. M. & WALKER, S. C. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* 67**,** 1-48.

BECCACECE, H. M., ZEBALLOS, S. R. & ZAPATA, A. I. 2016. Changes in species richness and composition of Tiger Moths (Lepidoptera: Erebidae: Arctiinae) among three Neotropical ecoregions. *PLoS One,* 11**,** e0162661.

BERGMANN, C. 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse: Göttinger Studien, v. 3.

BERNER, D. & BLANCKENHORN, W. U. 2006. Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *J Anim Ecol,* 75**,** 130-9.

BERNER, D., KÖRNER, C. & BLANCKENHORN, W. U. 2004. Grasshopper populations across 2000 m of altitude: is there life history adaptation? *Ecography,* 27**,** 733-740.

BIDAU, C. J. & MARTÍ, D. A. 2007. Clinal variation of body size in *Dichroplus pratensis* (Orthoptera: Acrididae): Inversion of Bergmann's and Rensch's Rules. *Annals of the Entomological Society of America,* 100**,** 850-860.

BIDAU, C. J., TAFFAREL, A. & CASTILLO, E. R. 2016. Breaking the rule: multiple patterns of scaling of sexual size dimorphism with body size in Orthopteroid insects. *Revista De La Sociedad Entomologica Argentina,* 75**,** 11-36.

BIRKETT, A. J., BLACKBURN, G. A. & MENÉNDEZ, R. 2018. Linking species thermal tolerance to elevational range shifts in upland dung beetles. *Ecography,* 41**,** 1510-1519.

BLANCKENHORN, W. U. & DEMONT, M. 2004. Bergmann and converse bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr Comp Biol,* 44**,** 413-24.

BLANCKENHORN, W. U., DIXON, A. F. G., FAIRBAIRN, D. J., FOELLMER, M. W., GIBERT, P., LINDE, K. V. D., MEIER, R., NYLIN, S., PITNICK, S. & SCHOFF, C. 2006a. Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *The American Naturalist,* 169**,** 245-257.

BLANCKENHORN, W. U., STILLWELL, R. C., YOUNG, K. A., FOX, C. W. & ASHTON, K. G. 2006b. When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution,* 60**,** 2004-2011.

BREHM, G. & FIEDLER, K. 2003. Faunal composition of geometrid moths changes with altitude in an Andean montane rain forest. *Journal of Biogeography,* 30**,** 431-440.

BREHM, G. & FIEDLER, K. 2004. Bergmann's rule does not apply to geometrid moths along an elevational gradient in an Andean montane rain forest. *Global Ecology Biogeography,* 13**,** 7-14.

BREHM, G., ZEUSS, D. & COLWELL, R. K. 2018. Moth body size increases with elevation along a complete tropical elevational gradient for two hyperdiverse clades. *Ecography*.

BUCKLEY, L. B., MILLER, E. F. & KINGSOLVER, J. G. 2013. Ectotherm thermal stress and specialization across altitude and latitude. *Integrative comparative biology* 53**,** 571-581.

BUCKLEY, L. B. & NUFIO, C. R. 2014. Elevational clines in the temperature dependence of insect performance and implications for ecological responses to climate change. *Conservation physiology,* 2.

BUCKLEY, L. B., NUFIO, C. R. & KINGSOLVER, J. G. 2014. Phenotypic clines, energy balances and ecological responses to climate change. *J Anim Ecol,* 83**,** 41-50.

CARDILINI, A. P., BUCHANAN, K. L., SHERMAN, C. D., CASSEY, P., & SYMONDS, M. R. 2016. Tests of ecogeographical relationships in a non-native species: what rules avian morphology?. *Oecologia,* 181, 783-793.

CAMPBELL, N. & DEARN, J. 1980. Altitudinal Variation in, and Morphological Divergence between, Three related species of grasshopper, *Praxibulus* sp., *Kosciuscola cognatus* and *K. usitatus* (Orthoptera: Acrididae). *Australian Journal of Zoology,* 28**,** 103-118.

CHAPPELL, M. & WHITMAN, D. 1990. Grasshopper thermoregulation. *Biology of grasshoppers. Wiley, New York***,** 143-172.

CHOWN, S. L. & GASTON, K. J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society,* 74**,** 87-120.

CHOWN, S. L. & GASTON, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biol Rev Camb Philos Soc,* 85**,** 139-69.

CIPLAK, B., SIRIN, D., TAYLAN, M. S. & KAYA, S. 2008. Altitudinal size clines, species richness and population density: case studies in Orthoptera. *Journal of Orthoptera Research,* 17**,** 157-164.

CLARK, D. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.

COLWELL, R. K., RAHBEK, C. & GOTELLI, N. J. 2004. The mid-domain effect and species richness patterns: what have we learned so far? *The American Naturalist,* 163**,** E1-E23.

CURRIE, D. J. & KERR, J. T. 2008. Tests of the mid-domain hypothesis: a review of the evidence. *Ecological Monographs,* 78**,** 3-18.

DEARN, J. 1977. Variable life history characteristics along an altitudinal gradient in three species of Australian grasshopper. *Oecologia,* 28**,** 67-85.

DEARN, J. 1990. Color pattern polymorphism. *Biology of grasshoppers***,** 517-549.

DIRNBÖCK, T., ESSL, F. & RABITSCH, W. 2011. Disproportional risk for habitat loss of high-altitude endemic species under climate change. *Global Change Biology,* 17**,** 990-996.

EWELEIT, L. & REINHOLD, K. 2014. Body size and elevation: do Bergmann's and Rensch's rule apply in the polytypic bushcricket *Poecilimon veluchianus*? *Ecological Entomology,* 39**,** 133-136.

FAIRBAIRN, D. J. 1997. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics,* 28**,** 659-687.

FAIRBAIRN, D. J. 2005. Allometry for sexual size dimorphism: testing two hypotheses for Rensch's rule in the water strider *Aquarius remigis*. *The American Naturalist,* 166**,** S69-S84.

FAIRBAIRN, D. J. 2013. *Odd couples: extraordinary differences between the sexes in the animal kingdom*, Princeton University Press.

FAIRBAIRN, D. J., BLANCKENHORN, W. U. & SZÉKELY, T. 2007. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*, Oxford University Press.

FOX, J. & WEISBERG, S. 2011. Multivariate linear models in R. An R companion to applied regression. *Los Angeles: Thousand Oaks*.

GARCÍA-NAVAS, V., NOGUERALES, V., CORDERO, P. J. & ORTEGO, J. 2017. Ecological drivers of body size evolution and sexual size dimorphism in short-horned grasshoppers (Orthoptera: Acrididae). *Journal of evolutionary biology,* 30**,** 1592-1608.

GOBIET, A., KOTLARSKI, S., BENISTON, M., HEINRICH, G., RAJCZAK, J. & STOFFEL, M. 2014. 21st century climate change in the European Alps—a review. *Science of the Total Environment,* 493**,** 1138-1151.

GREEN, K. 2010. Alpine taxa exhibit differing responses to climate warming in the snowy mountains of Australia. *Journal of Mountain Science,* 7**,** 167-175.
GREEN, K. & PICKERING, C. M. 2002. Scenario for mammal and bird diversity in the Snowy Mountains of Australia in relation to climate change. In 'Global Mountain Biodiversity: Changes and Threats'.(Eds C. Korner and E. Spehn.) pp. 241–249. Springer-Verlag, Berlin.
GREENVILLE, A. C., BURNS, E., DICKMAN, C. R., KEITH, D. A., LINDENMAYER, D. B., MORGAN, J. W., HEINZE, D., MANSERGH, I., GILLESPIE, G. R., EINODER, L., FISHER, A., RUSSELL-SMITH, J., METCALFE, D. J., GREEN, P. T., HOFFMANN, A. A. & WARDLE, G. M. 2018. Biodiversity responds to increasing climatic extremes in a biome-specific manner. *Sci Total Environ,* 634**,** 382-393.
HENNESSY, K., WHETTON, P., SMITH, I., BATHOLS, J., HUTCHINSON, M. & SHARPLES, J. 2003. The impact of climate change on snow conditions in mainland Australia. *Aspendale: CSIRO Atmospheric Research*.
HERZOG, S. K., HAMEL-LEIGUE, A. C., LARSEN, T. H., MANN, D. J., SORIA-AUZA, R. W., GILL, B. D., EDMONDS, W. D. & SPECTOR, S. 2013. Elevational distribution and conservation biogeography of phanaeine dung beetles (Coleoptera: Scarabaeinae) in Bolivia. *PLoS One,* 8**,** e64963.
HIJMANS, R. J. & VAN ETTEN, J. 2014. raster: Geographic data analysis and modeling. *R package version,* 2.
HILT, N. & FIEDLER, K. 2005. Diversity and composition of Arctiidae moth ensembles along a successional gradient in the Ecuadorian Andes. *Diversity and Distributions,* 11**,** 387-398.
HOCHKIRCH, A. & GRÖNING, J. 2008. Sexual size dimorphism in Orthoptera (sens. Str.): a review. *Journal of Orthoptera Research***,** 189-196.
HODKINSON, I. D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol Rev Camb Philos Soc,* 80**,** 489-513.
HOFFMANN, A. A., RYMER, P. D., BYRNE, M., RUTHROF, K. X., WHINAM, J., MCGEOCH, M., BERGSTROM, D. M., GUERIN, G. R., SPARROW, B. & JOSEPH, L. 2018. Impacts of recent climate change on terrestrial flora and fauna: Some emerging Australian examples. *Austral Ecology*.
HONĚK, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos***,** 483-492.
HORNE, C. R., HIRST, A. G. & ATKINSON, D. 2018. Insect temperature–body size trends common to laboratory, latitudinal and seasonal gradients are not found across altitudes. *Functional Ecology,* 32**,** 948-957.
IPCC 2014. Climate Change 2014 Synthesis Report.
JOERN, A. & GAINES, S. 1990. Population dynamics and regulation in grasshoppers. *Biology of grasshoppers***,** 415-482.
KEARNEY, M., SHINE, R. & PORTER, W. P. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences,* 106**,** 3835-3840.
KEMP, W. P., HARVEY, S. J. & O'NEILL, K. M. 1990. Patterns of vegetation and grasshopper community composition. *Oecologia,* 83**,** 299-308.
KLEIBER, M. 1947. Body size and metabolic rate. *Physiol Rev,* 27**,** 511-41.
KÖHLER, G., SAMIETZ, J. & SCHIELZETH, H. 2017. Morphological and colour morph clines along an altitudinal gradient in the meadow grasshopper *Pseudochorthippus parallelus*. *PloS one,* 12**,** e0189815.
KÖRNER, C. 2007. The use of 'altitude'in ecological research. *Trends in ecology evolution* 22**,** 569-574.
KOZŁOWSKI, J., CZARNOŁĘSKI, M. & DAŃKO, M. 2004. Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integrative Comparative Biology* 44**,** 480-493.

KUBOTA, U., LOYOLA, R. D., ALMEIDA, A. M., CARVALHO, D. A. & LEWINSOHN, T. M. 2007. Body size and host range co-determine the altitudinal distribution of Neotropical tephritid flies. *Global Ecology and Biogeography,* 16**,** 632-639.

LA SORTE, F. A. & JETZ, W. 2010. Projected range contractions of montane biodiversity under global warming. *Proc Biol Sci,* 277**,** 3401-10.

LAIOLO, P., ILLERA, J. C. & OBESO, J. R. 2013. Local climate determines intra- and interspecific variation in sexual size dimorphism in mountain grasshopper communities. *J Evol Biol,* 26**,** 2171-83.

LAURANCE, W. F., CAROLINA USECHE, D., SHOO, L. P., HERZOG, S. K., KESSLER, M., ESCOBAR, F., BREHM, G., AXMACHER, J. C., CHEN, I. C., GÁMEZ, L. A., HIETZ, P., FIEDLER, K., PYRCZ, T., WOLF, J., MERKORD, C. L., CARDELUS, C., MARSHALL, A. R., AH-PENG, C., APLET, G. H., DEL CORO ARIZMENDI, M., BAKER, W. J., BARONE, J., BRÜHL, C. A., BUSSMANN, R. W., CICUZZA, D., EILU, G., FAVILA, M. E., HEMP, A., HEMP, C., HOMEIER, J., HURTADO, J., JANKOWSKI, J., KATTÁN, G., KLUGE, J., KRÖMER, T., LEES, D. C., LEHNERT, M., LONGINO, J. T., LOVETT, J., MARTIN, P. H., PATTERSON, B. D., PEARSON, R. G., PEH, K. S. H., RICHARDSON, B., RICHARDSON, M., SAMWAYS, M. J., SENBETA, F., SMITH, T. B., UTTERIDGE, T. M. A., WATKINS, J. E., WILSON, R., WILLIAMS, S. E. & THOMAS, C. D. 2011. Global warming, elevational ranges and the vulnerability of tropical biota. *Biological Conservation,* 144**,** 548-557.

LEE, J. E., SOMERS, M. J. & CHOWN, S. L. 2011. Density, body size and sex ratio of an indigenous spider along an altitudinal gradient in the sub-Antarctic. *Antarctic Science,* 24**,** 15-22.

LINDERHOLM, H. W. (2006). Growing season changes in the last century. *Agricultural and forest meteorology,* 137, 1-14.

LOMOLINO, M. V. 2001. Elevation gradients of species-density: historical and prospective views. *Global Ecology and biogeography,* 10**,** 3-13.

LYONS, M. B., FOSTER, S. D. & KEITH, D. A. 2017. Simultaneous vegetation classification and mapping at large spatial scales. *Journal of biogeography,* 44**,** 2891-2902.

MARTIN-REGALADO, C. N., BRIONES-SALAS, M., LAVARIEGA, M. C. & MORENO, C. E. 2019. Spatial incongruence in the species richness and functional diversity of cricetid rodents. *PLoS One,* 14**,** e0217154.

MASAKI, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution,* 21**,** 725-741.

MAVEETY, S. A. & BROWNE, R. A. 2014. Patterns of morphology in carabid beetles (Coleoptera: Carabidae) along a Neotropical altitudinal gradient. *International Journal of Tropical Insect Science,* 34**,** 157-171.

MCCOY, E. D. 1990. The distribution of insects long elevational gradients. *Oikos,* 58**,** 313-322.

MONASTERIO, C., SALVADOR, A., IRAETA, P. & DÍAZ, J. A. 2009. The effects of thermal biology and refuge availability on the restricted distribution of an alpine lizard. *Journal of Biogeography,* 36**,** 1673-1684.

MOTA, G. S., LUZ, G. R., MOTA, N. M., COUTINHO, E. S., VELOSO, M. D. M., FERNANDES, G. W. & NUNES, Y. R. F. 2018. Changes in species composition, vegetation structure, and life forms along an altitudinal gradient of rupestrian grasslands in south-eastern Brazil. *Flora,* 238**,** 32-42.

MOUSSEAU, T. A. 1997. Ectotherms follow the converse to Bergmann's Rule. *Evolution,* 51**,** 630-632.

MOUSSEAU, T. A. & ROFF, D. A. 1989. Adaptation to seasonality in a Cricket: Patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution,* 43**,** 1483-1496.

NICHOLLS, N. 2005. Climate variability, climate change and the Australian snow season. *Australian Meteorological Magazine,* 54**,** 177-185.

OLIVER, I., DORROUGH, J., DOHERTY, H., & ANDREW, N. R. 2016. Additive and synergistic effects of land cover, land use and climate on insect biodiversity. *Landscape Ecology,* 31, 2415-2431.
PETERS, R. H. & PETERS, R. H. 1986. *The ecological implications of body size*, Cambridge University Press.
RAHBEK, C. 1997. The relationship among area, elevation, and regional species richness in neotropical birds. *Am Nat,* 149**,** 875-902.
RAMIREZ-BAUTISTA, A. & WILLIAMS, J. N. 2019. The importance of productivity and seasonality for structuring small rodent diversity across a tropical elevation gradient. *Oecologia,* 190**,** 275-286.
REHN, J. 1957. The grasshoppers and locusts (Acridoidea) of Australia. *Melbourn, Australia: CSIRO.*
ROBESON, S. M. (2002). Increasing growing-season length in Illinois during the 20th century. *Climatic Change*, 52, 219-238.
RÖDER, J., DETSCH, F., OTTE, I., APPELHANS, T., NAUSS, T., PETERS, M. K. & BRANDL, R. 2017. Heterogeneous patterns of abundance of epigeic arthropod taxa along a major elevation gradient. *Biotropica,* 49**,** 217-228.
ROFF, D. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. *Oecologia,* 45**,** 202-208.
ROLAND, J. & MATTER, S. F. 2016. Pivotal effect of early-winter temperatures and snowfall on population growth of alpine *Parnassius smintheus* butterflies. *Ecological Monographs,* 86**,** 412-428.
SÁNCHEZ-BAYO, F. & GREEN, K. 2018. Australian snowpack disappearing under the influence of global warming and solar activity. *Arctic, Antarctic, and Alpine Research,* 45**,** 107-118.
SHELOMI, M. 2012. Where are we now? Bergmann's rule sensu lato in insects. *The American Naturalist,* 180**,** 511-519.
SHERIDAN, J. A. & BICKFORD, D. 2011. Shrinking body size as an ecological response to climate change. *Nature Climate Change,* 1**,** 401-406.
SLATYER, R. A., NASH, M. A. & HOFFMANN, A. A. 2016. Scale-dependent thermal tolerance variation in Australian mountain grasshoppers. *Ecography,* 39**,** 572-582.
SLATYER, R. A., NASH, M. A., MILLER, A. D., ENDO, Y., UMBERS, K. D. & HOFFMANN, A. A. 2014. Strong genetic structure corresponds to small-scale geographic breaks in the Australian alpine grasshopper *Kosciuscola tristis*. *BMC Evol Biol,* 14**,** 204.
STAMPS, J. 1993. Sexual size dimorphism in species with asymptotic growth after maturity. *Biological Journal of the Linnean Society,* 50**,** 123-145.
STILLWELL, R. C., MORSE, G. E. & FOX, C. W. 2007. Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *The American Naturalist,* 170**,** 358-369.
SUNDQVIST, M. K., SANDERS, N. J. & WARDLE, D. A. 2013. Community and ecosystem responses to elevational gradients: Processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics,* 44**,** 261-280.
TATARNIC, N. J., UMBERS, K. D. L. & SONG, H. 2013. Molecular phylogeny of the *Kosciuscola* grasshoppers endemic to the Australian alpine and montane regions. *Invertebrate Systematics,* 27**,** 307-316.
TEAM, R.C. 2017. R*: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. [Online]*. Available: https://www.R-project.org/[Accessed].
TEDER, T. 2014. Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. *Functional Ecology,* 28**,** 479-486.
TEDER, T. & TAMMARU, T. 2005. Sexual size dimorphism within species increases with body size in insects. *Oikos,* 108**,** 321-334.

TELFER, M. G. & HASSALL, M. 1999. Ecotypic differentiation in the grasshopper *Chorthippus brunneus*: life history varies in relation to climate. *Oecologia,* 121**,** 245-254.
TERNAUSCOVER. 2011. *Woody extent and Foliage Projective Cover - SPOT, OEH algorithm, NSW coverage* [Online]. Available: http://www.auscover.org.au/purl/spot-woody-extent-fpc-nsw-2011 [Accessed].
TRULLAS, S. C., VAN WYK, J. H. & SPOTILA, J. R. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology,* 32**,** 235-245.
UMBERS, K. D. 2011. Turn the temperature to turquoise: cues for colour change in the male chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae). *J Insect Physiol,* 57**,** 1198-204.
UMBERS, K. D., HERBERSTEIN, M. E. & MADIN, J. S. 2013. Colour in insect thermoregulation: empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*. *J Insect Physiol,* 59**,** 81-90.
VALVERDE, J. P. & SCHIELZETH, H. 2015. What triggers colour change? Effects of background colour and temperature on the development of an alpine grasshopper. *BMC evolutionary biology,* 15**,** 168.
VINARSKI, M. 2014. On the applicability of Bergmann's rule to ectotherms: the state of the art. *Biology Bulletin Reviews,* 4**,** 232-242.
WACHTER, D. H., O'NEILL, K. M. & KEMP, W. P. 1998. Grasshopper (Orthoptera: Acrididae) communities on an elevational gradient in southwestern Montana. *Journal of the Kansas Entomological Society***,** 35-43.
WAHREN, C. H., CAMAC, J. S., JARRAD, F. C., WILLIAMS, R. J., PAPST, W. A. & HOFFMANN, A. A. 2013. Experimental warming and long-term vegetation dynamics in an alpine heathland. *Australian Journal of Botany,* 61**,** 36-51.
WERENKRAUT, V. & RUGGIERO, A. 2014. The richness and abundance of epigaeic mountain beetles in north-western Patagonia, Argentina: assessment of patterns and environmental correlates. *Journal of biogeography,* 41**,** 561-573.
WHITMAN, D. W. 2008. The significance of body size in the Orthoptera: a review. *Journal of Orthoptera research,* 17**,** 117-134.
WILLIAMS, S. E., SHOO, L. P., ISAAC, J. L., HOFFMANN, A. A. & LANGHAM, G. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol,* 6**,** 2621-6.
WILLOTT, S. & HASSALL, M. 1998. Life-history responses of British grasshoppers (Orthoptera: Acrididae) to temperature change. *Functional Ecology,* 12**,** 232-241.
XU, T. B. & HUTCHINSON, M. F. 2013. New developments and applications in the ANUCLIM spatial climatic and bioclimatic modelling package. *Environmental Modelling & Software,* 40**,** 267-279.
YADAV, S., STOW, A. J. & DUDANIEC, R. Y. 2019. Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (*Phaulacridium vittatum*). *Mol Ecol*.
YADAV, S., STOW, A. J., HARRIS, R. M. B. & DUDANIEC, R. Y. 2018. Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae). *J Insect Sci,* 18**,** 13.
ZENKER, M. M., DEVRIES, P. J., PENZ, C. M., TESTON, J. A., FREITAS, A. V. L. & PIE, M. R. 2015. Diversity and composition of *Arctiinae* moth assemblages along elevational and spatial dimensions in Brazilian Atlantic Forest. *Journal of Insect Conservation,* 19**,** 129-140.

(A)

(B)

(A) *Kosciuscola usitatus* female; (B) *Kosciuscola usitatus* juvenile female (photos: Sonu Yadav)

CHAPTER 5

Selection signatures associate with elevational niche in two co-occurring alpine grasshoppers (genus *Kosciuscola*)

Authors

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Author contributions

This chapter was conceived by Sonu Yadav and Rachael Dudaniec. I carried out the fieldwork with the help of Kate Umbers and Justin McNab. I collected datasets, carried out species identification, lab work, data analysis and interpretation of results. I discussed my findings with Rachael Dudaniec and Adam Stow. I wrote the chapter, which was greatly improved by comments and editing from Rachael Dudaniec and Adam Stow.

Abstract

Alpine ecosystems are strongly associated with steep temperature shifts with changes in elevation, which are being modified under anthropogenic climate change. Local adaptation might be expected to be more pronounced in species with narrow ecological niches, thus making these species more vulnerable to environmental change. Here we examine how genetic structure and patterns of selection vary across elevational gradients in two co-occurring grasshopper species (*Kosciuscola* genus) endemic to the Australian Alps, which differ in elevational niche breadth *(K. usitatus* = ~1400-2200m, *K. tristis* = ~1500-2000). With Single Nucleotide Polymorphisms (SNPs) derived from double digest restriction-site associated DNA (ddRAD) sequencing we conduct independent analyses for each species (9,017 SNPs *K. usitatus*, 7,363 SNPs *K. tristis*) and for a dataset with common loci (3,058 SNPs) to test for species-specific and common signatures of selection. We examine selection associated with environmental variables across two mountain regions using Fst outlier tests, univariate and multivariate environmental association analyses (EAA). Genetic structure was weaker for the more broadly distributed *K. usitatu*s (global Fst=0.026) compared to *K. tristis* (global Fst=0.05) across the two mountain regions. Detection of SNPs under putative selection revealed two fold unique outliers in the more narrowly distributed *K. tristis* (10.3%) compared to *K. usitatus* (5.1%). Climatic variables were more strongly associated with selection signatures in both species compared to terrain or soil variables. For the common dataset, 8.3% of SNPs under putative selection were shared by both species and were strongly associated with elevation, indicating parallel selection patterns and climatic drivers in both species. Additive polygenic scores were positively and non-linearly correlated with elevation in both species. The highest number of SNP annotations across all datasets was to a gene involved in lipid metabolism and development. Our study suggests that alpine insects with a wider elevational niche are likely to maintain higher gene flow and reduced local adaptation, and therefore be less sensitive to climate change-induced temperature shifts than species with a narrower elevational niche. Furthermore, closely related species sharing an overlapping environmental niche may show signatures of parallel local adaptation

Keywords

Local adaptation, parallel adaptation, landscape genomics, *Kosciuscola,* elevational gradient

Introduction

Alpine environments are subject to rapid changes in temperature, precipitation, vegetation cover and other abiotic factors, making them sensitive to shifting thermal regimes under climate change (Gobiet et al., 2014, IPCC, 2014, Beniston, 2003). Species in alpine areas are often specialists with high rates of local endemism (La Sorte and Jetz, 2010, Laurance et al., 2011, McCain and Colwell, 2011, Steinbauer et al., 2016) and have limited opportunities to shift their ranges due to narrow thermal tolerances, local physiological or behavioural adaptations, and limited habitat suitability (Elsen and Tingley, 2015, Colwell et al., 2008, Dahlhoff et al., 2019). Furthermore, the topographic complexity of alpine areas can isolate populations over fine geographic scales and confine species to specific elevational zones, with negative consequences for gene flow (Polato et al., 2017, Jackson et al., 2018, Polato et al., 2018, Steinbauer et al., 2016). Studying fine scale geographic patterns of genetic differentiation and local adaptation will contribute towards predicting the resilience of Alpine species to climate change.

Elevation gradients in alpine regions are characterised by sudden changes in environmental variables (Whiteman, 2000) and the study of adaptation along altitudinal gradients has a long history in evolutionary biology (Endler, 1977, Mayr, 1963). The relatively strong and spatially varying selection pressures at higher elevations where there is high solar radiation intensity, lower atmospheric oxygen pressure and lower temperature (Körner, 2007), and highly variables microclimate niches (Scherrer and Koerner, 2010) may maintain adaptive genetic variation across elevational gradients and lead to local adaptation (Keller et al., 2013, Kawecki and Ebert, 2004, Rank and Dahlhoff, 2002). Theoretical models and empirical studies have shown that local adaptation can occur when selection overcomes the homogenising effects of gene flow, while at finer scales gene flow is more likely to hinder local adaptation (reviewed in Richardson et al.,

2014). However, there is increasing evidence for environment-driven selection at fine spatial scales (hereafter 'microgeographic scales') in both alpine (Guo et al., 2016, Waterhouse et al., 2018, Eldon et al., 2019, Frachon et al., 2018) and non-alpine systems (e.g., Richardson and Urban, 2013, Kubota et al., 2015, Barney et al., 2017), challenging notions that environmental selection is generally observable over large spatial scales (several hundreds to thousands of kilometres).

Describing local adaptation at microgeographic scales (i.e. a few metres to kilometres) could reveal fine scale patterns of adaptive genetic responses, which in turn can increase the reliability of climate change response predictions (Manel et al., 2010). Microgeographic studies of local adaptation can efficiently minimize some of the shortcomings of large-scale studies such as the confounding effects of population structure, allelic heterogeneity and rare alleles, and may be more interpretable with regard to a focal species' mean migration distance, which could be a few kilometres per decade (Frachon et al., 2018, Chen et al., 2011). Yet local adaptation studies at microgeographic scales have received little attention (Richardson et al., 2014). Sharp climatic gradients observed at very fine scales (i.e. within a few metres) within alpine regions make them an excellent landscape to study fine scale signatures of selection.

The genetic architecture underlying environmental adaptation (i.e. gene numbers, allelic effects and the genomic location of genes) can influence a population’s evolutionary response to environmental conditions via increasing or decreasing the rate of adaptation (Gagnaire and Gaggiotti, 2016). For example, if multiple traits (several genes) contribute towards adaptation then possible pleiotropic effects and linkage disequilibrium between genes may influence the selection response. Thus, to better understand species' evolutionary responses to climatic conditions it is important to understand genomic architecture. Climatic adaptation appears to have a polygenic genomic architecture, with the involvement of hundreds to thousands of small-effect alleles scattered across the genome (reviewed in Bay et al., 2017). Recently, advancements

have been made to examine polygenic effects without knowing allelic effects (Lasky et al., 2015, Babin et al., 2017). Understanding gene relationships is a complex yet important step to for ascertaining whether adaptive traits are polygenic (i.e. involve several genes of small effect) or oligogenic (i.e. involve a few genes of large effect).

Environmental and intra-specific interactions may influence patterns of adaptive genomic variation (Hand et al., 2015), therefore a species' adaptive capacity may be better understood by comparing adaptive variation across co-occurring species (Gugerli et al., 2013). Patterns of gene flow and local adaptation may differ in closely related species due to differences in their ecological niche and thermal preference. Species with a narrow elevational niche and greater cold tolerance may have a lower potential to adapt to rapidly increasing temperatures than species with lower cold tolerance and a broader elevational distribution (for e.g., Reich et al., 2015, Deutsch et al., 2008, Birkett et al., 2018). Furthermore, closely related species may show similar adaptive genomic responses when sharing a common environment (Yeaman et al., 2016). However, most studies of local adaptation examine a single species in isolation, thus ignoring environmental effects that simultaneously affect co-occurring species that experience similar environmental challenges (Hand et al., 2015). Thus, studying co-occurring species simultaneously is required to characterise common or divergent patterns of local environmental selection pressures, or to detect climate-driven shifts in hybridisation (Gamboa and Watanabe, 2019). Understanding how species differ or overlap in their adaptive capacity is a key focus of multi-species conservation planning for climate change mitigation (Schwenk and Donovan, 2011, Nicholson and Possingham, 2006).

Like many other alpine ecosystems, the Australian Alps are susceptible to climate change. The Australian Alps have experienced a decline in precipitation by 6%, an increase in temperature at an average of 0.4°C since 1979 and a substantial decline in snow depth since 1954 (Nicholls, 2005, Hennessy et al., 2003, Sánchez-Bayo and Green, 2018, Wahren et al., 2013). Apart from

climate change, sub-alpine vegetation encroachment and increased impacts of invasive species (Hoffmann et al., 2018) are other important threats. Such impacts have been linked with alterations to the survival rate, distribution and population dynamics of endemic alpine species (Green, 2010, Nicholls, 2005). For instance, reduced snow cover may considerably influence survival rate of overwintering species due to the increase in temperature fluctuations, which would otherwise be buffered by snow cover (Bale and Hayward, 2010). Studies of environmental adaptation and genetic differentiation of alpine restricted species are thus needed to understand the adaptive potential of alpine species.

To examine genetic differentiation and the extent of local adaptation in co-occurring species of the Australian Alps, we compare two grasshopper species from the genus *Kosciuscola* (*K. usitatus* and *K. tristis*). The two species have been observed to have overlapping elevational distributions, with *K. usitatus* occupying a wider elevation zones (~1400-2200 m) than *K. tristis* (~1500-2000 m) (Slatyer et al., 2014, Campbell and Dearn, 1980, Slatyer et al., 2016) and is empirically tested in Yadav et al., Chapter 4. The two species also differ in their relative abundances with elevation, whereby the relative abundance of *K. usitatus* decreases and *K. tristis* relative abundance increases with increasing elevation, indicating elevation-associated niche partitioning (Yadav et al., Chapter 4). Apart from these fine scale elevational differences, both species differ in cold tolerances (but not in CTmax), with *K. usitatus* having a slightly higher cold tolerance (CTmin= -3 °C) than *K. tristis* (CTmin= -2 °C). Such differences in cold tolerance limits and elevational distributions are predicted to influence patterns of local adaptation and gene flow even at fine spatial scales in co-occurring species.

Here we conduct a landsape genomics analysis of two co-occurring alpine grasshoppers, *K. usitatus* and *K. tristis* with broader and narrower elevational niches, respectively, using a transect-based sampling design in two mountain regions of Mt Kosciuszko National Park in south-eastern Australia. We specifically examine, 1) whether the two co-occurring species show

contrasting patterns of neutral genetic structure and adaptive genetic variation along elevational gradients, 2) whether the two species show common selection signatures, indicating parallel local environmental adaptation, and 3) whether similar signals of polygenic local adaptation along elevational gradients are evident in both species.

Materials and methods

Study species and sampling

Grasshoppers of the genus *Kosciuscola* are endemic to Australia and the genus is an assemblage of six known species. The two focal species: *K. usitatus* and *K. tristis* are commonly distributed in the Kosciuszko alpine region ranging from montane woodlands to alpine areas and in some locations have overlapping elevational distributions (Slatyer et al., 2016). Our focal species were chosen based on observed differences in their elevational distributions and thermal tolerance breadths in the study area (Slatyer et al., 2016, Yadav et al., Chapter 4), Campbell and Dearn, 1980). Both the species are wingless and have non-overlapping generations (Green and Osborne, 1994). Sexual dimorphism is present in both species, whereby females have a larger body size than males (Slatyer et al., 2016, Umbers et al., 2013).

Sampling was undertaken for two weeks in late February to early March 2017. Three transects ranging from 1400 m a.s.l. to 2,200 m a.s.l. were laid in each of the regions Thredbo (36.5 °S, 148.31 °S) and Guthega (36.38 °S, 148.38 °S) within Kosciuszko National Park in New South Wales. However, *K. tristis* was not present in substantial number in the two transects (T2 and T3) in Guthega (Table 2). Samples were searched and collected at approximately 50 m intervals of elevation along each transect (Fig. 1; more details on sampling is in Chapter 4), however, some sites in the transects lacked the presence of one or both the species. The two mountain regions are located ~25km apart. Samples were collected from a total of 32 points across the transects in both regions, out of which 29 and 20 points were represented by *K. usitatus* and *K. tristis*, respectively (Table 1 and Table 2).

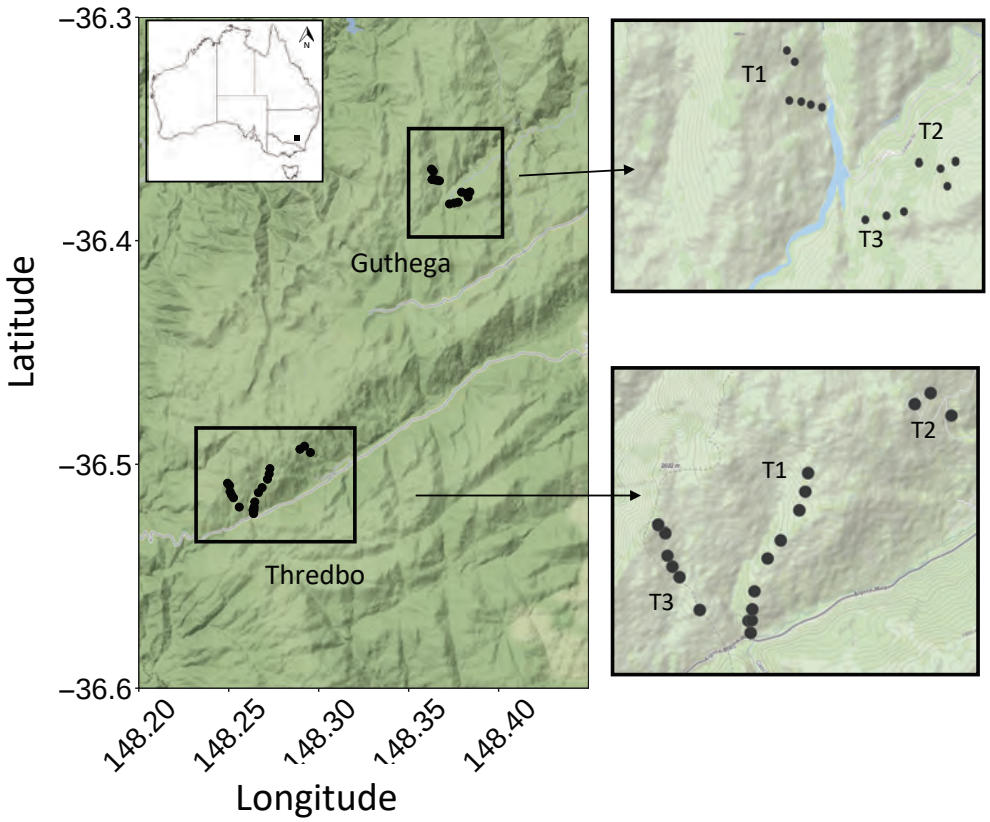


Figure 1. Map of sampling sites in two mountain regions- Thredbo and Guthega for both the species. Black points indicate sample sites were at least one species present along transects.

Table 1: Sample site information of only analysed samples for *K. usitatus*, a total of 130 individuals were analysed after removing poorly genotyped individuals.

Transect	Region	Elevation	Individuals
T1	Thredbo	1549	9
T1	Thredbo	1600	4
T1	Thredbo	1650	2
T1	Thredbo	1697	5
T1	Thredbo	1741	5
T1	Thredbo	1797	5
T1	Thredbo	1849	4
T1	Thredbo	1896	3
T1	Thredbo	1937	7
T2	Thredbo	1672	5
T2	Thredbo	1802	2
T2	Thredbo	1869	2
T3	Thredbo	1746	5
T3	Thredbo	1796	5
T3	Thredbo	1845	4

T3	Thredbo	1890	2
T3	Thredbo	1940	5
T1	Guthega	1591	6
T1	Guthega	1648	10
T1	Guthega	1688	5
T1	Guthega	1734	5
T1	Guthega	1767	6
T2	Guthega	1699	3
T2	Guthega	1797	1
T2	Guthega	1846	7
T2	Guthega	1847	2
T3	Guthega	1669	5
T3	Guthega	1732	5
T3	Guthega	1788	2

Table 2: Sample site information of only analysed samples for *K. tristis*, a total of 135 individuals were analysed after the elimination of poorly genotyped individuals.

Transect	Region	Elevation	Individuals
T1	Thredbo	1596	10
T1	Thredbo	1650	6
T1	Thredbo	1697	9
T1	Thredbo	1797	5
T1	Thredbo	1849	5
T1	Thredbo	1896	8
T1	Thredbo	1937	5
T2	Thredbo	1802	3
T2	Thredbo	1869	4
T3	Thredbo	1746	2
T3	Thredbo	1796	4
T3	Thredbo	1845	5
T3	Thredbo	1890	4
T3	Thredbo	1940	8
T3	Thredbo	1990	9
T1	Guthega	1648	7
T1	Guthega	1688	12
T1	Guthega	1728	16
T1	Guthega	1734	5
T1	Guthega	1767	8

The vegetation type along transects ranged from wet tussock grasslands, subalpine woodlands, Eucalyptus woodlands, and alpine herb lands. With increasing elevation along the transects

examined, mean annual temperature decreases rapidly from 6 to 4.3 °C (mean=5.29 °C), annual precipitation increases from 1788 to 2,500 mm (mean= 2127.9 mm) and foliage cover decreases from ~65% to 0%. Spatial coordinates (latitude, longitude) were recorded for each site using a handheld GPS device (Garmin Etrex10). All three species were searched for in a small plot of ~30m^2 for 20 mins by two people (40 minutes total search time). Samples were captured using small plastic jars, and species name and sex were recorded from live specimens upon processing. To further confirm species identification, features such as eye colour, cheek pattern, pronotum shape, body shape and size were compared against type specimens stored in the Australian National Insect Collection, CSIRO, Canberra. The left hind femur length was measured for each live individual using vernier calipers (accurate to 0.02 mm), which is a commonly-used proxy for body size in grasshoppers and Orthoptera (Masaki, 1967, Mousseau and Roff, 1989, Davidwitz, 2008). All samples were stored in 95% ethanol for DNA preservation.

DNA extraction and genotyping

DNA for both species (141 individuals of *K. usitatus* and 141 individuals of *K. tristis*) was extracted from femur tissue at Diversity Arrays Technology sequencing facility (DArTseq), Canberra (more detail in Supplementary Information Text S1). A modified double-digest RAD sequencing protocol described in Kilian et al. (2012) was used with a combination of SpfI-HpaII restriction enzymes used for library preparation. The SpfI enzyme adaptor was modified to contain an Illumina adaptor sequence, primer sequence and a variable-length barcode as described by Elshire et al. (2011), whereas the HpaII adaptor contained an Illumina flow cell attachment and overhang sequence. Digested and ligated DNA fragments were sequenced on Illumina HiSeq2500 and 65bp DNA reads were obtained, each with a unique barcode. To call SNPs, DNA sequences were aligned via BLAST using the *Locusta migratoria* reference genome with an E-value: 5e-05 and minimum sequence identity of >80%. Quality checking was undertaken by DArTseq using proprietary software (DArTsoft14) and cross contamination was examined by performing a BLAST search on bacterial, fungal and human DNA. More detail on

SNP calling and filtering by DArTseq is in supplementary information (Text S1). A SNP dataset was obtained for each species separately and a third dataset was obtained for both species combined. The third dataset was produced by analysing sequences from both *K. tristis* and *K. usitatus* combined by only calling SNPs that were common in both of the focal species.

Species SNP data filtering

To remove unreliable and low-quality SNPs, data were further filtered using the *filter_dart* function of the Radiator package (Gosselin, 2017) in R (Team, 2017). The following filtering parameters were applied to the individual species datasets: monomorphic SNPs were excluded, SNPs with 100% reproducibility were retained (i.e. the proportion of technical replicate assay pairs for which the marker score is consistent), SNPs with a call rate ≥ 0.95 (i.e., the genotype is called in a minimum of 95% of the samples) and a minimum depth coverage of ≥ 10 were retained. Individuals genotyped at less than 95% of SNPs were removed from the datasets. To further exclude alleles potentially a result of sequencing error, a minor allele frequency (MAF) threshold of ≥ 0.02 was applied in order to both retain rare alleles and remove alleles potentially a result of sequencing error.

For both the individual species datasets, we produced a dataset for which the effects of linkage disequilibrium (LD) were minimised by retaining one SNP per RAD tag (i.e. the SNP with highest the MAF), hereafter referred to as 'unlinked' datasets. To further assess the extent of linkage between 'unlinked' SNPs we tested for SNP pairs with a linkage correlation R^2 >50% using PLINK v1.9 (Purcell et al., 2007). These datasets was used to detect population structure after removing Fst outliers (mentioned below) and loci significantly deviating from Hardy-Weinberg Equilibrium (HWE). Loci deviating from HWE in both the locations were identified using *pegas* R package (Paradis, 2010), at a significance level of 0.05 with 1,000 simulations. The second full dataset, which included all SNPs per RAD tag, was used for analyses of adaptive genetic variation. However, to reduce possible effects of strong LD between markers in the full

dataset, SNP pairs with a R^2 >50% were identified using PLINK v1.9 (Purcell et al., 2007) and one of the markers from the pair was randomly removed.

Common dataset filtering

For the combined dataset (containing SNPs common in both *K. usitatus* and *K. tristis*), slightly different filtering parameters were applied due to the difference in the quality of the dataset. SNPs with a reproducibility of 90%, a call rate ≥ 0.70, minimum depth of coverage ≥ 8 and a MAF of ≥ 0.02 were retained. Individuals genotyped at less than 75% of SNPs were removed from the dataset. As above, two datasets were derived; an "unlinked" dataset with only one SNP per RAD tag and a second dataset including all SNPs. The unlinked dataset was used to detect potential hybridisation between species after excluding SNPs under HWE (undertaken as explained above). The full dataset was used for detecting adaptive genetic variation after accounting for strong LD (R^2 >50%). The final filtered datasets were converted to necessary formats for further analysis using PGDSpider (Lischer and Excoffier, 2011) and PLINK v 1.9 (Purcell et al., 2007).

Fst outlier SNP detection

To create datasets that excluded SNPs that were likely under selection and therefore was putatively neutral, outliers were detected from the 'unlinked' datasets using BayeScan (Foll and Gaggiotti, 2008), which uses a Bayesian framework to detect outliers with high Fst while accounting for samples size, demographic processes and hierarchical genetic structure (de Villemereuil et al., 2014). BayeScan was run for 50,000 pilot runs, with a 500,000 burn-in and 50,000 total iterations. We set the prior odds to 10,000 as it has been shown to lower the error rates (Lotterhos and Whitlock, 2015) and false discovery rate (FDR) threshold of 0.05. Log-likelihood traces were plotted in R to confirm model convergence.

Genetic structure

We calculated pairwise Fst between regions for each species (Reynolds et al., 1983) in Arlequin 3.5 (Excoffier and Lischer, 2010) with a 0.05 significance level and 1,000 permutations. Isolation by distance (IBD) within regions was assessed using a Mantel test of individual genetic distance generated in GenAlEx (Peakall and Smouse, 2006) and geographic distance (in Km) (number of permutations=9999) in the vegan R package (Oksanen et al., 2013). Spatial autocorrelation analysis examining spatial distribution of genetic variation was carried out in GenAlEx. The spatial autocorrelation coefficient (*r*) was calculated using the geographic distance between sites within the regions and the genetic distance between individuals. The size of the distance classes for each spatial autocorrelation run were decided based on the pairwise geographic distance between the sites. The spatial autocorrelation (*r)* was considered significant if it falls beyond the upper 95% bound of the distribution. Under significant IBD, the values of *r* divert from zero for geographically closer sites and decrease with increase in geographic distance between samples (Peakall et al., 2003).

Using the reduced 'unlinked' dataset (all three datasets) genetic structure was analysed between and among the two sampling regions using STRUCTURE 2.3.2 (Pritchard et al., 2000), which uses a Bayesian algorithm to assign individuals into groups based on shared ancestry. The analysis was performed without providing prior location information, using the admixture model with correlated allele frequencies. STRUCTURE runs were computed in parallel using StrAuto (Chhatre and Emerson, 2017). The analysis was performed with a 500,000 burn-in, 500,000 MCMC repeats, a range of *K* from 1- 4 for between region structure and for within region structure the range of *K* was set to the number of transects per mountain plus two.
We ran the analysis for ten iterations per *K*. The optimal *K* was selected using STRUCTURESELECTOR (Li and Liu, 2018), which calculates the optimal K using conventional the ΔK method (Evanno et al., 2005) and four other different parameters. STRUCTURESELECTOR assigns a subpopulation to a cluster if the membership coefficient mean MedMeaK (median of means), MaxMeaK (maximum of means) or median MedMedK

(median of medians) and 'MaxMedK' (maximum of medians) to a particular cluster is greater than the threshold value of 0.5. In addition, STRUCTURESELECTOR also performs the ΔK method, which compares the rate of change in log likelihood between each *K* and selects *K* with the maximum value of *ΔK* (Evanno et al., 2005).

For the common SNP dataset, between region genetic structure was analysed using STRUCTURE and the range of *K* was set to 1-5 (all other parameters same as above). To save computational time, we examined within region (Thredbo and Guthega) admixture between the two species using *snmf* function within LEA (Frichot and Francois, 2015) R package with the value of *K* ranging from 1 to 10 and 100 repetitions per *K*.

Environmental variables

Environmental variables deemed relevant for movement and local adaptation in the two study species were identified based on expert opinion and knowledge of the ecology and thermal tolerance of *Kosciuscola* (Slatyer et al., 2016, Slatyer et al., 2014, Umbers et al., 2013). Temperature, precipitation (including rainfall or snow cover) and topography are known to affect reproduction, growth, and distribution of alpine restricted insects including grasshoppers (Hodkinson, 2005, Roland and Matter, 2016, Joern and Gaines, 1990, Buckley et al., 2013). Temperature clines along elevational gradients in particular affect performance, feeding behaviour and movement of ectothermic grasshoppers (Buckley and Nufio, 2014) and can act as an important selection agent in ectotherms (For eg. Dudaniec et al., 2018). Terrain roughness and habitat properties such as foliage cover have an important contribution to thermoregulation, movement and oviposition site selection in grasshoppers (Yadav et al., 2018, Kearney et al., 2009, Clark, 1967) and have been shown to exert selection pressures in grasshoppers (Yadav et al., 2019). Furthermore, soil properties such as pH and soil bulk density can significantly influence grasshopper egg laying, survival and population dynamics via controlling distribution of food resources and nematode parasites (Miao et al., 2018, Macfadyen et al., 2018).

We extracted data for climatic variables such as annual rainfall, precipitation seasonality ("Precseas" hereafter), mean temperature in the warmest quarter, mean temperature in the coldest quarter, number of frost days (< -2°C); soil variables: soil pH ("pH" now onwards), soil bulk density (g/cm^3; "BD" now onwards), and terrain variables: roughness and Foliage Projective Cover (FPC %) for each sampling site. All data except FPC and elevation were extracted from satellite remote sensing and resampled in ANUCLIM 6.1 (Xu and Hutchinson, 2013, Lyons et al., 2017). Climate variables and terrain variables were extracted at a 30m resolution whereas soil variables were extracted at a 90 m resolution. Data for elevation at 30m were derived from a Digital Elevation Model (DEM) obtained from Geoscience Australia. Data for Foliage Projective Cover (FPC) were obtained from the Terrestrial Ecosystems Research Network AusCover database (TERN AusCover 2011) at a resolution of 5x5m. FPC is the percentage of land obscured by woody vegetation and trees and was extracted from a buffer of 30m around each sampling site from which cell values were averaged using the *raster* R package (Hijmans and van Etten, 2014). As high collinearity between variables may lead to erroneous results and restrict the ability to distinguish effects of individual variables (Rellstab et al., 2015), we assessed the extent of collinearity between all extracted variables. We performed a pairwise Pearson's correlation analyses of the site-based environmental data in R and removed highly correlated variables ($r| > 0.80$).

SNP associations: univariate analysis

In order to identify loci under putative selection, we conducted independent analyses of the three data sets (i.e. the datasets for each of the two species and the *common dataset*). We used the latent factor mixed modelling approach of LFMM (Frichot et al., 2013) within the LEA package in R (Frichot and Francois, 2015) to identify an association between allele frequency and environmental variables. In addition to environmental variables, body size (i.e. femur length used as proxy) was examined for significant SNP x morphology associations in LFMM. Body size is highly correlated with sex in both species, therefore sex was also included as a variable

(binary coded: Male=0, Female=1) in LFMM to identify loci that were uniquely correlated with body size (i.e. did not overlap with sex outliers). LFMM performs well for polygenic adaptation as it can identify many loci of small effect compared to Fst outlier tests (de Villemereuil et al., 2014). By introducing population structure as a latent factor LFMM can also efficiently account for genetic structure (Frichot et al., 2013). LFMM analysis was performed using 100,000 iterations, 10,000 burnin, with five repetitions for each variable. The number of *K* for LFMM for both species and for the common dataset was based on the outcome of their respective genetic structure analyses (described above). Adjusted p-values were calculated as mentioned in Frichot and Francois (2015) after combining median z-scores for each variable across five runs. Candidate loci with <1% FDR were obtained by applying the Benjamini-Hochberg procedure. The genomic inflation factor (GIF) was calculated using the formula λ=median (z^2)/0·456 (Devlin and Roeder, 1999) and was examined for its proximity to 1 to assess the inflation in test statistics. In case of detection of more than one outlier from the same RAD tag, only one outlier was retained randomly to minimize the effect of linkage disequilibrium.

SNP associations: multivariate analysis

We assessed the variance inflation factor (VIF) between the variables prior to fitting constrained ordination analysis using *vif.cca* function in the VEGAN R package (Oksanen et al., 2013). In genetically structured populations, neutral processes may confound signatures of selection and may lead to false interpretation of selection signatures (Excoffier et al., 2009). Thus, accounting for the neutral genetic structure is recommended while performing selection detection tests (Forester et al., 2018). We performed two constrained ordination analyses (i.e. redundancy analyses, RDA), 1) where population structure was not accounted for (standard RDA), and 2) where population structure was accounted for (using partial redundancy analysis, pRDA). The RDA approach has been shown to better detect weak multilocus signatures of selection (Forester et al., 2018) and performs PCA on response (genotype matrix) and predictor variables (environmental variables) simultaneously (Legendre & Legendre, 2012). The RDA was

performed on Hellinger-transformed SNP datasets and predictor variables using the *rda* function in the VEGAN R package (Oksanen et al., 2013). Statistical significance of global RDA and the number of significant axes to be used for candidate outlier detection was examined via an Analysis of Variance (ANOVA) with 999 permutations. Furthermore, the proportion of the genetic variance in the dataset explained by the predictors was examined from the adjusted R^2. RDA does not allow missing data in SNP genotypes therefore, missing data were imputed using LinkImpute (Money et al., 2015), which is based on a *k* - nearest neighbour genotype imputation method. This method was used because it is specifically designed to impute non-model species' genomes with unordered markers (Money et al., 2015). We excluded sex from the multivariate analysis as sex is highly correlated (R^2 = >0.85) with body size in both species, however due to this high correlation the confidence in detecting body size outliers was reduced. RDA candidate outliers for single species datasets and the common dataset were detected from SNP loadings (i.e. the loading of each SNP in ordination space) using a cut-off of ±3 *SD* from the mean loading following Forester et al. (2018).

For pRDA we used the spatial eigenfunction approach to account for population genetic structure following Xuereb et al. (2018) and Forester et al. (2018). To obtain spatial variables we calculated distance based Moran's eigenvector maps (dbMEMs) from the Euclidean distances between spatial coordinates of the sampling locations using adespatial V 0.1-0 (Dray et al., 2017) R package. We then carried out a PCA on Hellinger-transformed SNP datasets and retained PC axes based on screeplots. Another RDA analysis was performed using selected PC axes as response variables and all dbMEMs as predictors. To keep only dbMEMs, which significantly explain variance in the SNP dataset, we performed forward selection on all dbMEMs as recommended for spatial analysis (Blanchet et al., 2008). The selected dbMEMs were then included in the pRDA analysis as conditioning variables, with SNP datasets as response and environmental variables as predictors. Candidate SNPs corrected for population structure were then detected following the same approach as described above.

Fst Outlier detection

We performed a second BayeScan run corrected for population structure with the same parameters described earlier in both of the individual datasets for each species, however this run was performed to detect and retain loci under selection. The number of populations was based on the optimal *K* identified by STRUCTURE.

Additive polygenic scores

We examined for a cumulative effect of the candidate loci under selection by calculating additive polygenic scores following Babin et al. (2017) and Xuereb et al. (2018). The additive polygenic score approach is useful in order to understand the change in frequencies of alleles inferred to be favoured in a particular environment, when selective effects of an individual allele is not known (Hancock et al., 2011, Babin et al., 2017). Prior to calculating polygenic scores, we ensured SNPs were not tightly linked by examining the extent of correlation between SNP pairs. We first identified alleles associated with increasing values of elevation across all candidate loci based on the nature of the correlation (negative or positive) between allele frequencies and elevation. Then individual based scores were calculated by summing up the copies of favoured alleles across all candidate loci. Lastly, we tested the relationship between individual additive polygenic scores and elevation by fitting a linear model and a quadratic model. We determined the best fit model based on the lowest Akaike Information Criterion (AIC) score. We analysed polygenic scores with candidate loci corrected for population structure (LFMM, pRDA and BayeScan for individual species datasets and LFMM and pRDA for the common dataset)

Gene annotation: BLAST search

Annotation of all unique (i.e. non-overlapping) RAD tags (65 bp) that contained SNPs under putative selection (identified via BayeScan and EAA) was undertaken using the NCBI BLAST platform (Johnson et al., 2008). Due to the unavailability of a reference genome for *Kosciuscola*,

sequences were annotated to genes classified as ‘insects’ (Taxid:6960), with an E-value threshold of 0.00001 and BLASTN mode.

Results

SNP Filtering and sequencing

For *K. usitatus* a dataset of 125,513 SNPs (polymorphic and monomorphic) was obtained from DArTseq. After filtering out low confidence, tightly linked SNPs and poorly genotyped individuals (<75%), a total of 130 individuals of *K. usitatus* were analysed at 9,017 SNPs out of which 4,437 SNPs were unique per DNA fragment and represent an ‘unlinked dataset’. In the 4,437 ‘unlinked’ SNPs, further removal of SNPs significantly deviating from HWE (n=1,114), and Fst outliers identified by BayeScan (*n*=2) resulted in a total of 3,321 SNPs for neutral genetic analysis. A negligible number of SNPs in the unlinked dataset (8 SNPs out of 3,321) showed linkage correlation R^2 >50% suggesting no physical linkage.

For *Kosciuscola tristis* an initial dataset of 38,984 SNPs (polymorphic and monomorphic) was obtained. After excluding SNPs based on the filtering parameters described in the methods, LD cut-offs, and poorly genotyped individuals, a total of 135 individuals were analysed at 7,363 SNPs, with 4,410 ‘unlinked’ SNPs (i.e. one SNP per RAD tag). In the reduced 4,410 dataset we further removed SNPs significantly deviating from HWE (*n*= 842) and Fst outliers identified by BayeScan (*n*=2) and retained 3,566 SNPs for neutral genetic analysis. There were no SNPs with >50% linkage correlation within the ‘unlinked’ dataset for *K. tristis* indicating absence of physical linkage.

The common dataset (common SNPs identified across the two species) yielded an initial 58,063 SNPs (polymorphic and monomorphic) and after applying filtering parameters, LD thresholds (*n* =2,155 with R^2 >50%), and excluding poorly genotyped individuals (n=10) we analysed 266

individuals at 3,058 SNPs, with 2,143 “unlinked” SNPs (after removing SNPs deviating from HWE). Furthermore, none of the “unlinked” SNPs had a linkage correlation >50% suggesting no likelihood of physical linkage.

Environmental variables

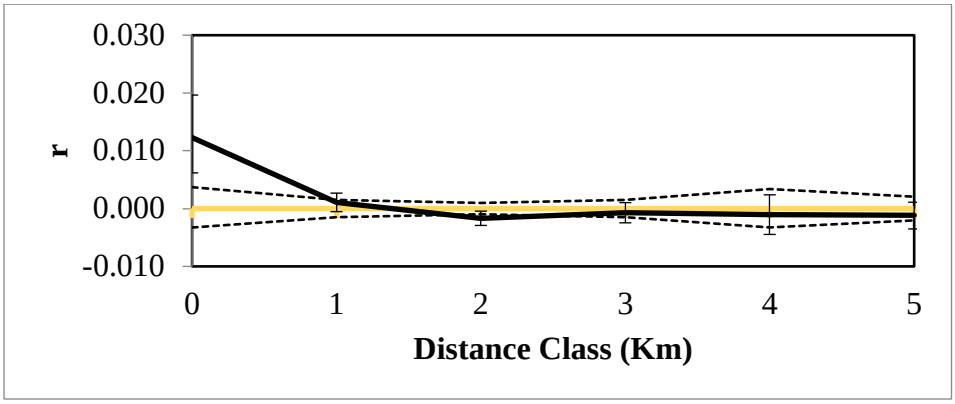
Significant correlations were observed between environmental variables and elevation (Fig. S1). Based on a correlation cut-off of R^2 >0.80 we excluded the following environmental variables: annual rainfall, mean temperature in warmest period, and mean temperature in coldest period. After discarding highly correlated variables, elevation and six variables were retained for subsequent analysis: 1) Precseas, 2) number of frost days, 3) FPC, 4) terrain roughness, 5) soil pH, and 6) BD. We divided these final variables into three categories for ease of interpretation: 1) climate variables, which included elevation (a direct proxy of temperature R^2 = -0.98), Precseas and number of frost days; 2) Surface terrain, which included FPC and Roughness, and 3) soil, which included soil pH and BD.

Genetic structure

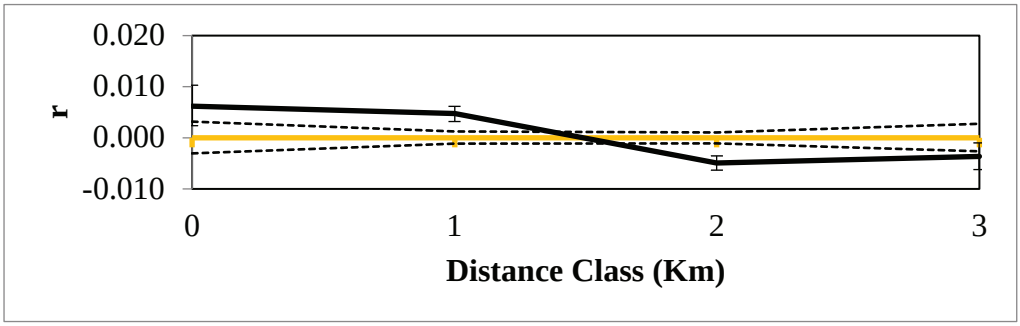
Putatively neutral and ‘unlinked’ datasets were used for genetic structure analysis of each species (*K. usitatus*= 3,321 SNPs and *K. tristis*= 3,566 SNPs) and the common dataset (SNPs = 2,143). Pairwise Fst between Thredbo and Guthega was significantly lower in *K. usitatus* (Fst = 0.026) than *K. tristis* (Fst = 0.050). A within region Mantel test between individuals’ genetic distance and geographic distance indicated no significant IBD for both the species (Fig. S2). However, spatial autocorrelation was detected with a significant non-linear pattern in Thredbo and Guthega for *K. usitatus* (Fig. 2A, B) and in Thredbo for *K. tristis* (Fig. 2C). In both Thredbo and Guthega for *K. usitatus*, individuals were significantly spatially autocorrelated for the first distance class (0-1 Km), with the pattern declining to zero at ~1.5-3 km. In *K. tristis*, significant spatial autocorrelation was observed between individuals for the first distance class (0-1 km),

however the relation was insignificant after the first class. No significant spatial correlation was observed in Guthega for *K. tristis*.

(A)



(B)



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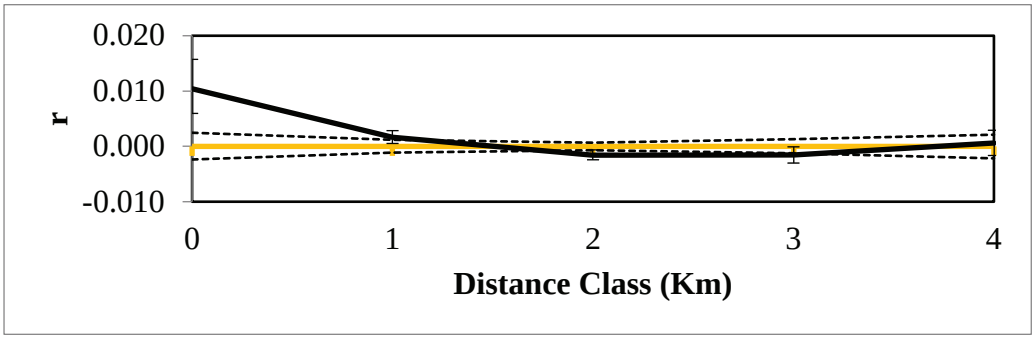


Figure 2: Correlograms showing the influence of distance (Km) on spatial autocorrelation (r). Dotted lines represent upper and lower bounds of 95% CI. (A) *K. usitatus* in Thredbo and B) *K. usitatus* in Guthega; (C) *K. tristis* in Thredbo.

Based on the agreement between the ΔK method and four estimators of STRUCTURESELECTOR (Li and Liu, 2018), the most likely number of clusters between regions was two (*K*=2) for both of the species (Fig. S3A and S3B). However, genetic structuring was stronger in *K. tristis* than *K. usitatus* suggesting a higher degree of genetic admixture in *K. usitatus* across the two mountains as compared to *K. tristis* (Fig. 3A, Fig. 4A). Furthermore, within region analysis of genetic structure in Thredbo and Guthega for *K. usitatus* indicated the most likely number of clusters to be two (*K*=2) per region. However, the extent of admixture was greater in Guthega than Thredbo and K=1 is more likely to be an optimal value in Thredbo (Fig. 3B). In *K. tristis*, within region analysis indicated two clusters in Thredbo but only one cluster in Guthega (Fig. 4B). The presence of only one cluster in Guthega for *K. tristis* is likely to be affected by the absence of individuals in the other two transects (T2 and T3) and further collection efforts may help to better understand the genetic structure of this in this region.

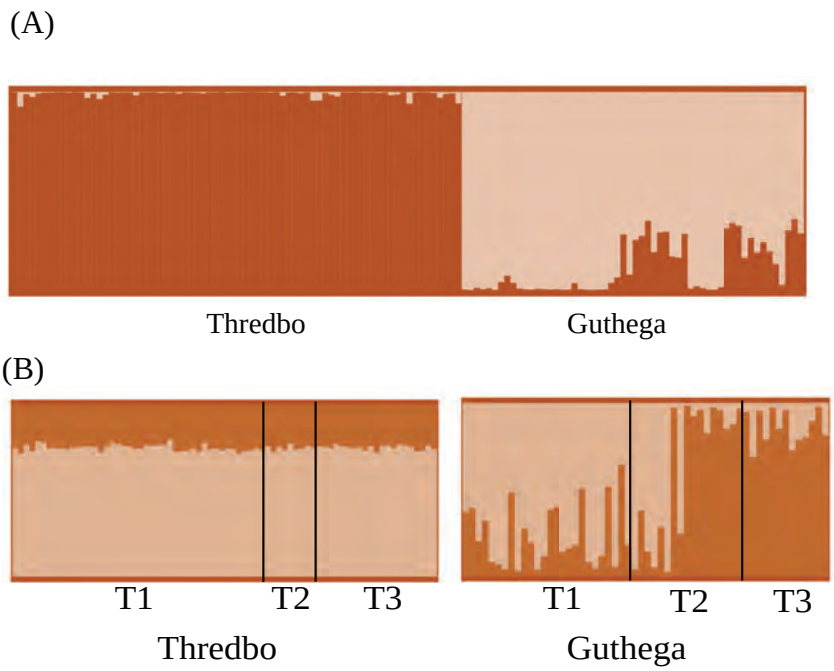


Figure 3: Bar plot of assignments of *K. usitatus* individuals to each cluster, the proportion of colour represents the proportion of individual assignment to each of the two genetic clusters. (A) Genetic structure between the two regions: Thredbo and Guthega, with two clusters; (B) within-region genetic structure analysis with transects indicated (T1= Transect 1, T2= Transect 2 and T3= Transect 3).

(A)

(B)

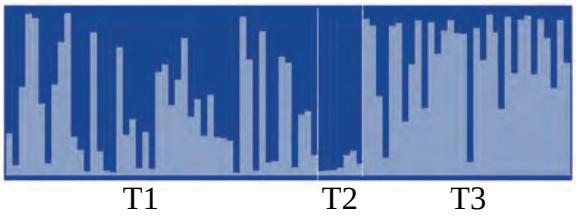


Figure 4: Bar plot of assignments of *K. tristis* individuals to each cluster, the proportion of colour represents the proportion of individual assignment to each of the two genetic clusters. (A) Genetic structure between the two regions: Thredbo and Guthega, with two clusters; (B) genetic structure within regions with transects indicated within Thredbo (T1= Transect 1, T2= Transect 2 and T3= Transect 3).

With the common dataset, two distinct clusters were indicated by STRUCTURE analysis (Fig. S3C). The presence of two clusters with no admixture (Fig. 5) indicated an absence of hybridisation between the two species. A within region admixture analysis using the common dataset also indicated no recent hybridisation between the two species (Fig. S4A and S4B).

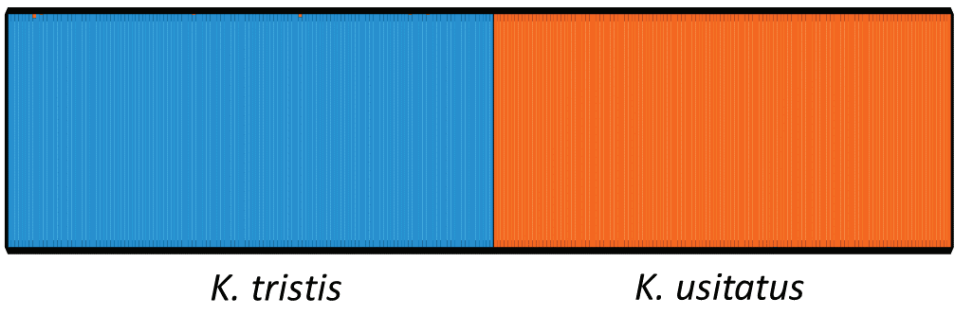


Figure 5: Bar plot showing no evidence of admixture between *K. tristis* and *K. usitatus* individuals across regions (Thredbo and Guthega).

Fst outlier test and Environment association analysis: univariate analysis

BayeScan detected five and two outlier SNPs for *K. usitatus* (Fig. S5A) and *K. tristis* (Fig.S5B) respectively under putative divergent selection in the full dataset (Table S1). LFMM analysis identified a total of 212 outlier SNPs (unique= 182 SNPs; 2%) for *K. usitatus* compared to 940 outliers (unique= 634; 8.6%) for *K. tristis.* For *K. usitatus*, the maximum number of SNP associations was found for elevation (n= 62 SNPs out of 9,017) followed by BD (n= 56 SNPs), pH (n= 35) and Precseas (n=22), whereas with *K. tristis* the maximum number of associations were found for Precseas (n= 179) followed by frost days (n=156), BD (n= 128) and FPC (n=93). In regards to the broader environmental variable classifications we implemented, climatic variables including elevation, Precseas and number of frost days together were correlated with the highest number of outliers in both the species (*K. usitatus n*=102; 1.1%, *K. tristis n*=422; 5.7%) followed by soil variables (pH, BD; n= 91; 1%) in *K. usitatus* and terrain variables (FPC, Roughness; n= 215; 2.92%) in *K. tristis*. For femur length (body size), only two candidates were detected in *K. usitatus*, out of which one did not overlap with sex, whereas 80 candidate loci were detected in *K. tristis*, out of which 45 associations did not overlap with sex.

When analysing the common dataset, a total of 331 common SNPs under putative selection were detected in both species across all tests (unique = 205; 6.7%). The maximum number of associations were detected in relation to sex (male and female, n=101) and body size (n= 84, non-overlap with sex = 9) followed by Precseas (n=39) and elevation (n=33). A large number of overlapping outliers detected for sex and body size indicates a limited ability to distinguish selection signatures between the two variables. Apart from body size outliers, the highest number of outliers was associated with climate variables (n=78; 2.5%), followed by soil (n=36; 1.1%) and terrain (n=32; 1%) characteristics. The closeness of lambda values (GIF) to 1 and *P*-distribution plots of each variable in all three tests indicated our model well accounted for genetic structure and defined FDR rates for all the datasets (Table 3).

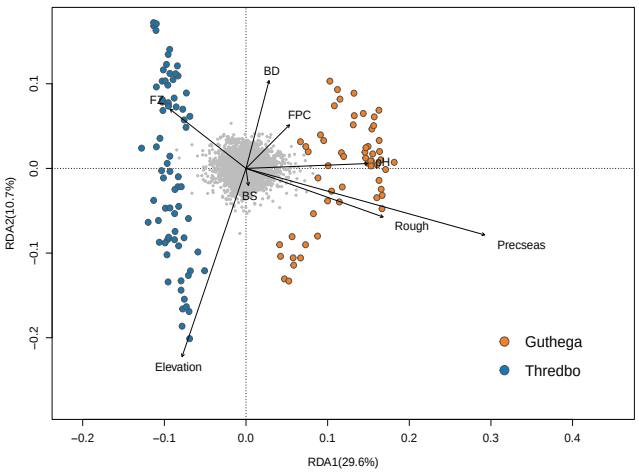
Environmental association analysis: multivariate constrained analysis

The VIF between predictor variables was <10 indicating no effect of collinearity between variables on the constrained analysis (Table S2). The global RDA analysis on all three full datasets (n= 9,017 for *K. usitatus,* n= 7,363 SNPs for *K. tristis,* and n= 3,058 SNPs for *Common dataset*) was significant (*K. usitatus P* =0.001; *K. tristis P*= 0.001 and *Common dataset P* = 0.001, Table S3, S4, S5) indicating a significant proportion of genetic variance in the SNPs is explained by the predictor variables. RDA explained 2.1% of the variance in the genomic data (adjusted R^2 = 0.021) in *K. usitatus,* compared to 3.71% of the variance (adjusted R^2 = 0.037) in *K. tristis.* Three axes were significant in both of the species and were used for candidate outlier detection. In *K. usitatus,* the first RDA axis explained 29.57% of the variance, the second axis explained 11.6% of the variance and the third axis explained 10.7% of the variance within SNPs (Fig. 6A). In *K. tristis,* the first axis explained 43.1% of the variance within SNPs, the second RDA axis explained 8.76% of the variance and the third axis explained 8.61% of the variance (Fig. 6). With the *Common dataset* RDA explained 5.2% of the variance (adjusted R^2 = 0.052), with the first axis showing significance (Table S5). However, we considered the first two RDA axes for outlier detection, which explained 64% of the variance and 15.7% of the variance, respectively (Fig. 6).

A total of 337 and 193 unique RDA outliers were detected for *K. usitatus* and *K. tristis* respectively. For *K. usitatus,* 139 candidate loci were detected on axis 1, 117 on axis 2 and 81 on axis 3, whereas for *K. tristis,* 82 candidate loci were detected on axis 1, 49 on axis 2 and 62 on axis 3. The maximum number of RDA outliers were associated with Precseas in both the species (*K. usitatus* = 157, 46.6%; *K. tristis*= 97, 50.2%), followed by elevation in *K. usitatus* (n=69, 20.4%) and roughness (n=31, 16.0%) in *K. tristis*. Overall the highest number of associations were found with climatic variables in both species (*K. usitatus*= 263, *K. tristis*= 141), followed by soil variables in *K. usitatus* (n=35) and terrain (n=38) in *K. tristis.*

Lastly, using the common dataset, 97 unique RDA outliers were detected with 36 candidate loci detected on axis 1 and 61 detected on axis 2. The maximum number of outliers was associated with elevation in the common dataset (n= 44, 45.3%) followed by Precseas (n=29, 29.8%). In terms of the broader classification, highest number of SNP associations were with climatic (n=74) and terrain (n=22) variables

(A)



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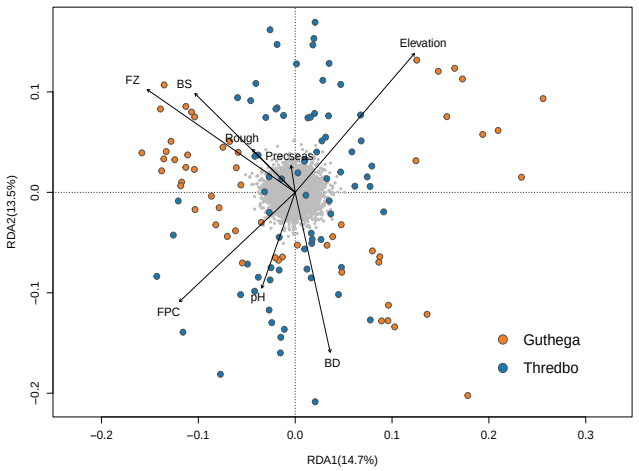

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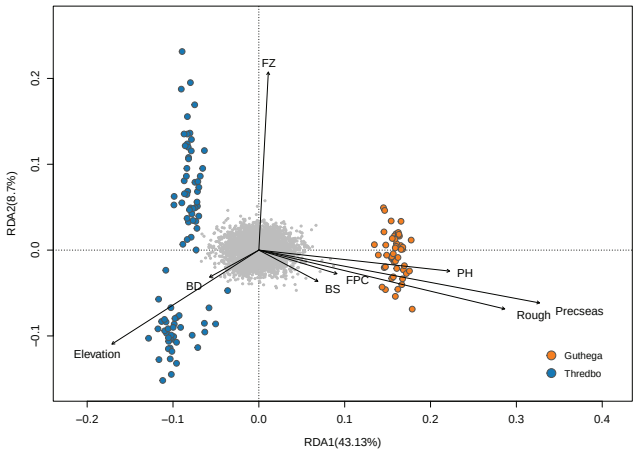
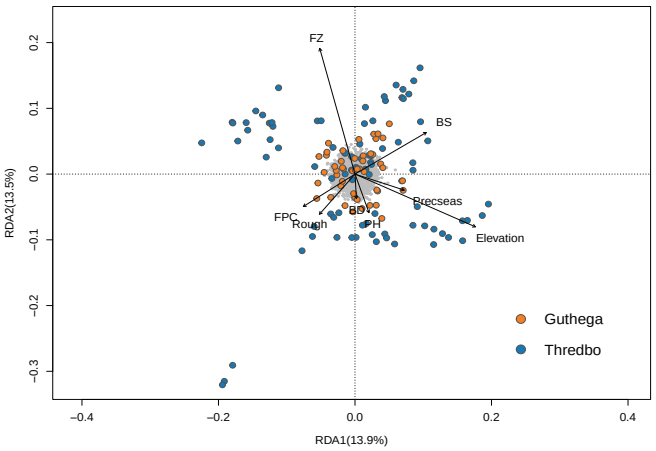


(D)



(E) (F)

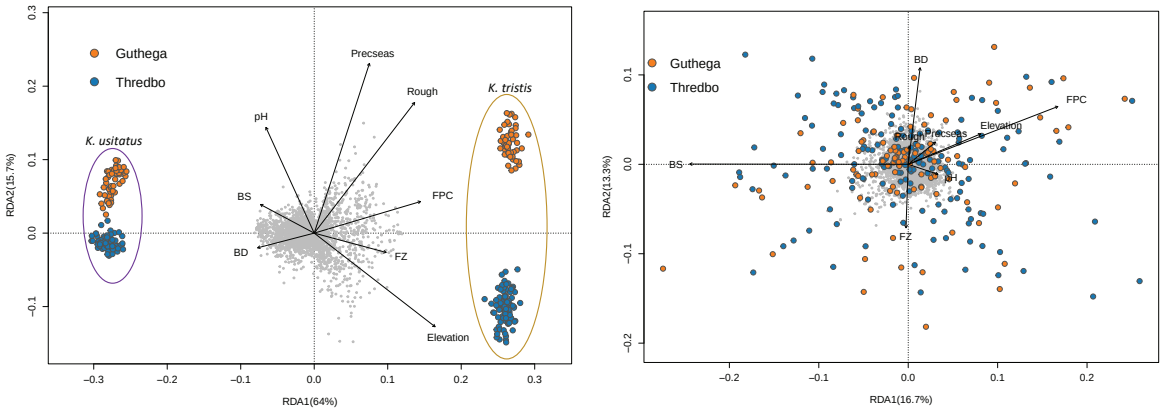


Figure 6. RDA and pRDA analysis of *K. usitatus* (A, B), *K. tristis,* (C, D) and in the common dataset (E, F) showing axes 1 and 2. Coloured dots indicate individuals, grey clusters indicate SNPs, arrows indicate environmental variables. Precipseas = Precipitation seasonality, FPC = Foliage Projective Cover, Rough = terrain roughness, FZ = number of frost days, BD = soil bulk density, pH = soil pH.

A total of six significant dbMEMs were identified for *K. usitatus*, 37 for *K. tristis* and 116 for whole dataset using forward selection, which were used as conditioning variables in pRDA. The overall pRDA was significant for *K. usitatus* and *K. tristis* (*K. usitatus P*= 0.001, adjusted R^2 = 0.005; *K. tristis P* = 0.04, adjusted R^2 = 0.004) but was insignificant for the common dataset (*P*= 0.12, adjusted R^2 = 0.001), likely because of the large number of neutral SNPs in the dataset. Using a ±3 *SD* cut-off from the mean loading and three axes to retain the consistency with RDA, we identified 292 candidate loci for *K. usitatus*, and 161 for *K. tristis* (Table 3).

Like RDA, the highest number of associations was detected for Precseas in both species (*K. usitatus* =66, 22.6%; *K. tristis* = 35, 21.7%) followed by body size (n=48, 16.4%) in *K. usitatus* and elevation (n=34, 21.1%) in *K. tristis*. Similar to RDA, with overall broader classification, the highest number of associations was detected with climatic variables using pRDA in both species (*K. usitatus* =136; *K. tristis* =103), followed by soil parameters in *K. usitatus* (n=68) and terrain (n=27) in *K. tristis.*

A total of 55 SNPs were detected on two axes for the common dataset using pRDA, with the maximum number of associations found for elevation (n=28, 50.9%) followed by roughness (n=10, 18.1%). Similar to RDA the highest number of associations was detected with climatic variables (n=35) followed by terrain (n=16) when analysing the common dataset.

All three methods (LFMM, pRDA and Bayescan) detected a higher number of unique outliers in *K. tristis* (n=763, 10.3%) compared to *K. usitatus* (n=462, 5.1%) (Fig. 7). A greater number of candidate outliers was detected in relation to climatic variables in both species (*K. usitatus* n= 238, *K. tristis* n=525) followed by soil variables in *K. usitatus* (n=159) and terrain variables in *K. tristis* (n=242). A small number of outliers overlapped between outlier detection methods in all of the datasets (*K usitatus*=17, *K tristis*= 34, common dataset=7), likely due to differences in the detection approach used by each analysis (Ahrens et al., 2018).

Table 3: Details of outliers detected using LFMM and partial redundancy (pRDA) approach for each species. GIF= Genomic Inflation Factor.

		K. usitatus		
Variable	LFMM GIF	LFMM Outliers	pRDA	Classification
Elevation	0.8	62	43	Climate
Precipitation seasonality	0.98	22	66	Climate
Number of frost days	0.76	18	27	Climate
Foliage projective cover	0.73	13	27	Terrain
Roughness	0.83	2	13	Terrain
Bulk density	0.61	56	34	Soil
pH	0.69	35	34	Soil
Femur length	0.84	2	48	Morph
Sex	0.8	2	-	Morph
	Total	212	292	
	Unique	182	-	
		K. tristis		
Elevation	0.76	87	34	Climate
Precipitation seasonality	1	179	35	Climate
Number of frost days	0.58	156	34	Climate

Foliage projective cover	0.61	93	7	Terrain
Roughness	1	122	20	Terrain
Bulk density	0.6	128	8	Soil
pH	0.94	30	10	Soil
Femur length	0.63	80	13	Morph
Sex	0.66	65	-	Morph
	Total	940	161	
	Unique	634	-	

Comparing RDA and pRDA, the number of outliers was lower in all three datasets after accounting for population structure. However, the same climatic predictor variables were detected as significant in both of the tests (before and after accounting for population structure). A total of 114 candidate SNPs (39%) and 47 SNPs (29%) overlapped between RDA and pRDA in *K. usitatus* and *K. tristis* respectively. A total of 56% SNPs associated with elevation were detected using both methods in *K. usitatus*, compared to 17.6% in *K. tristis*. Furthermore, 53% of Precseas candidates were identified by both methods in *K. usitatus* compared to 48.6% in *K. tristis*. Using the common dataset, only four SNPs (7%) overlapped between RDA and pRDA, yet elevation was detected as an important predictor by both methods.

Additive polygenic scores

To ensure the detected outliers were not in strong LD, we first assessed the extent of correlation between unique outliers. Pairwise LD between detected outlier loci was very low in both species, whereby 99% of candidate loci in *K. usitatus* had a pairwise correlation ≤ 20%, and this was similarly negligible for *K. tristis* with all outlier loci having a pairwise correlation ≤ 20%. The extent of correlation was lower in the common dataset, whereby 72.3% of outlier SNPs had a pairwise correlation ≤ 20%. In *K. usitatus*, additive polygenic scores derived from 436 candidate loci detected by three methods (LFMM, pRDA and BS) significantly increased with elevation (N = 130 individuals; Linear model: $F_{(1,128)}$ =111.5, *P*: < 2.2e-16, adjusted R^2 = 0.46,

AIC=1249.33). The quadratic model was also significant ($F_{(2,127)}$ = 65.82, *P*: < 2.2e-16, adjusted R^2 = 0.50, AIC= 1240.30) and had a lower AIC score than a linear model and explained a higher proportion of variation in allele frequencies (Fig. 7A). Similarly, for *K. tristis*, additive polygenic scores derived from 701 candidate loci significantly increased with elevation (N = 135 individuals; Linear model: $F_{(1,133)}$ =109.5, *P*: < 2.2e-16, adjusted R^2 = 0.45, AIC=1429.38) and the quadratic model was significant ($F_{(2,132)}$ = 72.17, *P*: < 2.2e-16, adjusted R^2 = 0.51, AIC= 1412.75) with a much lower AIC value and explained a higher proportion of the variance than a linear model (Fig. 7B). In the common dataset, additive polygenic scores derived from 234 candidate loci increased significantly with elevation (N = 266 individuals; Linear model: $F_{(1,264)}$= 11.88, *P*: 0.0006, adjusted R^2 = 0.04, AIC=2416.41) and the quadratic model was significant ($F_{(2,263)}$= 8.53, *P*: 0.0002, adjusted R^2 = 0.053, AIC=2413.39) with a slightly lower AIC than the linear model and explained a higher proportion of the variation (Fig. S6).

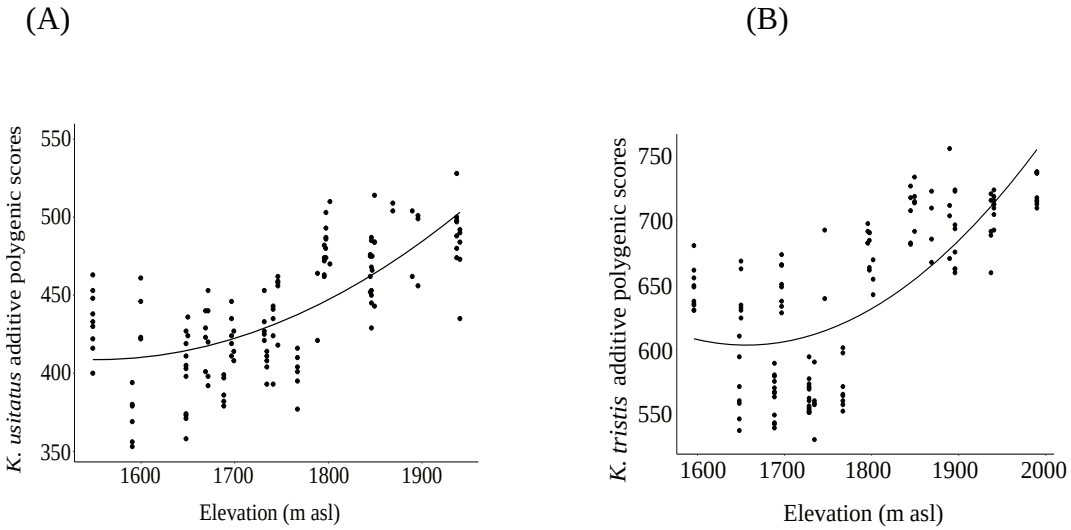
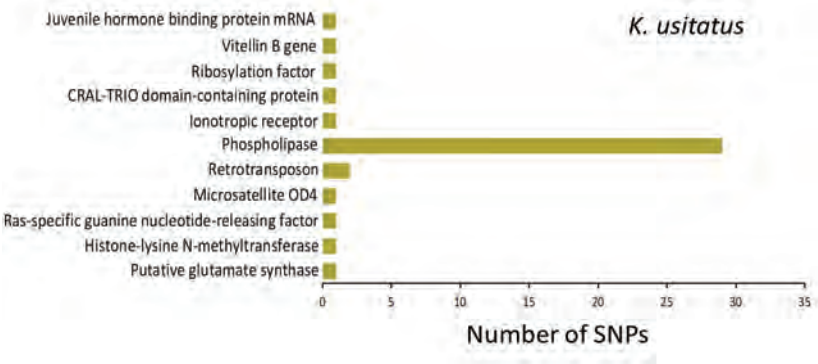


Figure 7: Correlation between individual additive polygenic scores and elevation. (A) *K. usitatus P*=<0.0001 and (B) *K. tristis P*=<0.0001. The solid line represents the regression line from the quadratic model. Each dots represents an individual grasshopper.

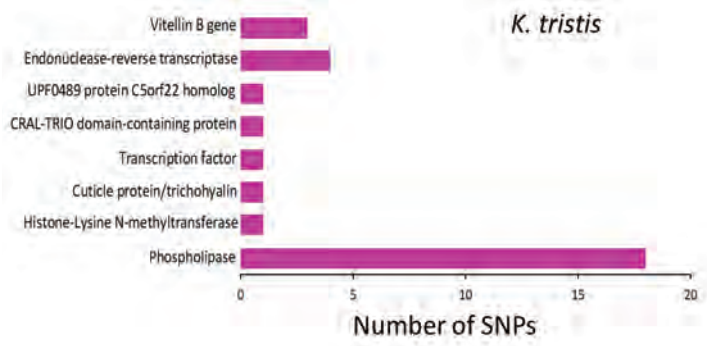
Gene annotation: BLAST search

Gene annotation of DNA fragments that contained SNPs identified via BayeScan and EAA resulted in 64 successful annotations for *K. usitatus*, 46 annotations for *K. tristis* and 34 for the common dataset (Table S6, S7, S8). All annotated SNPs were only identified by EAA (LFMM and pRDA). The most commonly annotated gene across all datasets was for phospholipase (PLA2G) mRNA (*K. usitatus*= 29 annotations, *K. tristis* =18, common dataset = 16). This enzyme is involved in lipid metabolic pathways including hydrolyses of phospholipids (GO 0002629). Another noteworthy match was obtained for the vitellin B gene (*K. usitatus* =1 SNP, *K. tristis*= 1 SNP and common data SNP=1). Vitellin proteins stored in yolk granules of insect eggs and are used as a food reserve for the developing embryo, and its synthesis in several insects is under hormonal control (Harnish and White, 1982). SNPs annotating to this protein were associated with climatic variables in both species: precipitation seasonality in *K. usitatus*, and FPC and frost days in *K. tristis*. Other annotations involved membrane receptors mediating cell signaling, olfaction and photoreception, endonucleases, and DNA repair proteins, with a complete list presented in Figure 8.

(A)



(B)



(C)

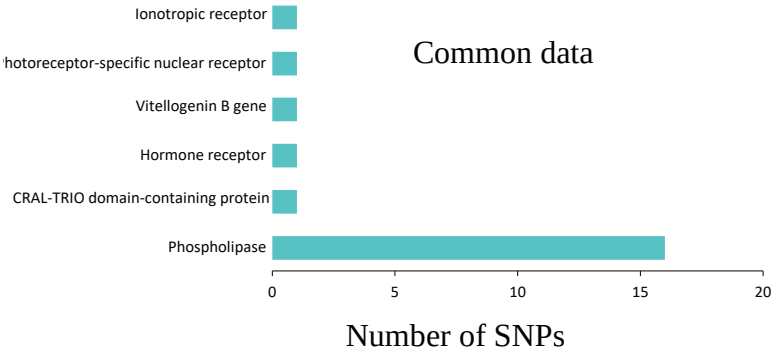


Figure 8: BLAST annotations of unique outlier SNPs with insect genome (Taxid:6960) using BLASTN mode in NCBI BLAST. (A) *K. usitatus*, (B) *K. tristis* and (C) common dataset.

Discussion

We find evidence that species with narrow elevational distributions and lower thermal tolerances show stronger genetic differentiation and local adaptation signals compared to species with broader elevational distributions and thermal tolerances (Kisdi, 2002, Atkins and Travis 2010). A large proportion of loci detected under selection in *K. tristis* compared to *K. usitatus* indicated stronger signatures of local adaptation in this species. Climatic variables including elevation (a proxy for temperature), precipitation seasonality, and the number of frost days strongly influenced adaptive genetic differentiation in both species compared to soil and terrain variables. A large number of common SNPs (i.e. present in both species) were detected putatively under selection with similar allele frequency changes indicating parallel evolutionary responses to environmental conditions in these co-occurring species, however it's not entirely possible to disentangle selection signals from shared alleles due to common ancestry. Furthermore, additive polygenic scores positively and non-linearly correlated with elevation in both species. A large difference in polygenic scores between low and high elevation indicates different genotypes (i.e. suitable in local conditions) are maintained at different elevational gradients. The higher polygenic scores at higher elevations in both species indicates there are stronger and steeper selection pressures at higher elevations, consistent with lower temperatures and higher

precipitation levels. SNP annotations in the individual species datasets and in the common dataset indicate the involvement of genes associated with lipid metabolism and development that may be relevant for adaptation along elevational gradients and colder environments. Our analyses find common and disparate signatures of selection that indicate both parallel and independent evolution among closely related species across elevational gradients. By comparing two co-occurring species we provide insights into how species with a slight difference in thermal tolerance and niche preferences may differ in fine scale genetic structure and the capacity to adapt to diverse local environments.

Broad and fine scale genetic structure

For montane species, steep environmental clines along elevational gradients often have patches of suitable habitat that are surrounded by unsuitable habitat and may play an important role in shaping genetic structure (Polato et al., 2017, Jackson et al., 2018). Our results found two genetic clusters across the two study regions (Thredbo and Guthega) separated by a short geographic distance (~25km) in both *K. usitatus* and *K. tristis*. Both of the study species are flightless (lack functional wings), are semelparous and have a short growing and mating season, and therefore, a limited dispersal capacity combined with a patchy habitat is likely to be important in driving genetic structure. Despite two genetic clusters in both species, we found stronger genetic differentiation in *K. tristis* with a global Fst of 0.05 between the regions as compared to 0.026 in *K. usitatus* suggesting more restricted gene flow in this species. These findings support the observations of a previous study on *K. tristis* by Slatyer et al. (2014), who found similar patterns of genetic structuring between Thredbo and Guthega with significant isolation by distance.

Habitat fragmentation, and topography can act as dispersal barriers even at micro geographic scales in alpine ecosystems. A fine scale examination of genetic differentiation within regions indicated the presence of two genetic clusters in Guthega for *K. usitatus*, whereby individuals at transect one (T1) are largely assigned to one cluster, a large proportion of individuals in transect

two (T2) are assigned to the second cluster and similarly individuals of transect three are largely assigned to the second cluster. Interestingly, T1 in Guthega is separated from T2 and T3 by a large water dam and a functional road (Fig. 1). The presence of large waterbodies and roads may act as significant barriers for dispersal of individuals in this species and may lead to significant genetic structuring as observed in our study. Our previous study on another grasshopper species have shown that water bodies provide strong resistance to genetic connectivity (Yadav et al., 2019). Similarly, in *K. tristis* two genetic clusters were supported in Thredbo, with a greater differentiation between T3, and T1 and T2. The transects T1 and T2 are geographically more proximal compared to T3. The limited dispersal ability can significantly impede movement and gene flow in *K. tristis* across microgeographic scales. Our results imply that the topographical challenges and anthropogenic habitat fragmentation (dams and roads) can act as barriers to gene flow and lead to genetic differentiation even at very short geographic distances. The unprecedented rate of climate change is leading to decline in snow cover, increased temperature and habitat fragmentation in the Australian alps is therefore likely to negatively affect these species by limiting the dispersal of individuals between populations and restricting gene flow.

Several studies on alpine insects have noted patterns of fine scale genetic structuring (i.e. within a few kilometres) due to isolation by distance (Finn et al., 2006, Garnier et al., 2004, Polato et al., 2017). Although linear isolation by distance within Thredbo and Guthega (micro geographic scale) is insignificant in both the species, spatial autocorrelation was detected, indicating restricted dispersal at short distances. In *K. usitatus* the pattern of spatial autocorrelation differed between Thredbo and Guthega, whereby in Thredbo individuals showed a stronger relationship up to a distance class of 0-1 km. In Guthega the pattern was consistent with results observed in the genetic structure analysis, whereby individuals showed a higher correlation for the first distance class (0-1 km), which is a likely an indication of the approximate distance of a transect and suggests that individuals within transects are not dispersing longer distances. The distance where significant correlation is absent, and *r* declines to zero may indicate the point where

genetic structure can be detected (Peakall et al., 2003). The insignificant correlation at ~1.5 km in Guthega for *K. usitatus* is probably an approximate point where population structure was detectable between the transects using STRUCTURE analysis between the transects. Furthermore, *K. usitatus* individuals were shown to be less spatially correlated than expected by random at the 2-3 km distance class, indicating high admixture between individuals in transects T2 and T3. For *K. tristis* in Thredbo, the greater spatial autocorrelation at the first distance class (0-1 km) indicates a 'micro-geographical' isolation by distance with insignificant correlation beyond the first distance. Taken together, the detection of micro-geographic signals of non-linear isolation by distance provides support to our genetic clustering analysis and suggest dispersal limitation of these specialist species likely within transects and at fine spatial scales.

Evidence of local adaptation

Notably a large proportion of loci were detected under selection in *K. tristis* compared to *K. usitatus*, indicating stronger signatures of local adaptation in this species. Furthermore, candidate loci under selection in *K. tristis* showed higher genetic differentiation between Thredbo and Guthega (mean Fst= 0.03) than *K. usitatus* (mean Fst= 0.02) indicating greater potential for local adaptation in *K. tristis* (Fig. 9), which may be aided by the species' more restricted thermal preference and elevational distribution (Slatyer et al., 2016). Increased numbers of SNPs under putative selection, and therefore, greater evidence for local adaptation in *K. tristis* aligns with our finding of stronger genetic structure in this species. Restricted gene flow between populations can limit the exchange of alleles in different environments and can promote stronger local adaptation in populations (Kawecki and Ebert, 2004). By showing greater genetic differentiation (restricted gene flow) and stronger signatures of local adaptation, our study implies that *K. tristis* is likely to be more sensitive to changing climatic and landscape conditions. On the other hand, *K. usitatus* is more likely to maintain a relatively higher gene flow and potential to exchange beneficial alleles across its range as it shows reduced local adaptation, and therefore may be less

sensitive to changing environmental conditions such as decrease in precipitation levels and increase in temperature as a consequence of climate change.

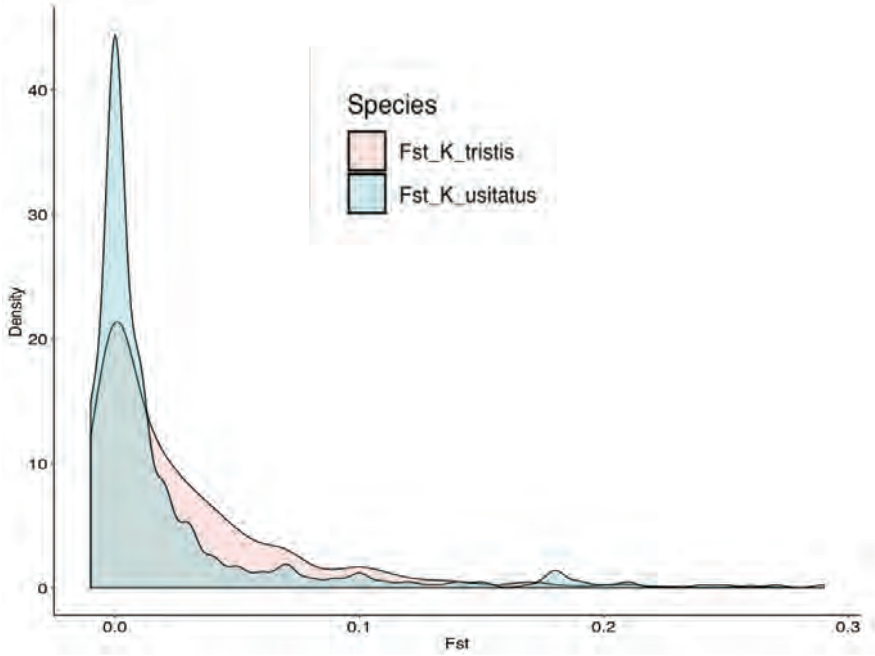


Figure 9: Fst distribution between candidate loci in the two regions (Thredbo and Guthega) for both *K. tristis* (pink) and *K. usitatus* (blue).

Similar genomic responses are likely in closely related species and independent populations, when confronted with challenging environmental pressures and similar selection pressures. For example, adaptive genetic convergence has been shown by independent stickleback populations to aquatic environments (Rennison et al., 2019), and similarly, two *Arabidopsis* species have shown convergence to adapt to soils with toxic levels of heavy metals (Preite et al., 2019). Common SNP markers (i.e. present in both species) identified as candidate loci under selection and a few common gene annotations in our study provide evidence for parallel evolutionary changes within these closely related, and co-occurring species that may act to deal with the challenging alpine environment. Furthermore, the greater association of these common SNPs with climatic variables establish the importance of local climates in driving common adaptive responses in closely related species. Such observations of shared genetic changes and parallel adaptation are not rare among closely related lineages (reviewed in Elmer and Meyer, 2011, Bolnick et al., 2018, Flood and Hancock, 2017). For instance, a review by Conte et al. (2012) on

published empirical data indicated that on average, the chances of usage of the same gene in adaptation is 0.32 to 0.55 in different lineages, which is evidently high and indicates that adaptive evolution is repeatable. Similarly, local adaptation can be more repeatable at the genomic level than expected as shown by Yeaman et al. (2016) using the distantly related conifers. However, studies have rarely used independent dataset to examine the generality of adaptive patterns using EAA (Rellstab et al., 2015). Further study with higher density markers or whole genome analysis may provide deeper insights in to the convergence of traits potentially locally adapted in these two closely related species.

We observed some species-specific environmental associations, for example, in *K. usitatus*, soil variables (including soil pH and soil bulk density) were associated with a greater number of candidate SNPs than terrain variables. Soil properties are important for controlling plant growth, viability of offspring in grasshoppers and the presence of parasitic nematodes and entomopathogenic fungi in grasshoppers (Herrmann et al., 2010, Milner and Prior, 1994). Our previous study on another grasshopper species also indicated an important role of soil in determining relative abundance and was also identified as a possible selection pressure (Yadav et al., 2018, 2019). For *K. tristis* terrain variables including terrain roughness and foliage cover is observed to be highly relevant selective pressure than soil variables including soil pH and soil bulk density. Terrain properties are important for species' fine scale distributions, movement, and in grasshoppers, foliage cover is relevant for thermoregulation (Kearney et al., 2009). Low foliage cover (i.e. bare ground at higher elevations) provide challenging habitats due to restricted thermoregulatory sites as grasshoppers thermoregulate via shuttling in sun and shade. Finally, we observed many more body size candidate SNPs for *K. tristis* compared to *K. usitatus*. This pattern may be more rigorously tested with a larger sample size. However, we found a large number of unique SNP associations that were not biased by sex in *K. tristis*, indicating potential for selection on body size due its correlation with fecundity, and efficiency of thermoregulation (Chown and Gaston, 2010).

Results combined from all outlier detection methods suggest that climatic variables are the most important predictors of adaptive genetic variation and are likely to be key drivers of spatially varying selection for both *K. usitatus* and *K. tristis*. Within the climatic variables, precipitation seasonality and elevation (a proxy for temperature) have a much higher number of outlier associations in both species and in the common dataset than the number of frost days. A key role of precipitation and temperature as major drivers of adaptive differentiation along elevational gradients is shown in a range of ectotherms (Keller et al., 2013, Muir et al., 2014, Macdonald et al., 2018) including insects (e.g. frutifly: Barker et al., 2011). Such observations are not unexpected as temperature and precipitation are strong selective pressures for ectotherms due to their direct link with physiological processes (Carey et al., 2003), growth rate, thermoregulation, and fitness of individuals in heterogeneous environments (Clusella-Trullas et al., 2011). Thus, like other ectotherms, adaptive divergence in grasshoppers is largely influenced by temperature and precipitation.

Environmental conditions associated with elevational gradients may facilitate adaptive divergence between populations at micro-geographic scales. Several studies consider large geographic scales (a few hundred to thousands of kilometres) to scan for selection signatures, however working at scales relevant for the dispersal and environmental tolerance of species is crucial for making informed conservation decisions (Stockwell et al., 2003). Adaptive divergence at fine geographic scales has been little studied due to an assumption that high gene flow at shorter distances may disrupt local adaptation (Barrett and Hoekstra, 2011). However recent work on alpine plants (Kubota et al., 2015, Frachon et al., 2018, Manel et al., 2010), alpine mammals (Waterhouse et al., 2018), and amphibians (Muir et al., 2014, Skelly, 2004, Richardson and Urban, 2013) have demonstrated that local adaptation can occur at fine spatial scales of less than a few kilometres. Our study is in agreement with these studies and implies that local adaptation can be detected at short geographic scales in invertebrates with limited dispersal ability, elevational distributions and thermal tolerances.

Correction for population structure in pRDA resulted in a decreased number of outliers in all three datasets. The influence of population structure correction is visible in ordination plots for each dataset (Fig. 6). A major concern in tests for selection detection is the discovery of true adaptive signals versus neutral signals. Genetic signatures of selection can be confounded by neutral demographic and historical processes in structured populations (Excoffier et al., 2009) and correction for population structure is recommended to avoid false interpretation of selection. However, these corrections can be overly conservative and may remove true signals of selection leading to loss of detection power as shown in a simulation-based study by Forester et al. (2018) because selection can also generate spatial pattern of population structure (Charlsworth et al., 1997). In our study, we identified a strong genetic structuring between the Thredbo and Guthega mountain regions, thus its challenging to separate true selection signals from neutral signals. However, results obtained from before and after correcting for population structure are not considerably different whereby a large number of outliers overlapped between the two methods. Further, the same environmental variables (elevation and precipitation) were detected as important predictors of genetic variation suggesting an important role of these predictors as drivers of local adaptation in the system.

Polygenic score along elevation gradients

The strong positive correlation between additive polygenic scores calculated for each individual and elevation indicates that the frequencies of multiple, different alleles (i.e. that are favorable in local environments) are being maintained along elevational gradients. A quadratic model described this relationship better than a linear model in both species, indicating selection pressures are not linear and alleles along the gradient are not following a clinal increase (Fig. 8). A relatively higher polygenic score at higher elevations suggests stronger and steeper selection pressures at higher elevations than at lower elevations. This is consistent with the challenging environmental conditions at higher elevations such as increasing coldness and precipitation. These results indicate a possible link between genotype and environmental pressures along

elevation and implies that locally favored alleles possibly affect the fitness of individuals at different elevational gradients. The lower extent of variance explained in the common data is possibly because this dataset encompasses two species with divergent allele frequencies. The pattern of relationship between polygenic scores and elevation is similar in *K. usitatus* and *K. tristis*, however the difference in polygenic scores between regions is more visible in *K. tristis* Thredbo (higher scores) and Guthega (lower scores; Fig. S7) than *K. usitatus*, indicating that *K. tristis* is more differentially locally adapted in the two regions.

Although it is not possible to completely rule out the possibility of physical linkage between the candidate loci due to the unavailability of a reference genome for our focal species, our LD correlation assessment indicates that the candidate loci are unlikely to be physically linked. The use of polygenic scores to characterise the strength of selection across candidate loci is a useful measure and is particularly useful to overcome limitations of single locus tests (Gagnaire and Gaggiotti, 2016), however a better characterization can be achieved via experimental confirmation of allelic effects and via examination of the phenotypic basis of loci under selection.

Candidate genes under selection along elevational gradient

A small number of outlier loci found across all three datasets (i.e. individual and combined) returned significant annotations to known insect genes. Several interesting annotations emerged including multiple matches to phospholipase gene (PLA2) and vitellin B gene with all three datasets, indicating evidence of positive selection on these genes. PL is an important gene involved in the hydrolyses of lipids, cell signaling, and can also assist in the synthesis of acetyl CoA (Groot et al., 2013). The latter is shown to be involved in maintaining lipid membrane fluidity in the lipid bilayer during cold acclimatization (Nozawa, 2011, Waterhouse et al., 2018). This highlights the potential role of PL enzymes in metabolic processes associated with cold

tolerance, which in turn can be highly beneficial for ectothermic grasshoppers that confront cooler temperatures.

Another common gene function in both species also found in the common dataset was annotated to the vitelline B gene. Vitellin is a major egg yolk protein and are present in large amount in females (Hagedorn and Kunkel, 1979, Tufail and Takeda, 2008). Vitellogenin the precursor of vitelline plays an important role as a food resource in developing embryo (Harnish and White, 1982), provide material for ovary development and is important for insect fecundity (Wu et al., 2018, Zhang et al., 2019). The involvement of vitellin gene in key reproductive pathways, suggest that this gene and its precursor proteins are putatively under strong selection and may provide a fitness advantage. Notably, a large number of independent candidate loci (LD <20%) across all datasets showed annotation to the PL gene and vitelline B gene, indicating involvement of common genes under selection in both species and the likelihood of parallel adaptation.

Conclusion

Alpine habitats make an excellent system to study fine scale gene flow and local adaptation. Comparing two co-occurring and closely related insect species in the Australian Alps, we have shown that the extent of local adaptation and genetic structure may differ in congeneric species based on their thermal preferences and elevational distributions. We demonstrate that species with a restricted thermal and elevational niche show strong genetic differentiation and local adaptation compared to a more widely distributed and tolerant species and is therefore likely to be more vulnerable to climate and landscape change. Our approach is therefore relevant for multispecies conservation inference (Nicholson and Possingham, 2006). By using several different outlier detection tests and polygenic score analyses we demonstrate that elevation and climatic variables are major drivers of adaptive divergence across fine spatial scales, and further changes in climate may negatively influence alpine restricted species. Our study provides

relevant information for understanding the adaptive capacity of two alpine restricted species and in assessing which species may be more or less susceptible to climate shifts. Given the predicted increases in temperature in the Australian Alps over the coming decades, it is critical to understand the responses and evolutionary trajectories of alpine-restricted species under climate change.

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References

AHRENS, C. W., RYMER, P. D., STOW, A., BRAGG, J., DILLON, S., UMBERS, K. D. L. & DUDANIEC, R. Y. 2018. The search for loci under selection: trends, biases and progress. *Mol Ecol,* 27**,** 1342-1356.

BABIN, C., GAGNAIRE, P. A., PAVEY, S. A. & BERNATCHEZ, L. 2017. RAD-Seq reveals patterns of additive polygenic variation caused by spatially-varying selection in the American Eel (*Anguilla rostrata*). *Genome Biol Evol,* 9**,** 2974-2986.

BALE, J. S. & HAYWARD, S. A. 2010. Insect overwintering in a changing climate. *J Exp Biol,* 213**,** 980-94.

BARKER, J. S., FRYDENBERG, J., SARUP, P. & LOESCHCKE, V. 2011. Altitudinal and seasonal variation in microsatellite allele frequencies of *Drosophila buzzatii*. *J Evol Biol,* 24**,** 430-9.

BARNEY, B. T., MUNKHOLM, C., WALT, D. R. & PALUMBI, S. R. 2017. Highly localized divergence within supergenes in Atlantic cod (*Gadus morhua*) within the Gulf of Maine. *BMC genomics,* 18**,** 271.

BARRETT, R. D. & HOEKSTRA, H. E. 2011. Molecular spandrels: tests of adaptation at the genetic level. *Nature Reviews Genetics,* 12**,** 767.

BAY, R. A., ROSE, N., BARRETT, R., BERNATCHEZ, L., GHALAMBOR, C. K., LASKY, J. R., BREM, R. B., PALUMBI, S. R. & RALPH, P. 2017. Predicting responses to contemporary environmental change using evolutionary response architectures. *The American Naturalist,* 189**,** 463-473.

BENISTON, M. 2003. Climatic change in mountain regions: a review of possible impacts. *Climate variability and change in high elevation regions: Past, present & future.* Springer.

BIRKETT, A. J., BLACKBURN, G. A. & MENÉNDEZ, R. 2018. Linking species thermal tolerance to elevational range shifts in upland dung beetles. *Ecography,* 41**,** 1510-1519.

BLANCHET, F. G., LEGENDRE, P. & BORCARD, D. 2008. Modelling directional spatial processes in ecological data. *Ecological modelling,* 215**,** 325-336.

BOLNICK, D. I., BARRETT, R. D., OKE, K. B., RENNISON, D. J. & STUART, Y. E. 2018. (Non) parallel evolution. *Annual Review of Ecology, Evolution, and Systematics,* 49**,** 303-330.

BUCKLEY, L. B., MILLER, E. F. & KINGSOLVER, J. G. 2013. Ectotherm thermal stress and specialization across altitude and latitude. *Integrative comparative biology* 53**,** 571-581.

BUCKLEY, L. B. & NUFIO, C. R. 2014. Elevational clines in the temperature dependence of insect performance and implications for ecological responses to climate change. *Conservation physiology,* 2.

CAMPBELL, N. & DEARN, J. 1980. Altitudinal Variation in, and Morphological Divergence between, Three Related Species of Grasshopper, *Praxibulus* sp., *Kosciuscola cognatu*s and *K. usitatus* (Orthoptera: Acrididae). *Australian Journal of Zoology,* 28**,** 103-118.

CAREY, C. & ALEXANDER, M. A. 2003. Climate change and amphibian declines: is there a link? *Diversity distributions,* 9**,** 111-121.

CHARLESWORTH, B., NORDBORG, M., & CHARLESWORTH, D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetics Research,* 70, 155-174.

CHEN, I.-C., HILL, J. K., OHLEMÜLLER, R., ROY, D. B. & THOMAS, C. D. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science,* 333**,** 1024-1026.

CHHATRE, V. E. & EMERSON, K. J. 2017. StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics,* 18**,** 192.

CHOWN, S. L. & GASTON, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biol Rev Camb Philos Soc,* 85**,** 139-69.

CLARK, D. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.
CLUSELLA-TRULLAS, S., BLACKBURN, T. M. & CHOWN, S. L. 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist,* 177**,** 738-751.
COLWELL, R. K., BREHM, G., CARDELÚS, C. L., GILMAN, A. C. & LONGINO, J. T. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science,* 322**,** 258-261.
CONTE, G. L., ARNEGARD, M. E., PEICHEL, C. L. & SCHLUTER, D. 2012. The probability of genetic parallelism and convergence in natural populations. *Proceedings of the Royal Society B: Biological Sciences,* 279**,** 5039-5047.
DAHLHOFF, E. P., DAHLHOFF, V. C., GRAINGER, C. A., ZAVALA, N. A., OTEPOLA-BELLO, D., SARGENT, B. A., ROBERTS, K. T., HEIDL, S. J., SMILEY, J. T. & RANK, N. E. 2019. Getting chased up the mountain: High elevation may limit performance and fitness characters in a montane insect. *Functional Ecology,* 33**,** 809-818.
DE VILLEMEREUIL, P., FRICHOT, É., BAZIN, É., FRANÇOIS, O. & GAGGIOTTI, O. E. 2014. Genome scan methods against more complex models: when and how much should we trust them? *Molecular Ecology,* 23**,** 2006-2019.

DEUTSCH, C. A., TEWKSBURY, J. J., HUEY, R. B., SHELDON, K. S., GHALAMBOR, C. K., HAAK, D. C. & MARTIN, P. R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences,* 105**,** 6668-6672.
DEVLIN, B. & ROEDER, K. 1999. Genomic control for association studies. *Biometrics,* 55**,** 997-1004.
DRAY, S., BLANCHET, G., BORCARD, D., GUENARD, G., JOMBART, T., LAROCQUE, G., LEGENDRE, P., MADI, N., WAGNER, H. H. & DRAY, M. S. 2017. Package 'adespatial'.
DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Molecular ecology,* 27**,** 2576-2593.
ELDON, J., BELLINGER, M. R. & PRICE, D. K. 2019. Hawaiian picture-winged Drosophila exhibit adaptive population divergence along a narrow climatic gradient on Hawaii Island. *Ecology evolution,* 9**,** 2436.
ELMER, K. R. & MEYER, A. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends Ecol Evol,* 26**,** 298-306.
ELSEN, P. R. & TINGLEY, M. W. 2015. Global mountain topography and the fate of montane species under climate change. *Nature Climate Change,* 5**,** 772.
ELSHIRE, R. J., GLAUBITZ, J. C., SUN, Q., POLAND, J. A., KAWAMOTO, K., BUCKLER, E. S. & MITCHELL, S. E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One,* 6**,** e19379.
ENDLER, J. A. 1977. *Geographic variation, speciation, and clines,* Princeton University Press.
EVANNO, G., REGNAUT, S. & GOUDET, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol,* 14**,** 2611-20.
EXCOFFIER, L., HOFER, T. & FOLL, M. 2009. Detecting loci under selection in a hierarchically structured population. *Heredity (Edinb),* 103**,** 285-98.
EXCOFFIER, L. & LISCHER, H. E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour,* 10**,** 564-7.
FINN, D. S., THEOBALD, D. M., BLACK, W. C. & POFF, N. L. 2006. Spatial population genetic structure and limited dispersal in a Rocky Mountain alpine stream insect. *Mol Ecol,* 15**,** 3553-66.

FLOOD, P. J. & HANCOCK, A. M. 2017. The genomic basis of adaptation in plants. *Current opinion in plant biology,* 36**,** 88-94.
FOLL, M. & GAGGIOTTI, O. E. 2008. A genome scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*.
FORESTER, B. R., LASKY, J. R., WAGNER, H. H. & URBAN, D. L. 2018. Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular ecology,* 27**,** 2215-2233.
FRACHON, L., BARTOLI, C., CARRÈRE, S., BOUCHEZ, O., CHAUBET, A., GAUTIER, M., ROBY, D. & ROUX, F. 2018. A genomic map of climate adaptation in *Arabidopsis thaliana* at a micro-geographic scale. *Frontiers in plant science,* 9**,** 967.
FRICHOT, E. & FRANCOIS, O. 2015. LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution,* 6**,** 925-929.
FRICHOT, E., SCHOVILLE, S. D., BOUCHARD, G. & FRANCOIS, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. *Mol Biol Evol,* 30**,** 1687-99.
GAGNAIRE, P. A. & GAGGIOTTI, O. E. 2016. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. *Current Zoology,* 62**,** 603-616.
GAMBOA, M. & WATANABE, K. 2019. Genome-wide signatures of local adaptation among seven stoneflies species along a nationwide latitudinal gradient in Japan. *BMC genomics,* 20**,** 84.
GARNIER, S., ALIBERT, P., AUDIOT, P., PRIEUR, B. & RASPLUS, J. Y. 2004. Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. *Molecular Ecology,* 13**,** 1883-1897.
GOBIET, A., KOTLARSKI, S., BENISTON, M., HEINRICH, G., RAJCZAK, J. & STOFFEL, M. 2014. 21st century climate change in the European Alps—a review. *Science of the Total Environment,* 493**,** 1138-1151.
GOSSELIN, T. 2017. radiator: RADseq data exploration, manipulation and visualization using R. R package version 0.0.5
GREEN, K. 2010. Alpine Taxa exhibit differing responses to climate warming in the snowy mountains of Australia. *Journal of Mountain Science,* 7**,** 167-175.
GREEN, K. & OSBORNE, W. S. 1994. *Wildlife of the Australian snow-country: a comprehensive guide to alpine fauna*, Reed.
GROOT, A. T., STAUDACHER, H., BARTHEL, A., INGLIS, O., SCHÖFL, G., SANTANGELO, R., GEBAUER-JUNG, S., VOGEL, H., EMERSON, J. & SCHAL, C. 2013. One quantitative trait locus for intra-and interspecific variation in a sex pheromone. *Molecular ecology,* 22**,** 1065-1080.
GUGERLI, F., BRANDL, R., CASTAGNEYROL, B., FRANC, A., JACTEL, H., KOELEWIJN, H. P., MARTIN, F., PETER, M., PRITSCH, K. & SCHRÖDER, H. 2013. Community genetics in the time of next-generation molecular technologies. *Molecular Ecology,* 22**,** 3198-3207.
GUO, B., LU, D., LIAO, W. B. & MERILÄ, J. 2016. Genomewide scan for adaptive differentiation along altitudinal gradient in the Andrew's toad *Bufo andrewsi*. *Molecular ecology,* 25**,** 3884-3900.
HAGEDORN, H. & KUNKEL, J. 1979. Vitellogenin and vitellin in insects. *Annual review of entomology,* 24**,** 475-505.
HANCOCK, A. M., BRACHI, B., FAURE, N., HORTON, M. W., JARYMOWYCZ, L. B., SPERONE, F. G., TOOMAJIAN, C., ROUX, F. & BERGELSON, J. 2011. Adaptation to climate across the *Arabidopsis thaliana* genome. *Science,* 334**,** 83-6.

HAND, B. K., LOWE, W. H., KOVACH, R. P., MUHLFELD, C. C. & LUIKART, G. 2015. Landscape community genomics: understanding eco-evolutionary processes in complex environments. *Trends in Ecology Evolution,* 30**,** 161-168.
HARNISH, D. G. & WHITE, B. N. 1982. Insect vitellins: identification, purification, and characterization from eight orders. *Journal of Experimental Zoology,* 220**,** 1-10.
HENNESSY, K., WHETTON, P., SMITH, I., BATHOLS, J., HUTCHINSON, M. & SHARPLES, J. 2003. The impact of climate change on snow conditions in mainland Australia. *Aspendale: CSIRO Atmospheric Research*.
HERRMANN, D. L., KO, A. E., BHATT, S., JANNOT, J. E. & JULIANO, S. A. 2010. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Annals of the Entomological Society of America,* 103**,** 227-235.
HIJMANS, R. J. & VAN ETTEN, J. 2014. raster: Geographic data analysis and modeling. *R package version,* 2.
HODKINSON, I. D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews,* 80**,** 489-513.
HOFFMANN, A. A., RYMER, P. D., BYRNE, M., RUTHROF, K. X., WHINAM, J., MCGEOCH, M., BERGSTROM, D. M., GUERIN, G. R., SPARROW, B. & JOSEPH, L. 2018. Impacts of recent climate change on terrestrial flora and fauna: Some emerging Australian examples. *Austral Ecology*.
IPCC 2014. Climate Change 2014 Synthesis Report.
JACKSON, J. M., PIMSLER, M. L., OYEN, K. J., KOCH-UHUAD, J. B., HERNDON, J. D., STRANGE, J. P., DILLON, M. E. & LOZIER, J. D. 2018. Distance, elevation and environment as drivers of diversity and divergence in bumble bees across latitude and altitude. *Molecular ecology,* 27**,** 2926-2942.
JOERN, A. & GAINES, S. 1990. Population dynamics and regulation in grasshoppers. *Biology of grasshoppers***,** 415-482.
JOHNSON, M., ZARETSKAYA, I., RAYTSELIS, Y., MEREZHUK, Y., MCGINNIS, S. & MADDEN, T. L. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res,* 36**,** W5-9.
KAWECKI, T. J. & EBERT, D. 2004. Conceptual issues in local adaptation. *Ecology Letters,* 7**,** 1225-1241.
KEARNEY, M., SHINE, R. & PORTER, W. P. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences,* 106**,** 3835-3840.
KELLER, I., ALEXANDER, J., HOLDEREGGER, R. & EDWARDS, P. 2013. Widespread phenotypic and genetic divergence along altitudinal gradients in animals. *Journal of evolutionary biology,* 26**,** 2527-2543.
KILIAN, A., WENZL, P., HUTTNER, E., CARLING, J., XIA, L., BLOIS, H., CAIG, V., HELLER-USZYNSKA, K., JACCOUD, D. & HOPPER, C. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. *Data production and analysis in population genomics.* Springer.
KÖRNER, C. 2007. The use of 'altitude'in ecological research. *Trends in ecology evolution* 22**,** 569-574.
KUBOTA, S., IWASAKI, T., HANADA, K., NAGANO, A. J., FUJIYAMA, A., TOYODA, A., SUGANO, S., SUZUKI, Y., HIKOSAKA, K. & ITO, M. 2015. Correction: A Genome can for genes underlying microgeographic-scale local adaptation in a wild *Arabidopsis* species. *PLoS genetics,* 11**,** e1005488.
LA SORTE, F. A. & JETZ, W. 2010. Projected range contractions of montane biodiversity under global warming. *Proceedings of the Royal Society B: Biological Sciences,* 277**,** 3401-3410.
LASKY, J. R., UPADHYAYA, H. D., RAMU, P., DESHPANDE, S., HASH, C. T., BONNETTE, J., JUENGER, T. E., HYMA, K., ACHARYA, C. & MITCHELL, S. E.

2015. Genome-environment associations in sorghum landraces predict adaptive traits. *Science Advances,* 1**,** e1400218.
LAURANCE, W. F., USECHE, D. C., SHOO, L. P., HERZOG, S. K., KESSLER, M., ESCOBAR, F., BREHM, G., AXMACHER, J. C., CHEN, I.-C. & GÁMEZ, L. A. 2011. Global warming, elevational ranges and the vulnerability of tropical biota. *Biological Conservation,* 144**,** 548-557.
LI, Y. L. & LIU, J. X. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular ecology resources,* 18**,** 176-177.
LISCHER, H. E. & EXCOFFIER, L. 2011. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics,* 28**,** 298-299.
LOTTERHOS, K. E. & WHITLOCK, M. C. 2015. The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Mol Ecol,* 24**,** 1031-46.
LYONS, M. B., FOSTER, S. D. & KEITH, D. A. 2017. Simultaneous vegetation classification and mapping at large spatial scales. *Journal of biogeography,* 44**,** 2891-2902.
MACDONALD, S. L., LLEWELYN, J. & PHILLIPS, B. L. 2018. Using connectivity to identify climatic drivers of local adaptation. *Ecology letters,* 21**,** 207-216.
MACFADYEN, S., MCDONALD, G. & HILL, M. P. 2018. From species distributions to climate change adaptation: Knowledge gaps in managing invertebrate pests in broad-acre grain crops. *Agriculture Ecosystems & Environment,* 253**,** 208-219.
MANEL, S., PONCET, B., LEGENDRE, P., GUGERLI, F. & HOLDEREGGER, R. 2010. Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. *Molecular Ecology,* 19**,** 3824-3835.
MASAKI, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution,* 21**,** 725-741.
MAYR, E. 1963. Animal species and evolution. *Animal species evolution*.
MCCAIN, C. M. & COLWELL, R. K. 2011. Assessing the threat to montane biodiversity from discordant shifts in temperature and precipitation in a changing climate. *Ecol Lett,* 14**,** 1236-45.
MIAO, H. T., LIU, Y., SHAN, L. Y. & WU, G. L. 2018. Linkages of plant-soil interface habitat and grasshopper occurrence of typical grassland ecosystem. *Ecological Indicators,* 90**,** 324-333.
MILNER, R. J. & PRIOR, C. 1994. Susceptibility of the Australian plague locust, *Chortoicetes terminifera,* and the wingless grasshopper, *Phaulacridium vittatum,* to the Fungi *Metarhizium* Spp. *Biological Control,* 4**,** 132-137.
MONEY, D., GARDNER, K., MIGICOVSKY, Z., SCHWANINGER, H., ZHONG, G.-Y. & MYLES, S. 2015. LinkImpute: fast and accurate genotype imputation for non-model organisms. *G3: Genes, Genomes, Genetics***,** g3. 115.021667.
MOUSSEAU, T. A. & ROFF, D. A. 1989. Adaptation to seasonality in a Cricket: Patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution,* 43**,** 1483-1496.
MUIR, A. P., BIEK, R., THOMAS, R. & MABLE, B. K. 2014. Local adaptation with high gene flow: temperature parazs drive adaptation to altitude in the common frog (*Rana temporaria*). *Molecular ecology,* 23**,** 561-574.
NICHOLLS, N. 2005. Climate variability, climate change and the Australian snow season. *Australian Meteorological Magazine,* 54**,** 177-185.
NICHOLSON, E. & POSSINGHAM, H. P. 2006. Objectives for multiple-species conservation planning. *Conservation Biology,* 20**,** 871-881.
NOZAWA, Y. 2011. Adaptive regulation of membrane lipids and fluidity during thermal acclimation in *Tetrahymena*. *Proc Jpn Acad Ser B Phys Biol Sci,* 87**,** 450-62.

OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R., SIMPSON, G. L., SOLYMOS, P., STEVENS, M. H. H. & WAGNER, H. 2013. Package 'vegan'. Community ecology package, version, 2(9).

PARADIS, E. 2010. pegas: an R package for population genetics with an integrated–modular approach. *Bioinformatics,* 26**,** 419-420.

PEAKALL, R., RUIBAL, M. & LINDENMAYER, D. B. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution,* 57**,** 1182-1195.

PEAKALL, R. & SMOUSE, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular ecology notes,* 6**,** 288-295.

POLATO, N. R., GILL, B. A., SHAH, A. A., GRAY, M. M., CASNER, K. L., BARTHELET, A., MESSER, P. W., SIMMONS, M. P., GUAYASAMIN, J. M., ENCALADA, A. C., KONDRATIEFF, B. C., FLECKER, A. S., THOMAS, S. A., GHALAMBOR, C. K., POFF, N. L., FUNK, W. C. & ZAMUDIO, K. R. 2018. Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proc Natl Acad Sci U S A,* 115**,** 12471-12476.

POLATO, N. R., GRAY, M., GILL, B., BECKER, C., CASNER, K., FLECKER, A., KONDRATIEFF, B., ENCALADA, A., POFF, N. & FUNK, W. 2017. Genetic diversity and gene flow decline with elevation in montane mayflies. *Heredity,* 119**,** 107.

PREITE, V., SAILER, C., SYLLWASSCHY, L., BRAY, S., AHMADI, H., KRÄMER, U. & YANT, L. 2019. Convergent evolution in *Arabidopsis halleri* and *Arabidopsis arenosa* on calamine metalliferous soils. *Philosophical Transactions of the Royal Society B,* 374**,** 20180243.

PRITCHARD, J. K., STEPHENS, M. & DONNELLY, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* 155**,** 945-959.

PURCELL, S., NEALE, B., TODD-BROWN, K., THOMAS, L., FERREIRA, M. A., BENDER, D., MALLER, J., SKLAR, P., DE BAKKER, P. I. & DALY, M. J. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics,* 81**,** 559-575.

RANK, N. E. & DAHLHOFF, E. P. 2002. Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect. *Evolution,* 56**,** 2278-89.

REICH, P. B., SENDALL, K. M., RICE, K., RICH, R. L., STEFANSKI, A., HOBBIE, S. E. & MONTGOMERY, R. A. 2015. Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nature Climate Change,* 5**,** 148.

RELLSTAB, C., GUGERLI, F., ECKERT, A. J., HANCOCK, A. M. & HOLDEREGGER, R. 2015. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology,* 24**,** 4348-4370.

RENNISON, D. J., STUART, Y. E., BOLNICK, D. I. & PEICHEL, C. L. 2019. Ecological factors and morphological traits are associated with repeated genomic differentiation between lake and stream stickleback. *Philosophical Transactions of the Royal Society B,* 374**,** 20180241.

REYNOLDS, J., WEIR, B. S. & COCKERHAM, C. C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics,* 105**,** 767-779.

RICHARDSON, J. L. & URBAN, M. C. 2013. Strong selection barriers explain microgeographic adaptation in wild salamander populations. *Evolution,* 67**,** 1729-1740.

RICHARDSON, J. L., URBAN, M. C., BOLNICK, D. I. & SKELLY, D. K. 2014. Microgeographic adaptation and the spatial scale of evolution. *Trends in ecology evolution*

29**,** 165-176.

ROLAND, J. & MATTER, S. F. 2016. Pivotal effect of early-winter temperatures and snowfall on population growth of alpine *Parnassius smintheus* butterflies. *Ecological Monographs,* 86**,** 412-428.

SÁNCHEZ-BAYO, F. & GREEN, K. 2018. Australian snowpack disappearing under the influence of global warming and solar activity. *Arctic, Antarctic, and Alpine Research,* 45**,** 107-118.
SCHERRER, D., & KOERNER, C. 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology,* 16**,** 2602-2613.
SCHWENK, W. S. & DONOVAN, T. M. 2011. A multispecies framework for landscape conservation planning. *Conservation Biology,* 25**,** 1010-1021.
SKELLY, D. K. 2004. Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. *Evolution,* 58**,** 160-165.
SLATYER, R. A., NASH, M. A. & HOFFMANN, A. A. 2016. Scale-dependent thermal tolerance variation in Australian mountain grasshoppers. *Ecography,* 39**,** 572-582.
SLATYER, R. A., NASH, M. A., MILLER, A. D., ENDO, Y., UMBERS, K. D. & HOFFMANN, A. A. 2014. Strong genetic structure corresponds to small-scale geographic breaks in the Australian alpine grasshopper *Kosciuscola tristis*. *BMC Evol Biol,* 14**,** 204.
STEINBAUER, M. J., FIELD, R., GRYTNES, J. A., TRIGAS, P., AH-PENG, C., ATTORRE, F., BIRKS, H. J. B., BORGES, P. A., CARDOSO, P. & CHOU, C. H. 2016. Topography-driven isolation, speciation and a global increase of endemism with elevation. *Global Ecology Biogeography,* 25**,** 1097-1107.
STOCKWELL, C. A., HENDRY, A. P. & KINNISON, M. T. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution,* 18**,** 94-101.
TATARNIC, N. J., UMBERS, K. & SONG, H. 2013. Molecular phylogeny of the *Kosciuscola* grasshoppers endemic to the Australian alpine and montane regions. *Invertebrate systematics,* 27**,** 307-316.
TEAM, R. C. 2017. *R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.* [Online]. Available: https://www.R-project.org/ [Accessed].
TUFAIL, M. & TAKEDA, M. 2008. Molecular characteristics of insect vitellogenins. *Journal of insect physiology,* 54**,** 1447-1458.
UMBERS, K. D., HERBERSTEIN, M. E. & MADIN, J. S. 2013. Colour in insect thermoregulation: empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*. *J Insect Physiol,* 59**,** 81-90.
WAHREN, C. H., CAMAC, J. S., JARRAD, F. C., WILLIAMS, R. J., PAPST, W. A. & HOFFMANN, A. A. 2013. Experimental warming and long-term vegetation dynamics in an alpine heathland. *Australian Journal of Botany,* 61**,** 36-51.
WATERHOUSE, M. D., ERB, L. P., BEEVER, E. A. & RUSSELLO, M. A. 2018. Adaptive population divergence and directional gene flow across steep elevational gradients in a climate-sensitive mammal. *Mol Ecol,* 27**,** 2512-2528.
WHITEMAN, C. D. 2000. *Mountain meteorology: fundamentals and applications,* Oxford University Press.
WU, H., JIANG, F.-Z., GUO, J.-X., YI, J.-Q., LIU, J.-B., CAO, Y.-S., LAI, X.-S. & ZHANG, G.-R. 2018. Molecular characterization and expression of vitellogenin and vitellogenin receptor of *Thitarodes pui* (Lepidoptera: hepialidae), an insect on the Tibetan plateau. *Journal of Insect Science,* 18**,** 23.
XU, T. B. & HUTCHINSON, M. F. 2013. New developments and applications in the ANUCLIM spatial climatic and bioclimatic modelling package. *Environmental Modelling & Software,* 40**,** 267-279.
XUEREB, A., KIMBER, C. M., CURTIS, J. M. R., BERNATCHEZ, L. & FORTIN, M. J. 2018. Putatively adaptive genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as revealed by environmental association analysis of restriction-site associated DNA sequencing data. *Molecular Ecology,* 27**,** 5035-5048.

YADAV, S., STOW, A. J. & DUDANIEC, R. Y. 2019. Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (*Phaulacridium vittatum*). *Mol Ecol*.

YADAV, S., STOW, A. J., HARRIS, R. M. B. & DUDANIEC, R. Y. 2018. Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae). *J Insect Sci,* 18**,** 13.

YEAMAN, S., HODGINS, K. A., LOTTERHOS, K. E., SUREN, H., NADEAU, S., DEGNER, J. C., NURKOWSKI, K. A., SMETS, P., WANG, T. & GRAY, L. K. 2016. Convergent local adaptation to climate in distantly related conifers. *Science,* 353**,** 1431-1433.

ZHANG, H., WANG, Y., LIU, Y., ZHAO, M., JIN, J., ZHOU, Z. & GUO, J. 2019. Identification and expression patterns of three Vitellogenin genes and their roles in reproduction of the alligatorweed flea beetle *Agasicles hygrophila* (Coleoptera: Chrysomelidae). *Frontiers in physiology,* 10**,** 368.

(A)

(B)

Photo (A): *Kosciuscola tristis* males; (B) *Kosciuscola tristis* females (photos: Sonu Yadav)

GENERAL DISCUSSION

The collection of chapters in this thesis provides a comprehensive understanding of the impact of environmental variables on the ecology and evolution of a generalist pest versus an alpine specialist grasshopper species assemblage along latitudinal and elevational gradients. This thesis showed that the environmental variables including climatic data, vegetation cover and soil properties have an important influence on the morphology, abundance, genetic structure and local adaptation of the two contrasting study systems. The widespread generalist species, *P. vittatum*, was found to have high gene flow between sites along a 900 km latitudinal gradient, yet signatures of selection were detected despite this high gene flow, indicating its potential to spread and adapt to changing climatic regimes. The independent landscape resistance analysis and local adaptation detection analyses identified temperature as a common variable influencing dispersal and evolutionary processes in this agricultural pest.

On the other hand, the two alpine specialist species, *Kosciuscola usitatus* and *Kosciuscola tristis* were found to have strong genetic structure at both broad and micro geographic scales and were shown to have strong signatures of local adaptation. Within the alpine specialist species, *K. tristis* showed a more pronounced genetic structure and stronger selection signatures of local adaptation as compared to *K. usitatus* and thus may be more vulnerable to changing climatic conditions. Temperature is identified as a dominant variable influencing abundance patterns, morphology, and local adaptation in the specialist grasshopper systems. Although annotated genomes of the study species were unavailable yet annotation of candidate loci putatively under environmental selection among all study systems to “insects” and *Locusta migratoria* genome (Wang et al., 2014) in NCBI BLAST database (Johnson et al., 2008) indicated genes involved in environmental cue detection, UV protection, detoxification, lipid metabolism, and development. These results suggest that environmental variables are important selective pressures and may lead to changes at a functional level.

By applying statistical modelling, and landscape genomics approach with newly developed statistical approaches within environmental association analysis framework (Rellstab et al., 2015, Forester et al., 2018, Balkenhol et al., 2017), the chapters in this thesis provide insights into species' morphological variations, genetic connectivity and adaptive capacity in relation to diverse environmental conditions. Such knowledge is crucial to predict species vulnerability, and responses to environmental changes and for implementing better management and conservation measures under increasing impacts of climate and land use change (de los Ríos et al., 2018; Hoffmann & Sgro, 2011).

Environmental influences on morphology, abundance and adaptation in Phaulacridium vittatum*: Chapters 2-3*

Insects can be divided into generalists and specialists based on their climatic distributions which is an important determinant of their resistance to climatic extremes and reproductive output (Hoffmann 2010). Environmental variation across wide geographic ranges can influence several aspects of an organisms' development and exert selection pressures that favour morphological variation. For instance, colour polymorphism in Orthoptera, including grasshoppers, is a well-known trait (Dearn, 1990) and is often regulated by environmental conditions (Rowell, 1972, Key, 1954). In the generalist agricultural pest, *P. vittatum*, solar radiation significantly influences body size variation and the presence of white stripes, whereby a higher frequency of larger, striped individuals are found at sites with higher solar radiation. The presence of two white dorsal stripes has potential benefit for thermoregulation in this species (Harris et al., 2013b, Harris et al., 2013a), while larger body size may be a consequence of a longer growth period at higher latitude sites with greater solar radiation (Blanckenhorn, 2000, Blanckenhorn and Demont, 2004). Furthermore, other results indicate that an increase in foliage cover promotes a higher frequency of winged individuals than wingless (Chapter 2), highlighting the importance of habitat type for polymorphic traits (e.g., as shown in planthoppers Denno et al., 1991). These

results demonstrate how environmental factors can impact the distribution of morphological variation which may confer fitness advantage for living in diverse environments.

The relative abundance of *Phaulacridium vittatum* is significantly positively correlated with latitude, wind speed, and soil moisture and negatively correlated with foliage cover. A greater relative abundance at sites with increased wind speed indicates that the presence of wings and wind-assisted movement in this species may aid their long distance dispersal. Furthermore, lower foliage cover supports a higher relative abundance and suggest that further clearing of landcover may result in greater availability of open lands and may support a higher population size of this agricultural pest. This finding is further supported by neutral analysis of landscape genetic connectivity (Chapter 3), whereby pasture, agricultural and rural areas provide negligible resistance to genetic connectivity as compared to water bodies and urban areas, suggesting that the spread of pasture will provide suitable habitat and sites for dispersal and may potentially lead to more serious outbreaks.

The molecular analysis of *Phaulacridium vittatum* in this thesis is the first broad scale genetic analysis of this species and improves our understanding of gene flow, environmental adaptation and the interplay between gene flow and local adaptation. The low genetic differentiation between sites indicates substantial admixture over long geographic distances in this species (Chapter 3). Landscape resistance modelling highlighted a significant effect of temperature on genetic connectivity, that was ten fold higher than resistance due to land cover. The mean annual temperatures of 19 °C provided negligible resistance to gene flow indicating that a further increase in temperature is likely to facilitate the spread of this species to new areas. In addition, greater signatures of local adaptation in relation to latitude, temperature, and body size indicate that local selection pressures can favour some genotypes more than others despite the homogenising effect of gene flow between sites. The annotation to genes that are involved in

neurotransmission, chemical signalling, olfaction (Dingledine et al., 1999, Usherwood, 1994) and metabolism (Ahn et al., 2012) and are relevant for widespread adaptation such as better environmental cue detection, UV shielding and digesting toxic plant secondary metabolites, indicates that this species is favourably dealing with heterogeneous environments via regulating underlying metabolic pathways. Taken together, Chapters 2 and 3 indicate strong potential for *P. vittatum* to adapt and spread within spatially variable and challenging environments.

Insights into Kosciuscola *ecology and evolution: Chapters 4-5*

Alpine ecosystems are under threat globally from increased temperature, declined precipitation levels as a result of climate change and in the Australian Alpine bioregion an increase in temperature, decrease in snow depth, and sub-alpine vegetation encroachment is already visible (Hoffmann et al., 2018; Sánchez-Bayo and Green, 2018), imposing negative consequences on the survival of alpine restricted species. For instance, the numbers of Pygmy-possums, *Burramys parvus*, which are Australian alpine specialists, have declined by 30% over 35 years (Greenville et al., 2018). Furthermore, an altitudinal shift in the distribution of avian and mammalian biodiversity in the Australian alpine bioregion was recorded over a 30 year period prior to 1999 (Green and Pickering, 2002). However, vital knowledge on the distribution and abundance of many invertebrate species inhabiting the Australian Alps is still missing.

Our data from on three co-occurring *Kosciuscola* (*K. usitatus, K. tristis,* and *K. cognatus*) grasshoppers across three alpine regions within the Mt. Kosciuszko National Park (Chapter 4), provides information on relative abundance and body size along elevation gradients. The dissimilar trends of relative abundances along elevation gradients across the three species (negative in *K. usitatus*, positive in *K. tristis* and insignificant in *K. cognatus*) indicates differences in physiological tolerance and elevational preference. A reduction in relative abundance at higher elevations as seen in *K. usitatus* can also be a consequence of reduced fecundity via a decline in egg production at higher elevations as shown by a previous study on

the species (Dearn, 1977). Furthermore, male *K. tristis* exhibits colour change in response to temperature, being turquoise blue at ambient temperature 25°C and black below an ambient temperature of 15°C (Umbers, 2011, Umbers et al., 2013). This may help to increase fitness and in turn, the relative abundance of this species at higher elevation via increased thermoregulation efficiency.

Furthermore, *Kosciuscola* body size showed a decline along an elevational gradient in all three species, however the change in body size was sex specific. The overall smaller body size at higher elevations in all three species possibly suggest strong selective pressures are acting at higher altitude where growth season is limited (highly important for univoltine species) (reviewed in Chown and Gaston, 2010, Horne et al., 2018, Honěk, 1993), and climatic conditions are challenging such as very low temperatures and higher precipitation (Whitman, 2008). Sex specific clines as observed in *K. tristis* (only female body size change with elevation) and *K. cognatus* (only male body size change), suggests that the evolution of male and female body size may follow a divergent evolutionary pathway within each species as a consequence of sexual selection on males or natural selection on female or sex specific plasticity (Fairbairn et al., 2007, Fairbairn, 2013, García-Navas et al., 2017).

With the sharp and steep variation in environmental conditions along elevational gradients, alpine ecosystems provide an ideal system to investigate fine scale genetic differentiation and local adaptation (Körner, 2007). Recent studies have shown that genetic structure and environmental adaptation can be visible at fine scales (reviewed in Richardson et al., 2014), opposing the common notion that environmental selection is likely to be visible at large geographic scales. By showing strong genetic structure in *K. usitatus* and *K. tristis* between sites separated by only ~25 km, a significant non-linear isolation by distance at fine scales and the presence of genetic differentiation between transects potentially shaped by geographic and anthropogenic barriers, the Chapter 5 point out that change in temperature and precipitation

levels due to climate change and habitat fragmentation will negatively impact dispersal and gene flow in specialist species. Similarly, detection of a high number of candidate SNPs putatively under selection across fine geographical scales (especially in *K. tristis*) indicates that both the species are adapted to local environmental conditions, whereby the strength of adaptive variation in relation to environmental conditions is greater in *K. tristis*. Results in Chapter 5 showed that *K. tristis,* which has a narrower elevational distribution and lower cold tolerance shows stronger genetic differentiation and local adaptation signals as compared to the more broadly distributed *K. usitatus,* which also has a wider thermal tolerance (Slatyer et al., 2016). This demonstrates that further change in climatic and habitat conditions are likely to more negatively impact *K. tristis* than *K. usitatus* and similar negative effects can be expected for other alpine specialist species with limited dispersal and higher levels of local adaptation. For instance, studies have reported declines in specialist species including insects, plants, birds and mammals as a consequence of habitat disturbances and climate change (reviewed in Clavel et al., 2011, Devictor et al., 2008). Due to their higher extent of local adaptation (Jones, 2013) to the contemporary environmental conditions and limited gene flow between populations, specialist species may show constrained evolutionary response to future climatic changes.

Interestingly, a large number of SNPs common in both species were detected under selection indicating that the same genes may be under selection in both species and climate adaptation may involve parallel adaptative pathways. Several common annotations to phospholipase and vitellin B gene indicate a high likelihood that these genes are under selection in both species. The relation of phospholipase gene with cold tolerance (Nozawa, 2011, Waterhouse et al., 2018) and vitellin B gene with growth and development (Harnish and White, 1982) make them vital candidate genes to be under selection and influence fitness. These similar annotations highlight that selection pressures appear to converge on a functional level in two co-occurring species diverged from each other around 2.78 MYA. Furthermore, using an additive polygenic approach, which is useful to examine cumulative effect of loci putatively under selection without knowing

their selective effects (Babin et al., 2017, Hancock et al., 2011), Chapter 5 results showed that locally favoured alleles are maintained at different frequencies and follow a non-linear increase in frequency along elevation gradients in both species, yet the extent of spatially variable adaptation was greater in *K. tristis* compared to *K. usitatus*. Notably, higher polygenic scores at a higher elevation in both species confirm that selection pressures are likely to be greater at higher elevations as compared to lower elevations, possibly due to harsher environmental conditions. Taken together, by applying a suite of genetic analysis approaches and comparing two co-occurring species, Chapter 5 provides insights into microgeographic gene flow and local adaptation, climate driven common adaptive responses (parallel adaptation) and genetic architecture of climate adaptation in specialist species

Temperature and insects

Notably in all chapters (Chapter 2,3,4,5), temperature appeared to be the most common climatic driver of ecological and evolutionary processes in both study systems. In *P. vittatum*, temperature is detected as a key driver regulating neutral landscape genetic connectivity despite no isolation by distance and local signatures of selection along the broad latitudinal scale. Similarly, in *Kosciuscola spp.*, elevation (used as a proxy for temperature; correlation= 98%) emerged as a significant driver of relative abundance in *K. usitatus* and *K. tristis,* and of body size change in all three species (*K. usitatus, K. tristis, K. cognatus).* Furthermore, a greater number of candidate loci putatively under selection correlated with elevation in both the species highlights its important role in driving adaptive divergence in populations separated by short geographic distances.

Such significant associations between the abundance, morphology, genetic connectivity, and local adaptation of ectothermic insects and temperature is however somewhat anticipated. A significant effect of temperature is well demonstrated in influencing important life history traits of insects such as hatching time in geometrid moths (Falt-Nardmann et al., 2016), diapause in

fruit flies (Moraiti et al., 2014), and voltinism in moths (Yamanaka et al., 2008). In addition, temperature is also shown to be a strong driver of adaptive divergence in insects such as damselflies (Dudaniec et al., 2018), bamboo locust (Feng et al., 2015) and harlequin flies (Waldvogel et al., 2018). Similarly, temperature is equally important to grasshoppers and there is possibly no phase in grasshopper biology that remains unaffected by temperature either directly or indirectly (Chappell and Whitman, 1990). Important biological activities such as locomotion, fecundity, survival of eggs and juveniles, habitat selection and food consumption are all influenced by thermal regimes in grasshoppers (Chappell and Whitman, 1990). Due to a vital association of temperature with important aspects of an insects' life cycle it is expected that a further increase in temperature as a consequence of anthropogenic climate change will substantially influence their survival and spread.

Conclusion and future directions

Climate change impacts on biological systems include a change in species distribution, abundance, and reduced genetic diversity and gene flow (Scheffers et al., 2016). In addition, climate change is altering interspecific interactions and species' interactions with their environment, and such alterations may hamper ecosystem functioning (Pecl et al., 2017). Generalist species are more likely to adapt to shifting climatic regimes than specialist species, making specialist species more vulnerable to climate change (Ofori et al., 2017). A decline in specialist species has already been documented by several studies in the past few decades (Clavel et al., 2011). Thus, estimating the extent of vulnerability of a species or community to climate change can provide crucial information on what is under increased threat and may require conservation attention (de los Ríos et al., 2018).

Ecological knowledge about abundance and morphological variation in the widespread pest *P. vittatum* and the alpine-restricted *Kosciuscola* species assemblage provides information that is important for understanding current and future species distributions under climate change.

Furthermore, observations made in Chapter 3 fill an important gap in regard to invertebrate species' response to climatic variables and foliage cover in the alpine ecosystems, with particular relevance to Australian Alps. The landscape genomics framework applied in Chapters 3 and 5, explores subtle effects of landscape and environmental variables on adaptive and neutral genetic variation. A further increase in temperature as a consequence of climate warming and an increase in open pasture are likely to facilitate wider distributions of *P. vittatum*. On the other hand, climate warming and habitat destruction are likely to negatively impact *K. tristis* and *K. usitatus* due to higher levels of localised adaptation and restricted gene flow. Taken together, Chapters 3 and 5 provide relevant knowledge for understanding the adaptive capacity and genetic connectivity of the two contrasting study systems.

While the ecological studies (Chapter 2 and 4) were carried out on the dataset collected in one season, the robust sampling design and strong statistical support permitted for strong conclusions to be drawn from studies, yet further sampling efforts over multiple seasons in the future would help to disentangle the spatial and temporal interactions and variations in abundance changes across seasons. A further expansion of the sampling area for *Kosciuscola* grasshoppers in other alpine regions of New South Wales and Victoria would further help to improve understanding of their population dynamics and abundances throughout their distributions. Availability of fine scale environmental data particularly in the alpine ecosystems will assist in examining impact of environmental variables on genetic connectivity of species at microgeographic scales.

Landscape genomics study using cost-effective SNP datasets provides useful information on species adaptive capacity and the genetic basis of local adaptation (reviewed in Bragg et al., 2015). An important aspect however, is to translate the involvement of the selected marker into biological functions, underlying functional pathways, and phenotypic changes. Functional validation of outlier SNPs is often not viable, especially when study species cannot be transformed or when selective pressures cannot be recreated experimentally and thus is a major

limitation to EAA studies (Rellstab et al., 2017). One feasible solution to understand the functional basis is to annotate identified outlier markers to the available genomes. Although reference genome of closely related species could be used for annotation with an assumption that they perform the same function, an essential requirement to understand functional basis is the availability of well annotated genomes of nonmodel species. Further advancements in genome sequencing, assembly, and functional characterization of genes will help to better understand the underlying genomic signatures of selection, the genetic architecture of selection and the functional relevance of genes that will assist species to persist under changing environments. Further, single loci tests are not always effective in identifying subtle signals of local adaptation (Yeaman and Whitlock 2011) and a multi-locus approach is useful to overcome this limitation (Gagnaire and Gaggiotti, 2016). By using recently available polygenic score analysis the study this thesis has explored signals of selection across multiple loci. However, further experimental confirmation of allelic effects can provide improved characterisation of strength and genomic architecture of selection.

References

AHN, S. J., VOGEL, H. & HECKEL, D. G. 2012. Comparative analysis of the UDP-glycosyltransferase multigene family in insects. *Insect Biochem Mol Biol,* 42**,** 133-47.

ATKINS, K. E., & TRAVIS, J. M. J. 2010. Local adaptation and the evolution of species' ranges under climate change. *Journal of Theoretical Biology,* 266**,** 449-457.

BABIN, C., GAGNAIRE, P. A., PAVEY, S. A. & BERNATCHEZ, L. 2017. RAD-Seq Reveals Patterns of Additive Polygenic Variation Caused by Spatially-Varying Selection in the American Eel (*Anguilla rostrata*). *Genome Biol Evol,* 9**,** 2974-2986.

BALKENHOL, N., DUDANIEC, R. Y., KRUTOVSKY, K. V., JOHNSON, J. S., CAIRNS, D. M., SEGELBACHER, G., SELKOE, K. A., VON DER HEYDEN, S., WANG, I. J. & SELMONI, O. 2017. *Landscape Genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations,* Springer.

BLANCKENHORN, W. U. 2000. The evolution of body size: what keeps organisms small? *The quarterly review of biology,* 75**,** 385-407.

BLANCKENHORN, W. U. & DEMONT, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integrative Comparative Biology* 44**,** 413-424.

BRAGG, J. G., SUPPLE, M. A., ANDREW, R. L., & BOREVITZ, J. O. 2015. Genomic variation across landscapes: insights and applications. *New Phytologist,* 207, 953-967.

CHAPPELL, M. & WHITMAN, D. 1990. Grasshopper thermoregulation. *Biology of grasshoppers. Wiley, New York***,** 143-172.

CHOWN, S. L. & GASTON, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biol Rev Camb Philos Soc,* 85**,** 139-69.

CLAVEL, J., JULLIARD, R. & DEVICTOR, V. 2011. Worldwide decline of specialist species: toward a global functional homogenization? *Frontiers in Ecology the Environment,* 9**,** 222-228.

DE LOS RÍOS, C., WATSON, J. E. & BUTT, N. 2018. Persistence of methodological, taxonomical, and geographical bias in assessments of species' vulnerability to climate change: A review. *Global ecology conservation,* 15**,** e00412.

DEARN, J. 1977. Variable life history characteristics along an altitudinal gradient in three species of Australian grasshopper. *Oecologia,* 28**,** 67-85.

DEARN, J. 1990. Color pattern polymorphism. *Biology of grasshoppers***,** 517-549.

DENNO, R. F., RODERICK, G. K., OLMSTEAD, K. L. & DOBEL, H. G. 1991. Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. *The American Naturalist,* 138**,** 1513-1541.

DEVICTOR, V., JULLIARD, R. & JIGUET, F. 2008. Distribution of specialist and generalist species along spatial gradients of habitat disturbance and fragmentation. *Oikos,* 117**,** 507-514.

DINGLEDINE, R., BORGES, K., BOWIE, D. & TRAYNELIS, S. F. 1999. The glutamate receptor ion channels. *Pharmacol Rev,* 51**,** 7-61.

DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Mol Ecol,* 27**,** 2576-2593.

FAIRBAIRN, D. J. 2013. *Odd couples: extraordinary differences between the sexes in the animal kingdom,* Princeton University Press.

FAIRBAIRN, D. J., BLANCKENHORN, W. U. & SZÉKELY, T. 2007. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism,* Oxford University Press.

FALT-NARDMANN, J., KLEMOLA, T., ROTH, M., RUOHOMAKI, K. & SAIKKONEN, K. 2016. Northern geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures than their southern conspecifics: Implications of climate change. *European Journal of Entomology,* 113**,** 337-343.

FENG, X. J., JIANG, G. F. & FAN, Z. 2015. Identification of outliers in a genomic scan for selection along environmental gradients in the bamboo locust, *Ceracris kiangsu*. *Sci Rep,* 5**,** 13758.

FORESTER, B. R., LASKY, J. R., WAGNER, H. H. & URBAN, D. L. 2018. Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular ecology,* 27**,** 2215-2233.

GAGNAIRE, P. A. & GAGGIOTTI, O. E. 2016. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. *Current Zoology,* 62**,** 603-616.

GARCÍA-NAVAS, V., NOGUERALES, V., CORDERO, P. J. & ORTEGO, J. 2017. Ecological drivers of body size evolution and sexual size dimorphism in short-horned grasshoppers (Orthoptera: Acrididae). *Journal of evolutionary biology,* 30**,** 1592-1608.

GREEN, K. & PICKERING, C. M. 2002. Scenario for mammal and bird diversity in the Snowy Mountains of Australia in relation to climate change. In 'Global Mountain Biodiversity: Changes and Threats'.(Eds C. Korner and E. Spehn.) pp. 241–249. Springer-Verlag, Berlin.

GREENVILLE, A. C., BURNS, E., DICKMAN, C. R., KEITH, D. A., LINDENMAYER, D. B., MORGAN, J. W., HEINZE, D., MANSERGH, I., GILLESPIE, G. R., EINODER, L., FISHER, A., RUSSELL-SMITH, J., METCALFE, D. J., GREEN, P. T., HOFFMANN, A. A. & WARDLE, G. M. 2018. Biodiversity responds to increasing climatic extremes in a biome-specific manner. *Sci Total Environ,* 634**,** 382-393.

HANCOCK, A. M., BRACHI, B., FAURE, N., HORTON, M. W., JARYMOWYCZ, L. B., SPERONE, F. G., TOOMAJIAN, C., ROUX, F. & BERGELSON, J. 2011. Adaptation to climate across the *Arabidopsis thaliana* genome. *Science,* 334**,** 83-6.

HARNISH, D. G. & WHITE, B. N. 1982. Insect vitellins: identification, purification, and characterization from eight orders. *Journal of Experimental Zoology,* 220**,** 1-10.

HARRIS, R. M., MCQUILLAN, P. & HUGHES, L. 2013a. Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). *PLoS One,* 8**,** e80243.

HARRIS, R. M. B., MCQUILLAN, P. & HUGHES, L. 2013b. A test of the thermal melanism hypothesis in the wingless grass-hopper *Phaulacridium vittatum*. *Journal of insect science,* 13.

HOFFMANN, A. A. 2010. A genetic perspective on insect climate specialists. *Australian Journal of Entomology,* 49**,** 93-103.

HOFFMANN, A. A., RYMER, P. D., BYRNE, M., RUTHROF, K. X., WHINAM, J., MCGEOCH, M., BERGSTROM, D. M., GUERIN, G. R., SPARROW, B. & JOSEPH, L. 2018. Impacts of recent climate change on terrestrial flora and fauna: Some emerging Australian examples. *Austral Ecology*.

HOFFMANN, A. A. & SGRO, C. M. 2011. Climate change and evolutionary adaptation. *Nature,* 470**,** 479-85.

HONĚK, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos***,** 483-492.

HORNE, C. R., HIRST, A. G. & ATKINSON, D. 2018. Insect temperature–body size trends common to laboratory, latitudinal and seasonal gradients are not found across altitudes. *Functional Ecology,* 32**,** 948-957.

JOHNSON, M., ZARETSKAYA, I., RAYTSELIS, Y., MEREZHUK, Y., MCGINNIS, S. & MADDEN, T. L. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res,* 36**,** W5-9.

JONES, T. A. 2013. When local isn't best. *Evolutionary applications,* 6**,** 1109-1118.

KEY, K. 1954. The Taxonomy, Phases, and Distribution of the Geneva *Chortoicetes Brunn*. and Austroicetes UY. (Orthoptera: Acrididae).

KISDI, É. (2002). Dispersal: risk spreading versus local adaptation. *The American Naturalist,* 159**,** 579-596.

KÖRNER, C. 2007. The use of 'altitude' in ecological research. *Trends in ecology evolution* 22**,** 569-574.

MORAITI, C., NAKAS, C. & PAPADOPOULOS, N. 2014. Diapause termination of *Rhagoletis cerasi* pupae is regulated by local adaptation and phenotypic plasticity: escape in time through bet-hedging strategies. *Journal of Evolutionary Biology,* 27**,** 43-54.

NOZAWA, Y. 2011. Adaptive regulation of membrane lipids and fluidity during thermal acclimation in Tetrahymena. *Proc Jpn Acad Ser B Phys Biol Sci,* 87**,** 450-62.

OFORI, B. Y., STOW, A. J., BAUMGARTNER, J. B. & BEAUMONT, L. J. 2017. Influence of adaptive capacity on the outcome of climate change vulnerability assessment. *Sci Rep,* 7**,** 12979.

PECL, G. T., ARAUJO, M. B., BELL, J. D., BLANCHARD, J., BONEBRAKE, T. C., CHEN, I. C., CLARK, T. D., COLWELL, R. K., DANIELSEN, F., EVENGARD, B., FALCONI, L., FERRIER, S., FRUSHER, S., GARCIA, R. A., GRIFFIS, R. B., HOBDAY, A. J., JANION-SCHEEPERS, C., JARZYNA, M. A., JENNINGS, S., LENOIR, J., LINNETVED, H. I., MARTIN, V. Y., MCCORMACK, P. C., MCDONALD, J., MITCHELL, N. J., MUSTONEN, T., PANDOLFI, J. M., PETTORELLI, N., POPOVA, E., ROBINSON, S. A., SCHEFFERS, B. R., SHAW, J. D., SORTE, C. J., STRUGNELL, J. M., SUNDAY, J. M., TUANMU, M. N., VERGES, A., VILLANUEVA, C., WERNBERG, T., WAPSTRA, E. & WILLIAMS, S. E. 2017. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science,* 355**,** eaai9214.

RELLSTAB, C., GUGERLI, F., ECKERT, A. J., HANCOCK, A. M. & HOLDEREGGER, R. 2015. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology,* 24**,** 4348-4370.

RELLSTAB, C., FISCHER, M. C., ZOLLER, S., GRAF, R., TEDDER, A., SHIMIZU, K. K., ... & GUGERLI, F. 2017. Local adaptation (mostly) remains local: reassessing environmental associations of climate-related candidate SNPs in *Arabidopsis halleri*. *Heredity,* 118, 193-201.

RICHARDSON, J. L., URBAN, M. C., BOLNICK, D. I. & SKELLY, D. K. 2014. Microgeographic adaptation and the spatial scale of evolution. *Trends in ecology evolution* 29**,** 165-176.

ROWELL, C. F. 1972. The variable coloration of the Acridoid grasshoppers. *Advances in insect physiology.* Elsevier.

SÁNCHEZ-BAYO, F. & GREEN, K. 2018. Australian Snowpack Disappearing Under the Influence of Global Warming and Solar Activity. *Arctic, Antarctic, and Alpine Research,* 45**,** 107-118.

SCHEFFERS, B. R., DE MEESTER, L., BRIDGE, T. C., HOFFMANN, A. A., PANDOLFI, J. M., CORLETT, R. T., BUTCHART, S. H., PEARCE-KELLY, P., KOVACS, K. M., DUDGEON, D., PACIFICI, M., RONDININI, C., FODEN, W. B., MARTIN, T. G., MORA, C., BICKFORD, D. & WATSON, J. E. 2016. The broad footprint of climate change from genes to biomes to people. *Science,* 354**,** aaf7671.

SLATYER, R. A., NASH, M. A. & HOFFMANN, A. A. 2016. Scale-dependent thermal tolerance variation in Australian mountain grasshoppers. *Ecography,* 39**,** 572-582.

UMBERS, K. D. 2011. Turn the temperature to turquoise: cues for colour change in the male chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae). *J Insect Physiol,* 57**,** 1198-204.

UMBERS, K. D., HERBERSTEIN, M. E. & MADIN, J. S. 2013. Colour in insect thermoregulation: empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*. *J Insect Physiol,* 59**,** 81-90.

USHERWOOD, P. 1994. Insect glutamate receptors. *Advances in Insect Physiology.* Elsevier.

WALDVOGEL, A. M., WIESER, A., SCHELL, T., PATEL, S., SCHMIDT, H., HANKELN, T., FELDMEYER, B. & PFENNINGER, M. 2018. The genomic footprint of climate adaptation in *Chironomus riparius*. *Mol Ecol,* 27**,** 1439-1456.

WANG, X. H., FANG, X. D., YANG, P. C., JIANG, X. T., JIANG, F., ZHAO, D. J., LI, B. L., CUI, F., WEI, J. N., MA, C. A., WANG, Y. D., HE, J., LUO, Y., WANG, Z. F., GUO, X. J., GUO, W., WANG, X. S., ZHANG, Y., YANG, M. L., HAO, S. G., CHEN, B., MA, Z. Y., YU, D., XIONG, Z. Q., ZHU, Y. B., FAN, D. D., HAN, L. J., WANG, B., CHEN, Y. X., WANG, J. W., YANG, L., ZHAO, W., FENG, Y., CHEN, G. X., LIAN, J. M., LI, Q. Y., HUANG, Z. Y., YAO, X. M., LV, N., ZHANG, G. J., LI, Y. R., WANG, J., WANG, J., ZHU, B. L. & KANG, L. 2014. The locust genome provides insight into swarm formation and long-distance flight. *Nature Communications,* 5**,** 1-9.

WATERHOUSE, M. D., ERB, L. P., BEEVER, E. A. & RUSSELLO, M. A. 2018. Adaptive population divergence and directional gene flow across steep elevational gradients in a climate-sensitive mammal. *Mol Ecol,* 27**,** 2512-2528.

WHITMAN, D. W. 2008. The significance of body size in the Orthoptera: a review. *Journal of Orthoptera research,* 17**,** 117-134.

YAMANAKA, T., TATSUKI, S. & SHIMADA, M. 2008. Adaptation to the new land or effect of global warming? An age-structured model for rapid voltinism change in an alien lepidopteran pest. *J Anim Ecol,* 77**,** 585-96.

YEAMAN, S., & WHITLOCK, M. C. 2011. The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution*, 65, 1897-1911.

SUPPLEMENTARY INFORMATION

Chapter 2 Supplementary material: Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae)

Sonu Yadav, Adam J. Stow, Rebecca M. B. Harris, Rachael Y. Dudaniec

Text S1. *Pearson correlation analysis*

Solar radiation showed a strong correlation with longitude (Pearson correlation coefficient, r = -0.89), and latitude (r = 0.81) whereas wind speed had a strong correlation with mean annual temperature (BIO1, r = -0.90) (Table S2). Mean annual precipitation (BIO12) was strongly correlated with mean annual temperature (BIO1, r = -0.83).

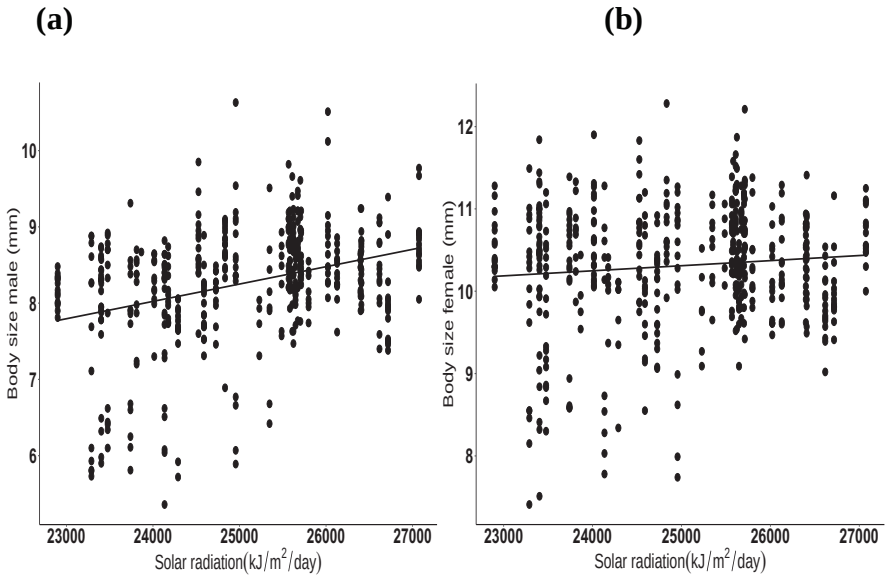


Figure. S1: Correlation between body size (mm) and solar radiation (kJ/m^2/day). An increase in femur length of both males and females is observed with increase in solar radiation, however the relation was stronger in males than females: (a) male (linear model P <0.0001) and (b) female (linear model P = 0.03).

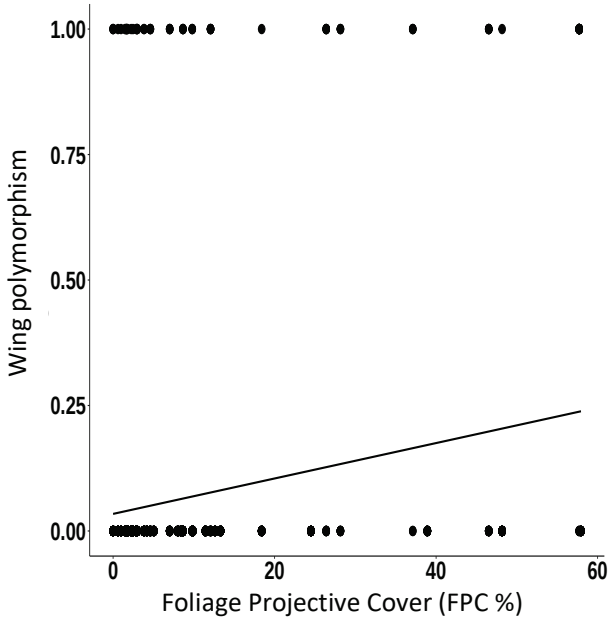


Figure. S2: Correlation between wing polymorphism measured as 0 (wingless morph) and 1 (winged morph) with Foliage Projective Cover (FPC %). A high number of winged individuals were observed at sites with high foliage cover.

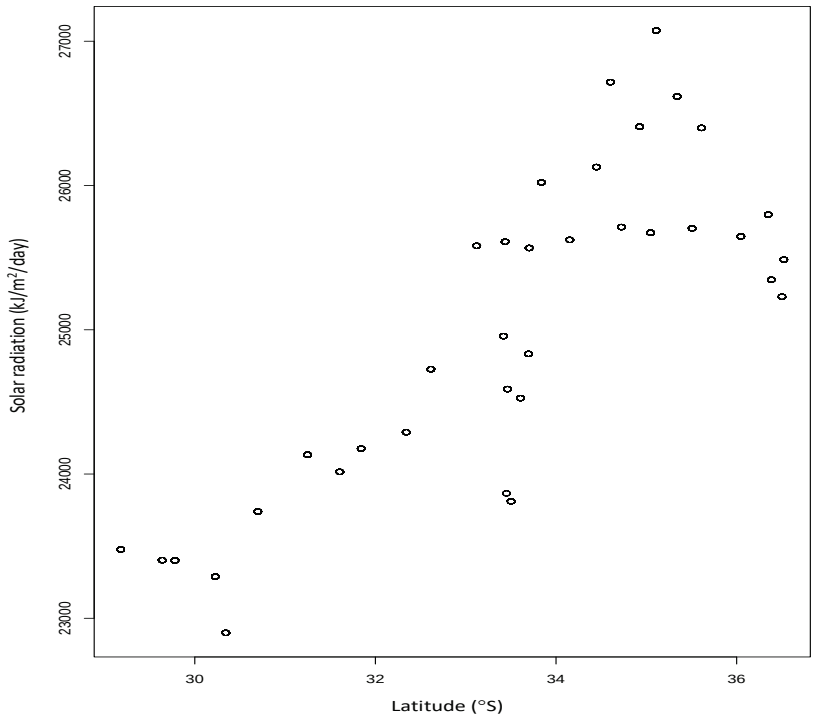


Figure. S3: Scatter plot of solar radiation along a latitudinal gradient covered by 36 sampled sites in the study. Sites towards more Southern latitudes (35-36 °S) receive high solar radiation.

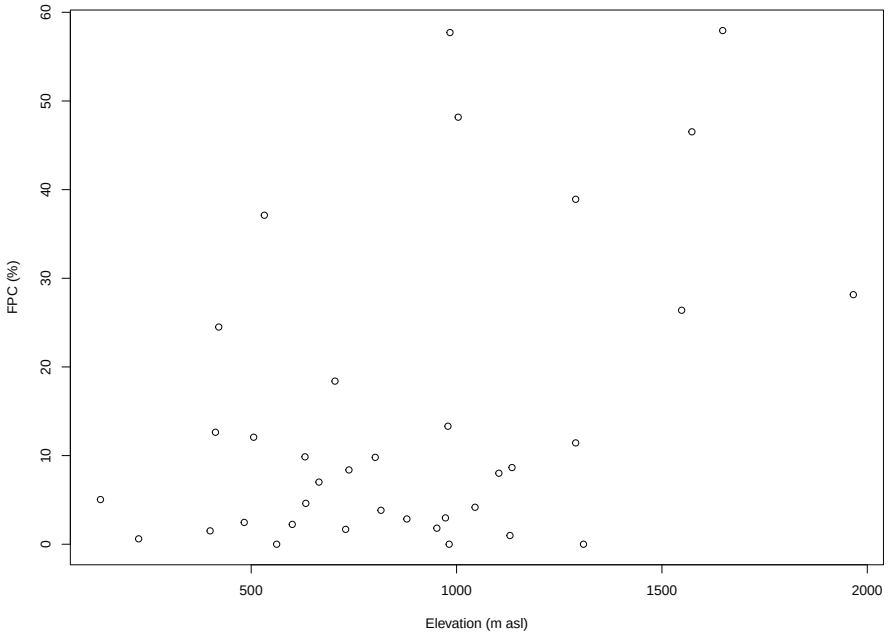


Figure S4: Scatterplot of elevation (m asl) and Foliage Projective Cover (FPC %) indicating sites above 1000 m asl have higher foliage cover than sites below 1000 m asl elevation.

Table S1. Spatial coordinates of sampled sites across the eastern coast of New South Wales and extracted values of environmental variables for each site. Data for mean annual temperature (BIO1) and mean annual precipitation (BIO12) were downloaded for a period of 1960-1990, wind speed and solar radiation data were downloaded for a period of 1970-2000. Lat= Latitude; Long=Longitude; FPC= Foliage Projective Cover (%).

Site ID	Lat (°S)	Long (°S)	Elevation (m asl)	BIO1 (°C)	BIO12 (mm)	Solar radiation ($kJ/m^2/day$)	Wind speed (m/s)	FPC (%)	Soil moisture
1	-29.18	152	802	15	929	23477	3.32	9.8	0
2	-29.64	151.95	1103	13.2	985	23403	3.7	8.01	0
3	-29.78	151.73	1130	13.1	880	23401	3.77	0.98	1
4	-30.23	151.82	1309	11.6	909	23289	3.81	0	1
5	-30.34	152.42	1290	11.8	1402	22899	3.69	11.43	1
6	-30.7	151.47	1045	12.8	813	23740	3.4	4.17	0
7	-31.25	150.91	483	16	722	24134	3.05	2.46	1
8	-31.61	150.78	631	14.9	832	24015	3.14	9.86	1
9	-31.84	150.91	413	15.9	804	24176	3	12.63	0
10	-32.34	150.58	133	17.4	625	24290	2.9	5.04	0
11	-32.61	150.07	421	15.5	657	24727	3.13	24.5	1
12	-33.12	148.91	506	14.4	760	25582	3.61	12.07	1
13	-33.44	149.33	973	11.5	864	25610	3.79	2.97	1
14	-33.42	149.72	816	12.5	703	24956	3.54	3.83	1
15	-33.46	150.12	979	12	901	24589	3.6	13.31	0
16	-33.5	150.39	984	11.8	1298	23811	2.92	57.72	1
17	-33.45	150.6	532	14.4	1309	23866	2.77	37.11	0
18	-33.61	150.12	952	11.8	998	24526	3.58	1.81	1
19	-33.7	149.84	1135	10.7	926	24833	3.81	8.65	1
20	-33.7	149.55	879	11.6	831	25567	3.8	2.85	1
21	-33.84	149.00	738	12.5	905	26021	3.78	8.38	0
22	-34.15	149.75	982	11.1	915	25623	3.8	0	1
23	-34.45	149.2	730	12.4	800	26128	3.99	1.68	0
24	-34.72	149.76	633	13.1	689	25712	3.58	4.61	0
25	-35.05	149.86	600	13	720	25673	3.71	2.24	0
26	-35.51	149.69	665	12.5	942	25703	3.46	7.01	0
27	-35.34	149.28	704	12.4	677	26617	3.99	18.4	0
28	-34.93	149.00	562	13.4	713	26408	3.54	0	0
29	-34.6	148.32	400	14.3	701	26716	3.86	1.51	1
30	-35.11	148.06	226	15.4	710	27074	3.73	0.61	0
31	-35.61	148.09	1004	10.4	1353	26399	4.57	48.17	1
32	-36.05	148.38	1573	6.7	1756	25647	5.1	46.52	0
33	-36.35	148.56	1290	8.5	1289	25798	4.74	38.91	1
34	-36.38	148.37	1648	5.9	1867	25347	5.26	57.94	0
35	-36.50	148.27	1966	4.6	1676	25229	5.38	28.15	0
36	-36.52	148.26	1548	6	1783	25486	5.1	26.39	0

Table S2. Pearson correlation analysis matrix between all environmental variables. Variables with r >0.80 were dropped from the further analysis, except latitude. FPC= Foliage Projective Cover; BIO1= mean annual temperature; BIO12= mean annual precipitation; Lat=latitude and Long= longitude. Final six variables chosen for analysis are indicated in bold.

Variables	**Solar radiation (kJ/m^2/day)**	**Wind speed (m/s)**	**Soil moisture**	**FPC (%)**	BIO1 (°C)	**BIO12 (mm)**	Elevation (m asl)	**Lat (°S)**	Long (°S)
Solar radiation	1.00								
Wind speed	0.41	1.00							
Soil moisture	-0.17	-0.14	1.00						
FPC	0.02	0.43	-0.06	1.00					
BIO1	-0.21	-0.90	0.07	-0.53	1.00				
BIO12	-0.07	0.71	-0.13	0.76	-0.83	1.00			
Elevation	-0.17	0.77	0.02	0.45	-0.91	0.80	1.00		
Latitude	0.81	0.61	-0.23	0.42	-0.58	0.39	0.20	1.00	
Longitude	-0.89	-0.61	0.12	-0.33	0.47	-0.28	-0.10	-0.92	1.00

Table S3. The relative importance is shown for four fixed effects used in the multiple linear regression analysis with relative abundance as a response variable using lmg method (R^2 partitioned by averaging over orders). Foliage Projective Cover (FPC %) shows the highest relative abundance followed by wind speed, soil moisture, and latitude.

Fixed effects	Relative importance (method=Lmg)
FPC (%)	0.57
Wind speed	0.27
Soil moisture	0.09
Latitude	0.07

Table S4. Details of soil type extracted for each site. Soils were classified based on Great Soil Group (GSG) and Australian Soil Classification (ASC) as low moisture (0) and high moisture (1).

Site ID	Latitude (°S)	Soil type (Great Soil Group/Australian Soil classification)	GSG code	Soil Moisture Assignment
1	-29.18	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
2	-29.64	Soloths	SH	0
3	-29.78	Black Earths/Vertosols	BE	1
4	-30.23	Kraznozems	K	1
5	-30.34	Gleyed Podzolic Soils/Hydrosols	GP	1
6	-30.70	Soloths	SH	0
7	-31.25	Red Brown Earths	RBE	1
8	-31.61	Chocolate Soils	C	1
9	-31.84	Soloths	SH	0
10	-32.34	Solodic Soils	SC	0
11	-32.61	Chernozems	CH	1
12	-33.12	Euchrozems	E	1
13	-33.44	Red Earths - less fertile (granites and metasediments)/Kandosols	REl	1
14	-33.42	Non Calcic Brown Soils	NKB	1
15	-33.46	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
16	-33.50	Kraznozems	K	1
17	-33.45	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
18	-33.61	Yellow Earths/Kandosols	YE	1
19	-33.70	Kraznozems	K	1
20	-33.70	Red Podzolic Soils - more fertile (volcanics and granodiorites)	RPm	1

21	-33.84	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
22	-34.15	Chocolate Soils	C	1
23	-34.45	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
24	-34.72	Soloths	SH	0
25	-35.05	Siliceous Sands/Rudosols	SS	0
26	-35.51	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
27	-35.34	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
28	-34.93	Yellow Podzolic Soils - less fertile (granites and metasediments)	YPl	0
29	-34.60	Red Brown Earths	RBE	1
30	-35.11	Red Podzolic Soils - less fertile (granites and metasediments)	RPl	0
31	-35.61	Red Podzolic Soils - more fertile (volcanics and granodiorites)	RPm	1
32	-36.05	Alpine Humus soils	AH	0
33	-36.35	Brown Earths/Dermosols	BRE	1
34	-36.38	Alpine Humus soils	AH	0
35	-36.50	Alpine Humus soils	AH	0
36	-36.52	Lithosols	L	0

Chapter 3 Supplementary material: Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (*Phaulacridium vittatum*)

Sonu Yadav, Adam J. Stow, Rachael Y. Dudaniec

Text S1. DArT extraction, SNP calling, filtering and quality check

To call the SNPs, DNA sequences were aligned via BLAST using the *Locusta migratoria* reference genome with an E-value: 5e-3 and minimum sequence identity of >70%. To check for contamination, sequences were also aligned to bacterial and fungal genomes (NCBI). Furthermore, to call SNPs all tags from all libraries were included in DArTsoft14 proprietary software and were clustered using DArT PL's C++ algorithm at the threshold distance of 3. This was followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were based on analysis of approximately 1,000 controlled cross populations. Technical parameters to call true allelic variants from paralogous sequences were facilitated by testing for Mendelian distribution of alleles in these populations. In addition multiple samples were processed from DNA to allelic calls as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers

DNA extraction was performed using the automated DNA extraction protocol on a 'TECAN freedom evo 100' liquid handling robot with a commercially available magnetic bead-based DNA extraction kit (Macherey-Nagel). DNA digestion and ligation reactions were performed as per Kilian et al. (2012). Digested and ligated samples were amplified for 30 cycles using the following PCR conditions: 94°C for 1 min, 94°C for 20 sec, 58°C for 30 sec, 72°C for 45 sec, 72°C for 7 min.

Proprietary analytical pipelines (DArTsoft14 – DArT's proprietary software) were used to process sequences generated from each lane. After quality filtering, approximately 2,500,000 (± 7%) sequences per sample were retained. DArT uses a reproducibility score (i.e. the proportion

of technical replicate assay pairs for which the marker score is consistent) and polymorphism information content (PIC: an index ranging from zero to one and inform allele variations of SNP marker) to assess the quality and information content of SNP calls. The threshold for the reproducibility score is set to 97%. Further quality control was undertaken by removing any bacterial or viral contaminant sequences using alignment to matching sequences within the GenBank and DArT databases.

Text S2. Justification of environmental variables used in EAA

Temperature and precipitation significantly affect the distribution, morphological variation and body size of *P. vittatum* (Harris et al., 2012, Harris et al., 2013). Wind speed can be relevant for insect passive dispersal (Anderson et al., 2010, Drake, 1994, Kennedy and Storer, 2000) and local adaptation (Dudaniec et al., 2018), and have a positive effect on the relative abundance of *P. vittatum*, whereby sites with high wind speed support higher site relative abundances (Yadav et al., 2018).

Furthermore, foliage cover and habitat type are known to influence dispersal, thermoregulation, and oviposition site selection in *P. vittatum* (Clark, 1967). Soil acidity and soil moisture were chosen because the physical properties of soil have been shown to have substantial effects on the survival and density of grasshoppers (Miao et al., 2018). This is likely due to the role of soil in determining the distribution of preferable food sources (e.g., particular plants), suitable oviposition sites for egg development (Miao et al., 2018), population growth (Macfadyen et al., 2018) and the distribution of nematode parasites (Barbercheck, 1992). Yadav et al. (2018) also found that soil moisture influenced site relative abundance of *P. vittatum* along the identical gradient sampled in the current study.

TABLE S1: Details of environmental variables used in LFMM analysis, shown per site. BIO1= Mean annual temperature; BIO18= mean precipitation of the warmest quarter; FPC= Foliage Projective Cover (%). Soil Moisture: 0= Low; 1= High to intermediate. Soil Acidity: 1= Acidic (pH <5.5), 0= Non- acidic (pH > 5.5). Soil moisture and soil acidity values were assigned to sites-based information from Great soil group and Australian Soil Classification.

Site ID	Latitude (°S)	Longitude (°S)	BIO1 (°C)	BIO18 (mm)	Wind speed (m/s)	FPC (%)	Soil Moisture	Soil Acidity
1	-29.18	152.00	15	365	3.32	9.8	0	1
2	-29.64	151.95	13.2	383	3.70	8.01	0	1
3	-30.23	151.82	11.6	342	3.81	0	1	1
4	-30.70	151.47	12.8	293	3.40	4.17	0	1
5	-31.25	150.91	16	254	3.05	2.46	1	0
6	-31.84	150.91	15.9	279	3.00	12.63	0	1
7	-32.34	150.58	17.4	238	2.90	5.04	0	0
8	-32.61	150.07	15.5	229	3.13	24.5	1	0
9	-33.42	149.72	12.5	209	3.54	3.83	1	0
10	-33.50	150.39	11.8	434	2.92	57.72	1	1
11	-33.84	149.00	12.5	209	3.78	8.37	0	1
12	-34.15	149.75	11.1	243	3.80	0	1	1
13	-34.60	148.32	14.3	157	3.86	1.51	1	0
14	-34.72	149.76	13.1	186	3.58	4.61	0	1
15	-35.11	148.06	15.4	147	3.73	0.61	0	1
16	-35.51	149.69	12.5	246	3.46	7.01	0	1
17	-35.61	148.09	10.4	220	4.57	48.17	1	0
18	-36.05	148.38	6.7	270	5.10	46.52	0	1
19	-36.52	148.26	6	283	5.10	26.39	0	1

Table S2: Pearson correlation analysis between variables used in the study. BIO1=Annual mean temperature, BIO12= Annual precipitation; BIO10= Mean temperature of the warmest quarter; BIO11= Mean temperature of coldest quarter; BIO18= mean precipitation of the warmest quarter, BIO19= Precipitation of the coldest quarter extracted from the WorldClim v1.4 database (Hijmans et al., 2005). Wind speed and Solar Radiation (SR) data was extracted from Worldclim v2.0 (Fick & Hijmans, 2017). FPC= Foliage Projective Cover (%). SoMo =Soil moisture and SoAc= soil acidity. Variables with a correlation **>0.80** were removed from further analysis except for wind speed. Seven environmental variables and three morphological variables used in further analyses are indicated in bold.

Variable	**Striped**	**Winged**	**Femur length**	**Latitude**	Longitude	Elevation	**BIO1**	BIO12	BOI10	BIO11	**BIO18**	BIO19	**Wind speed**	SR	**FPC**	**SoMo**
Striped	1															
Winged	-0.22	1														
Femur length	0.01	0.04	1													
Latitude	-0.08	-0.2	-0.47	1												
Longitude	-0.16	-0.09	-0.42	0.93	1											
Elevation	-0.06	0.21	-0.12	-0.11	-0.02	1										
BIO1	0.01	-0.26	-0.11	0.54	0.44	-0.89	1									
BIO12	-0.09	0.33	0.12	-0.45	-0.37	0.8	-0.88	1								
BOI10	0.06	-0.29	-0.07	0.43	0.29	-0.94	0.98	-0.88	1							
BIO11	-0.02	-0.23	-0.13	0.6	0.53	-0.85	0.99	-0.87	0.96	1						
BIO18	-0.22	0.25	-0.23	0.55	0.67	0.48	-0.15	0.35	-0.3	-0.06	1					
BIO19	0.01	0.23	0.19	-0.65	-0.63	0.68	-0.87	0.93	-0.81	-0.89	0.01	1				
Wind speed	0.07	0.06	0.1	-0.58	-0.62	0.7	-0.86	0.77	-0.78	-0.89	-0.17	0.88	1			
SR	0.19	-0.02	0.42	-0.85	-0.93	-0.18	-0.24	0.11	-0.08	-0.32	-0.81	0.4	0.48	1		
FPC	-0.05	0.4	0.21	-0.38	-0.32	0.42	-0.5	0.74	-0.49	-0.5	0.35	0.68	0.34	0.05	1	
SoMo	0.2	0	-0.04	0	-0.06	-0.03	0.04	-0.14	0.07	0.01	-0.03	-0.11	-0.12	0.01	0.14	1
SoAc	-0.16	0.16	0.07	0.05	0.2	0.44	-0.35	0.32	-0.43	-0.28	0.43	0.16	0.15	-0.17	0	-0.59

Table S3: Description of outliers identified by OutFLANK (Whitlock & Lotterhos, 2015) with P-values. Fst= locus based Fst calculated by OutFLANK.

SNP ID	He	Fst	P-values
3014	0.38	0.49	<0.0001
3197	0.11	0.41	<0.0001
8998	0.4	0.44	<0.0001

Table S4: Description of 17 Fst outliers identified by BayeScan (Foll & Gaggiotti, 2008) at Q value (qval) threshold = 0.05; one outlier is detected under putative balancing selection (in bold) while all other outliers are under putative divergent selection.

SNP ID	Probability	qval	Locus Fst
1354	0.85	0.04	0.22
2340	1.00	0.00	0.27
2341	1.00	0.00	0.31
3014	1.00	0.00	0.44
3197	0.92	0.01	0.34
3885	1.00	0.00	0.30
4737	0.83	0.05	0.22
7060	0.90	0.02	0.23
8998	1.00	0.00	0.37
9459	1.00	0.00	0.33
9703	0.98	0.00	0.22
9890	1.00	0.00	0.37
9993	0.88	0.03	0.27
10534	0.96	0.01	0.32
10677	0.98	0.00	0.26
11345	1.00	0.00	0.35
8981	**0.89**	**0.03**	**0.02**

Table S5: Pairwise Fst distance matrix generated from Arlequin (Excoffier & Lischer, 2010) among 19 sampled sites for *P. vittatum*. The Fst distance ranged from 0.0003 - 0.08. Significant difference shown in bold; significance level= 0.05.

Sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	-																	
2	0.02																	
3	**0.05**	**0.04**																
4	**0.03**	0.03	0.03															
5	0.03	0.03	**0.04**	0.03														
6	**0.05**	**0.05**	**0.06**	**0.04**	0.03													
7	**0.04**	0.03	**0.05**	**0.04**	0.03	0.04												
8	**0.06**	**0.06**	**0.08**	**0.06**	**0.05**	**0.06**	0.05											
9	**0.05**	**0.04**	**0.04**	0.03	0.02	**0.05**	**0.03**	**0.05**										
10	**0.04**	0.02	0.03	0.03	0.03	**0.05**	0.02	**0.05**	0.02									
11	**0.04**	**0.03**	0.03	0.02	0.03	**0.04**	0.03	**0.05**	0.02	0.03								
12	**0.06**	**0.03**	0.03	0.03	**0.05**	**0.06**	**0.05**	**0.07**	**0.04**	**0.04**	0.03							
13	0.00	0.00	0.01	-0.01	-0.02	0.00	-0.02	0.01	-0.01	0.00	0.00	0.01						
14	0.03	0.01	0.01	0.00	0.01	0.02	0.02	0.04	0.00	-0.01	0.00	0.00	0.04					
15	0.03	0.02	0.01	0.01	0.01	0.03	0.02	0.04	0.01	0.01	0.02	0.02	0.03	0.03				
16	**0.04**	0.02	0.02	0.02	0.02	0.04	0.02	**0.05**	0.02	0.02	0.01	0.02	0.03	0.02	0.03			
17	0.01	0.00	0.00	-0.01	0.00	0.01	-0.02	0.02	-0.02	-0.02	-0.01	-0.01	0.04	0.03	0.05	0.03		
18	**0.04**	0.03	0.03	0.02	**0.04**	**0.05**	0.04	**0.07**	0.02	0.02	0.03	0.02	0.03	0.03	**0.05**	**0.04**	0.04	
19	**0.07**	**0.06**	**0.05**	**0.04**	**0.05**	**0.06**	**0.05**	**0.06**	0.04	0.04	0.03	**0.04**	**0.05**	0.03	0.05	**0.05**	0.03	**0.06**

Table S6. Annotation of four outlier loci identified by environment association analysis (EAA) only to the *Locusta migratoria* genome, using the NCBI ntBLAST search and E-value threshold of 0.0001. GluR= Glutamate receptor; iGluR= Inotropic glutamate receptor; GT= Glucosyl glucuronosyl transferases; BIO1= mean annual temperature.

SNP ID	Fst	SNP LFMM association	Product annotation	E-value	GenBank Accession number
5439	0.184	Latitude	GluR 3	2E-24	KP843222.1
7776	0.176	Body size	iGluR 3	6E-05	KP843204.1
7777	0.165	Body size	iGluR 3	6E-05	KP843204.1
8890	0.246	BIO1	GT	8E-10	KC118981.1

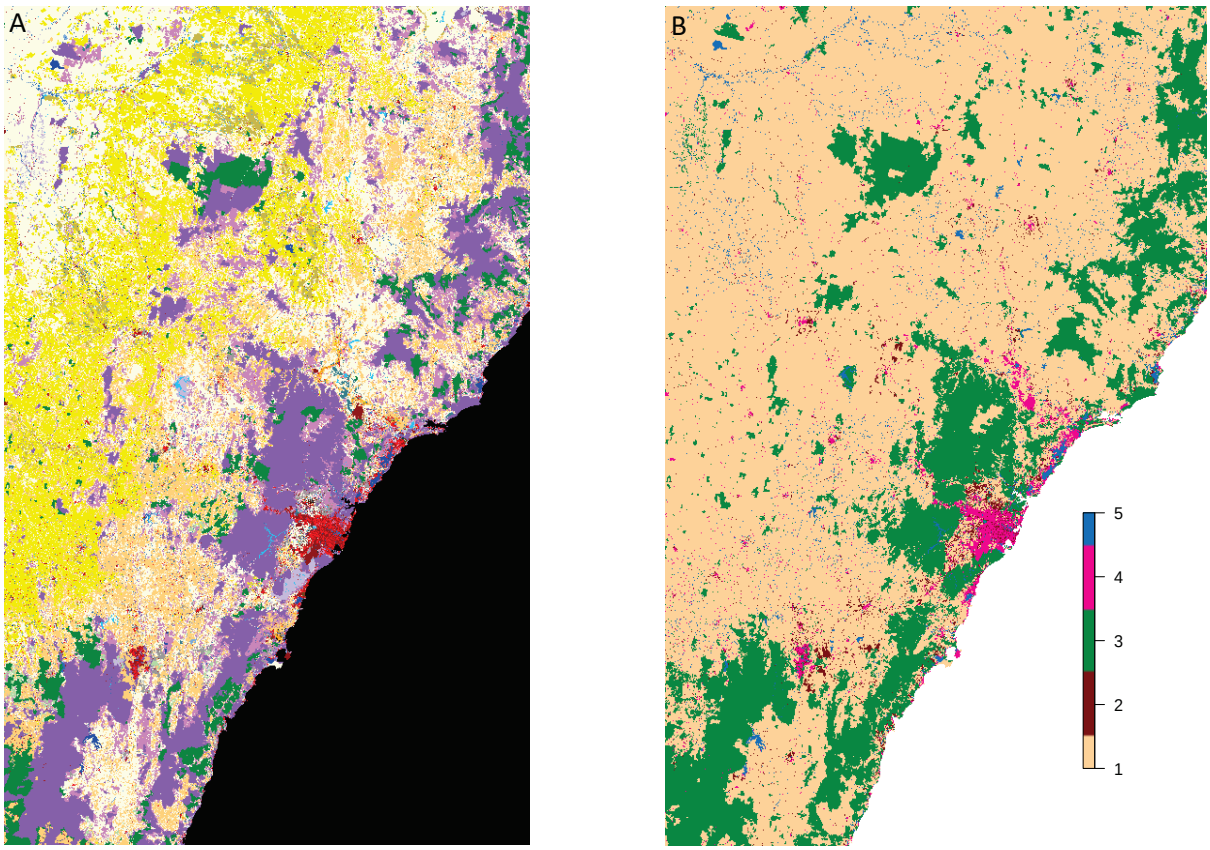


Figure S1. Landcover surfaces: (A) untransformed original raster layer downloaded from the Department of Agriculture and Water Resources, Australian Bureau of Agricultural and Resource Economics and Sciences, Land use management (ABARES 2017); (B) recategorized raster layer used for landscape resistance modeling analysis; 1= agricultural areas, grazing areas and pasture, 2= rural infrastructure, 3= forest and conservation areas, 4= urban infrastructure, 5= water bodies.

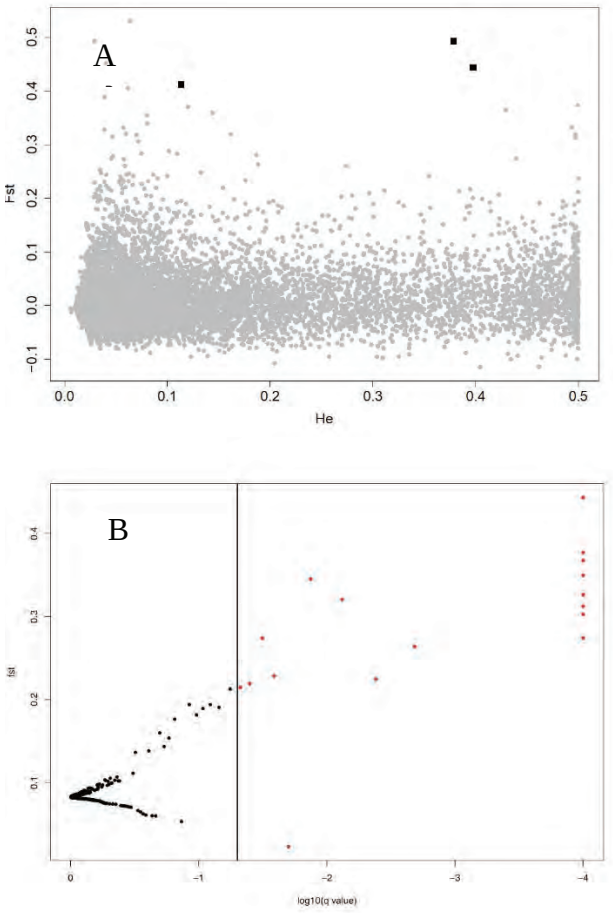


FIGURE S2. Outliers detected by Fst outlier methods. (A) Three outliers detected by OutFLANK with false discovery rate of ≤5% and expected heterozygosity ≥10%; (B) Outliers identified by BayeScan with the vertical line indicating the log10(q value) significance cut-off and red points indicating significant Fst outliers.

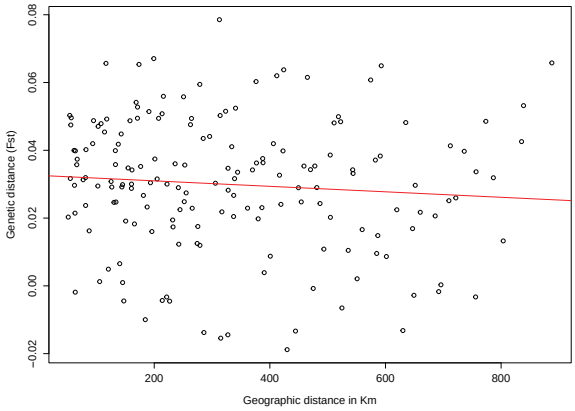


FIGURE S3: Scatterplot showing no isolation by distance (*P* value= 0.79, Mantel statistic *r* = -0.080) between pairwise Fst and geographic distance in km using the Mantel test in VEGAN R package (Oksanen et al., 2013).

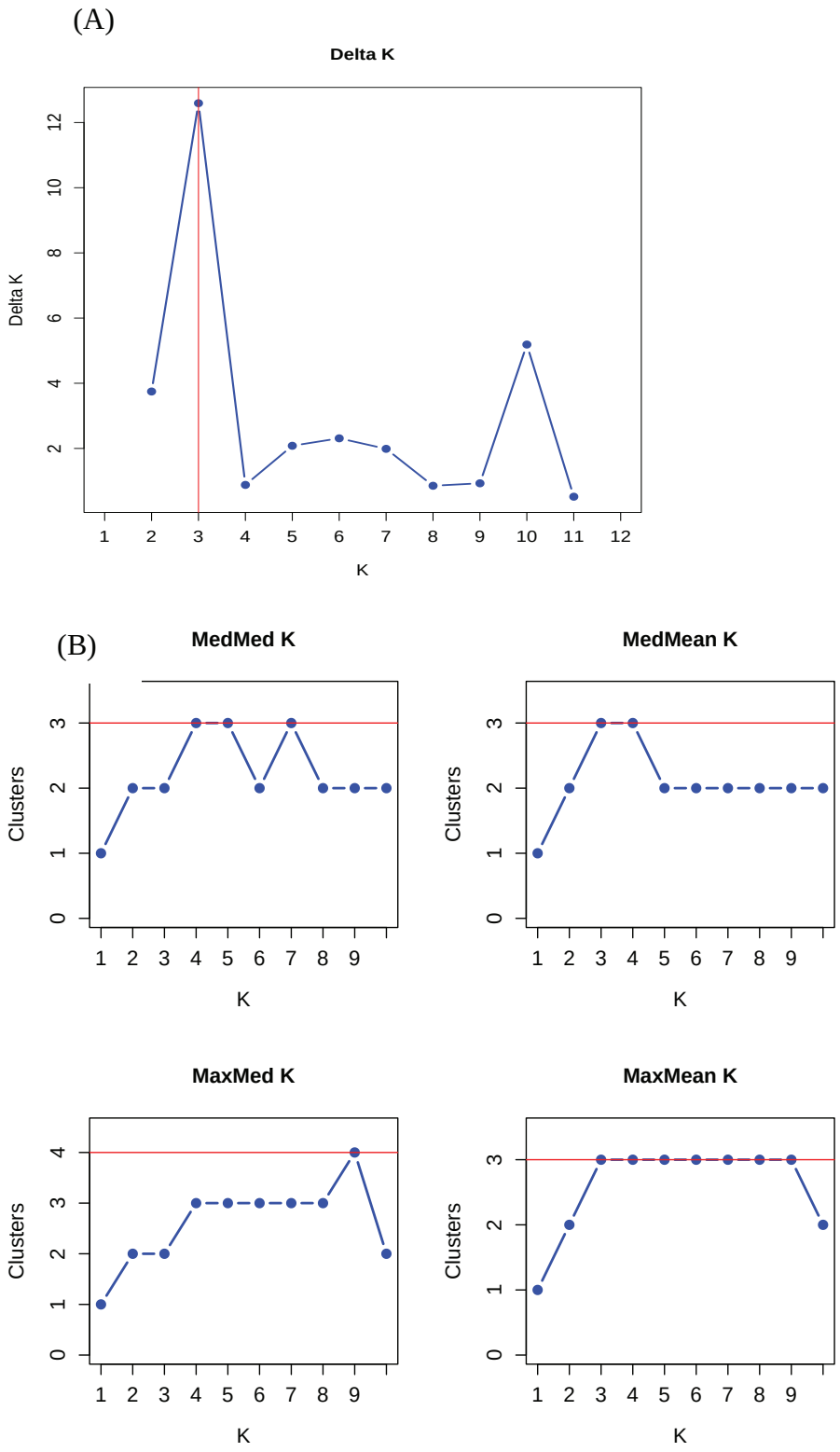


FIGURE S4: (A) Evanno plot indicating *K*=3 best fit the data based on delta K. (B) The robustness of detection of optimal K was also tested using other four estimators of STRUCTURESELECTOR (Li and Liu, 2018). The optimal K is indicated by the red line on Y-axis.

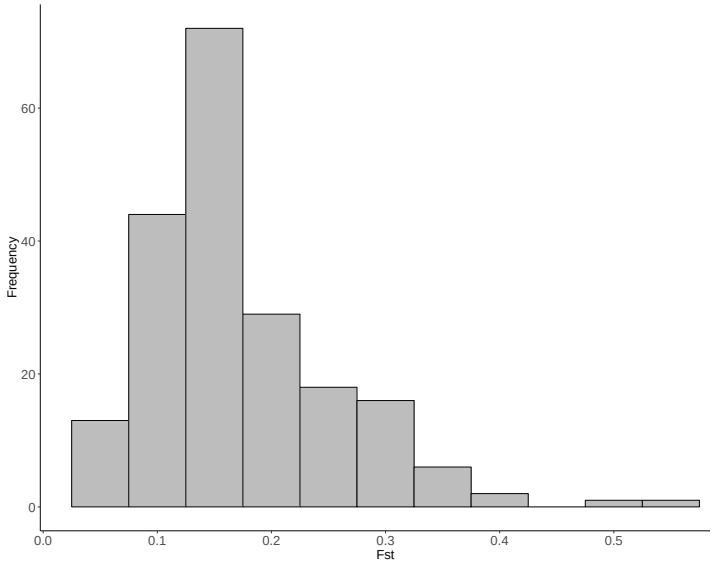


FIGURE S5. Fst distribution of outliers identified by LFMM (Frichot et al. 2013) ranged from 0.05-0.54 (mean= 0.17 ± 0.01).

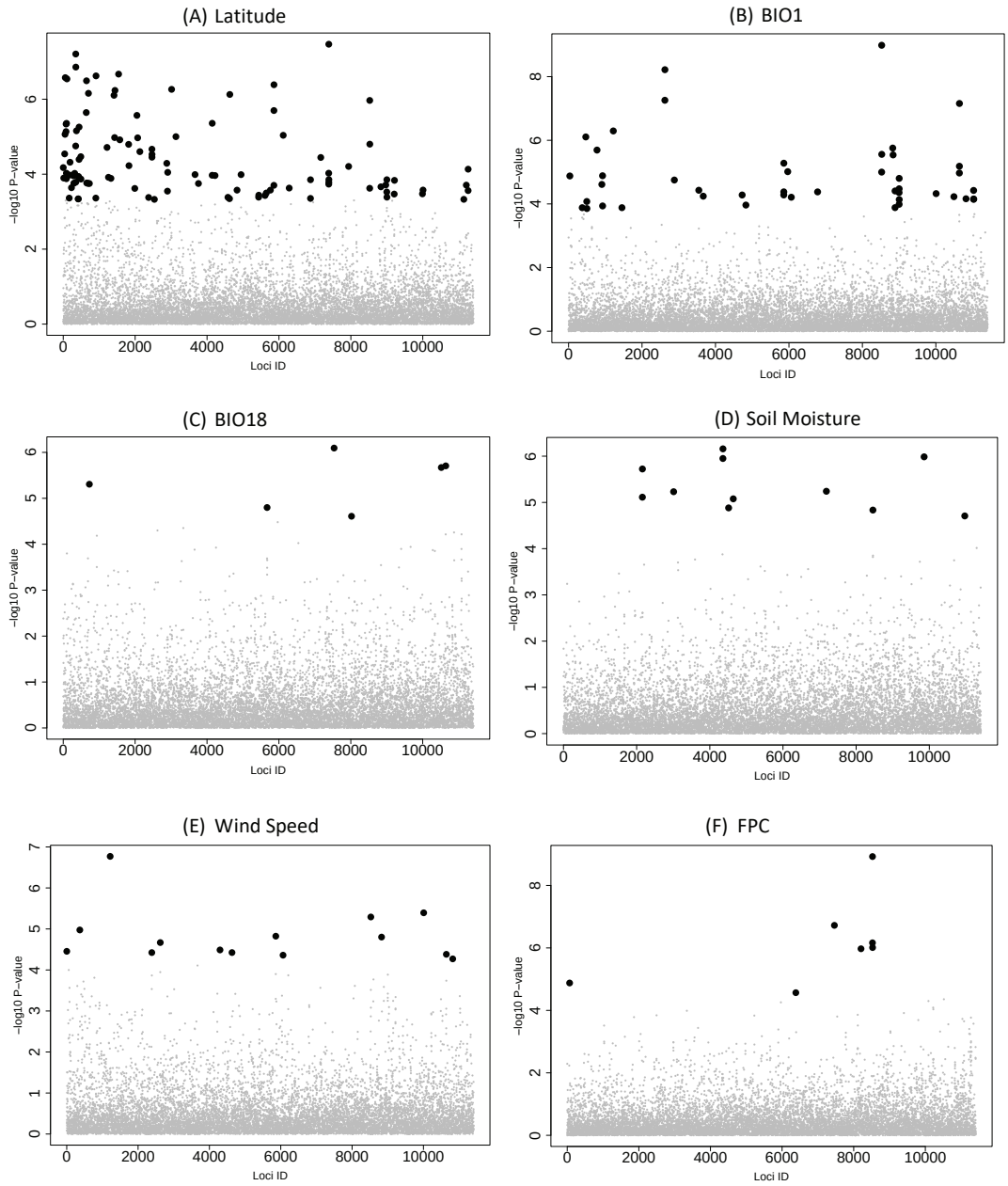


Figure S6: Outliers putatively associated with environmental variables in LFMM (Frichot et al. 2013) implemented in LEA R package (Frichot and Francois 2015). Associations are shown for variables: (A) Latitude, (B) Mean Annual Temperature (BIO1), (C) Precipitation of the warmest quarter (mm, BIO18), (D) Soil moisture, (E) Wind speed, (F) Foliage Projective Cover (FPC %). Significant outliers shown in black and insignificant SNPs in grey. X-axis= Loci ID, Y-axis= –log10 P-value threshold.

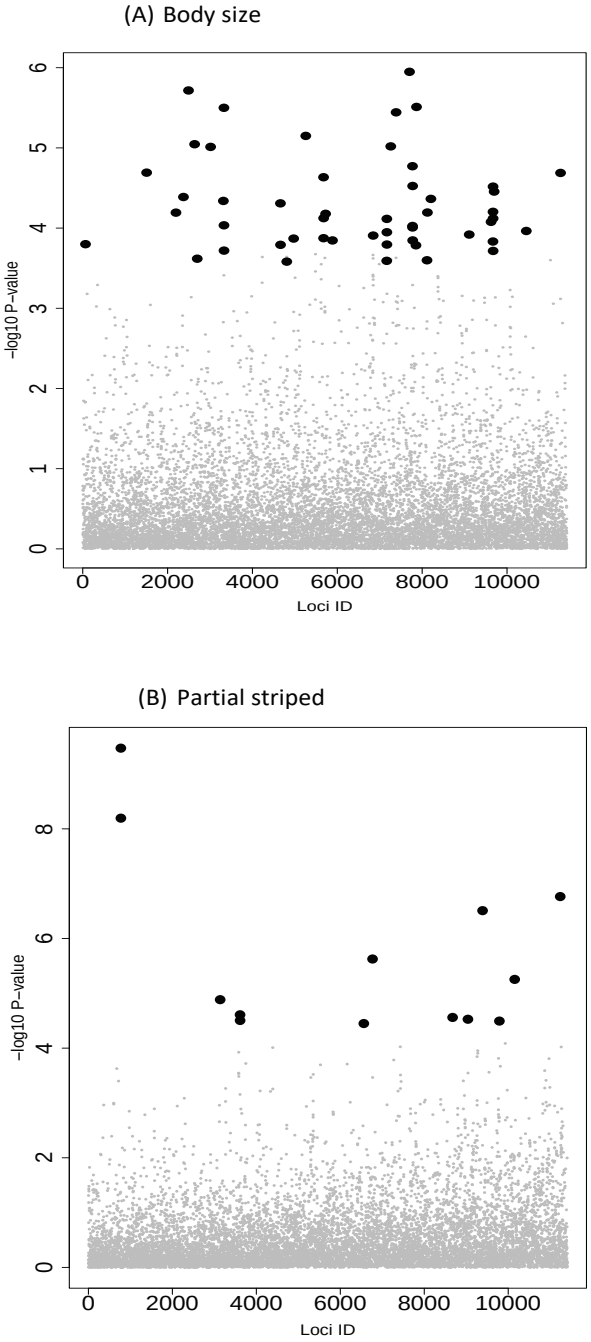
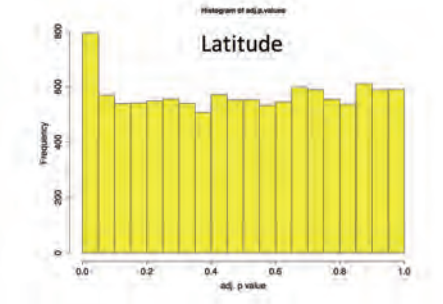
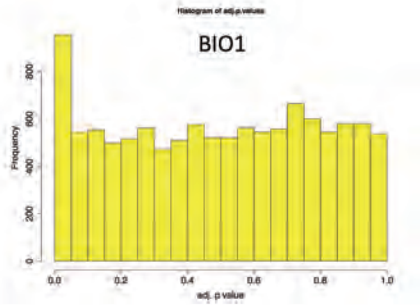
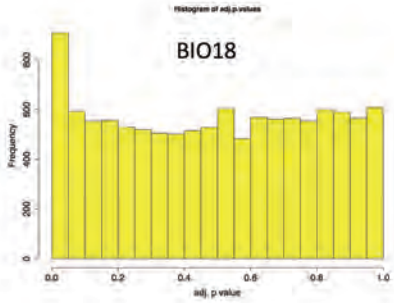
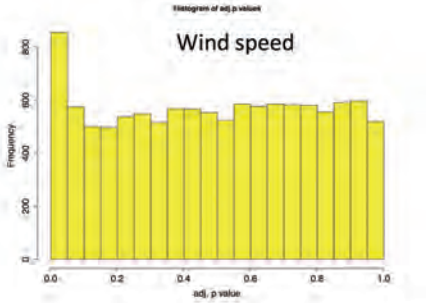


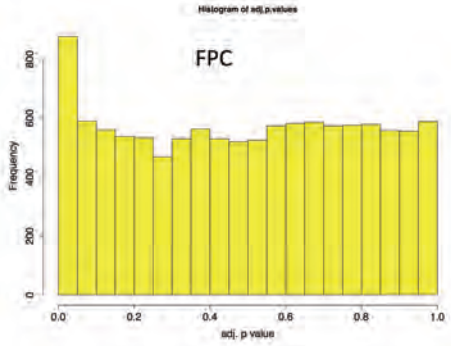
Figure S7: Outliers detected to be associated with morphological variables using LFMM (Frichot et al. 2013) implemented in LEA R package (Frichot and Francois 2015). Associations are shown for variables: (A) Body size (measured as femur length), (B) Partial striped pattern. Significant outliers shown in black and insignificant SNPs in grey. X-axis= Loci ID, Y-axis= –log10 P-value threshold.

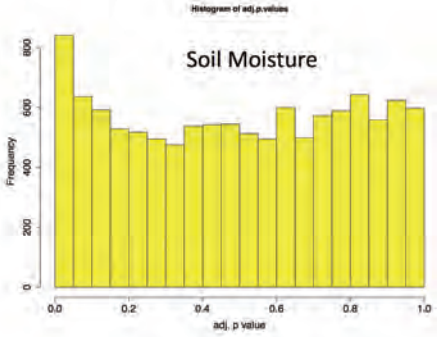

Latitude
Frequency
adj. p value

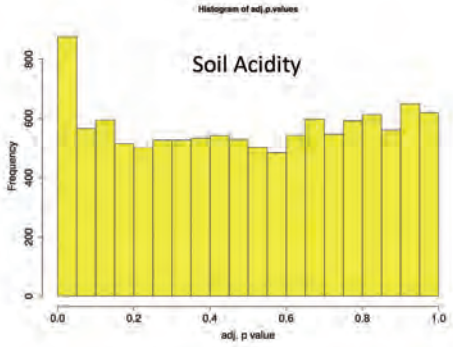

BIO1
Frequency
adj. p value

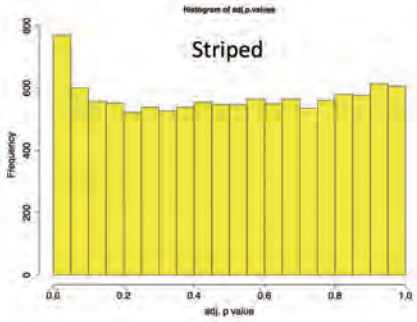

BIO18
Frequency
adj. p value


Wind speed
Frequency
adj. p value


FPC
Frequency
adj. p value


Soil Moisture
Frequency
adj. p value


Soil Acidity
Frequency
adj. p value


Striped
Frequency
adj. p value

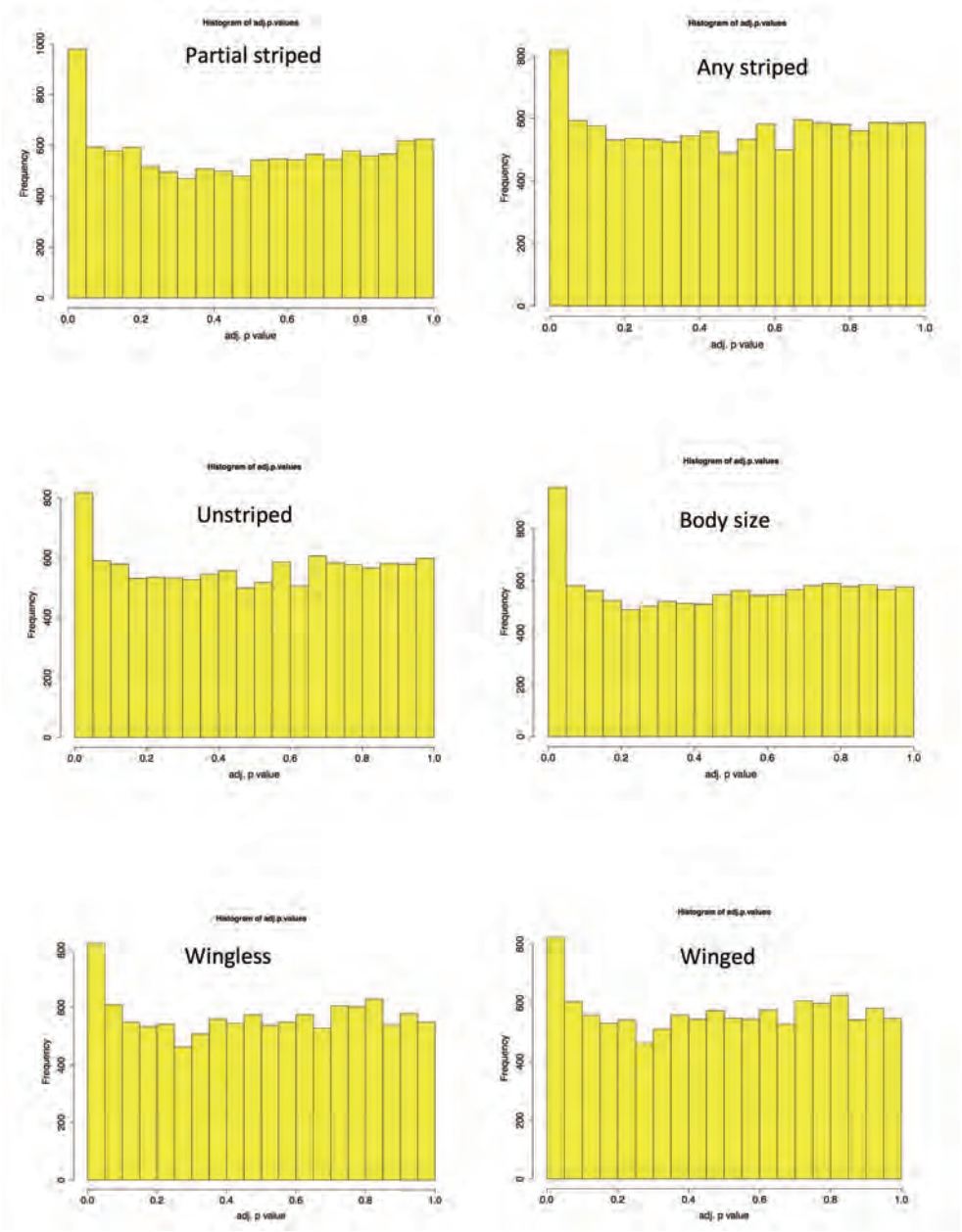


Figure S8: The P-value distributions of environmental and morphological variables tested in LFMM analysis. BIO1= Mean Annual Temperature, BIO18= Precipitation of the warmest quarter (mm), FPC= Foliage Projective Cover (%).

References cited in Supplementary material

ANDERSON, K. L., DEVESON, T. E., SALLAM, N. & CONGDON, B. C. 2010. Wind-assisted migration potential of the island sugarcane planthopper *Eumetopina flavipes* (Hemiptera: Delphacidae): implications for managing incursions across an Australian quarantine frontline. *Journal of Applied Ecology,* 47**,** 1310-1319.

BARBERCHECK, M. E. 1992. Effect of soil physical factors on biological-control agents of soil insect pests. *Florida Entomologist,* 75**,** 539-548.

CLARK, D. P. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology,* 15**,** 799-872.

DEARN, J. M. 1984. Colour pattern polymorphism in the grasshopper *Phaulacridium Vittatum.* I. Geographic variation in Victoria and evidence of habitat association. *Australian journal of zoology,* 32**,** 239-249.

DRAKE, V. A. 1994. The influence of weather and climate on agriculturally important insects - an Australian perspective. *Australian Journal of Agricultural Research,* 45**,** 487-509.

DUDANIEC, R. Y., YONG, C. J., LANCASTER, L. T., SVENSSON, E. I. & HANSSON, B. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Mol Ecol,* 27**,** 2576-2593.

HARRIS, R., MCQUILLAN, P. & HUGHES, L. 2012. Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *Journal of Biogeography,* 39**,** 1450-1461.

HARRIS, R. M. B., MCQUILLAN, P. & HUGHES, L. 2013. A test of the thermal melanism hypothesis in the wingless grass-hopper *Phaulacridium vittatum*. *Journal of insect science,* 13.

KENNEDY, G. G. & STORER, N. P. 2000. Life systems of polyphagous arthropod pests in temporally unstable cropping systems. *Annu Rev Entomol,* 45**,** 467-93.

KILIAN, A., WENZL, P., HUTTNER, E., CARLING, J., XIA, L., BLOIS, H., CAIG, V., HELLER-USZYNSKA, K., JACCOUD, D. & HOPPER, C. 2012. Diversity arrays technology: a generic genome profiling technology on open platforms. *Data production and analysis in population genomics.* Springer.

LI, Y. L. & LIU, J. X. 2018. StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular ecology resources,* 18**,** 176-177.

MACFADYEN, S., MCDONALD, G. & HILL, M. P. 2018. From species distributions to climate change adaptation: Knowledge gaps in managing invertebrate pests in broad-acre grain crops. *Agriculture Ecosystems & Environment,* 253**,** 208-219.

MIAO, H. T., LIU, Y., SHAN, L. Y. & WU, G. L. 2018. Linkages of plant-soil interface habitat and grasshopper occurrence of typical grassland ecosystem. *Ecological Indicators,* 90**,** 324-333.

YADAV, S., STOW, A. J., HARRIS, R. M. B. & DUDANIEC, R. Y. 2018. Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae). *J Insect Sci,* 18**,** 13.

Chapter 4 Supplementary material: Elevational partitioning in species distribution, abundance and body size in Australian alpine grasshoppers (*Kosciuscola*)

Sonu Yadav, Adam Stow, Rachael Y. Dudaniec

Table S1: Sample site information of *K. usitatus* with the number of individuals collected.

Latitude	Longitude	Region	Elevation	Individuals collected
-36.50037	148.30493	Thredbo	1413.51	22
-36.52205	148.26382	Thredbo	1548.53	28
-36.52415	148.26375	Thredbo	1550.96	10
-36.52738	148.26712	Thredbo	1599.58	46
-36.52044	148.26405	Thredbo	1599.71	32
-36.51909	148.26413	Thredbo	1649.99	5
-36.49474	148.29539	Thredbo	1671.56	17
-36.51681	148.2645	Thredbo	1696.61	18
-36.51267	148.26649	Thredbo	1741.03	17
-36.51912	148.25587	Thredbo	1746.12	29
-36.51498	148.25266	Thredbo	1795.61	28
-36.51041	148.26857	Thredbo	1797.42	16
-36.49192	148.29214	Thredbo	1801.71	2
-36.51372	148.25159	Thredbo	1844.74	12
-36.50668	148.27151	Thredbo	1849.29	7
-36.49326	148.28958	Thredbo	1868.84	5
-36.51228	148.25078	Thredbo	1889.57	5
-36.50432	148.27248	Thredbo	1895.72	13
-36.50193	148.2729	Thredbo	1937.04	14
-36.50948	148.25044	Thredbo	1940.47	17
-36.50848	148.24933	Thredbo	1989.78	3
-36.37323	148.36711	Guthega	1590.61	30
-36.38229	148.37073	Guthega	1599.92	16
-36.38437	148.36865	Guthega	1636.44	3
-36.37298	148.36568	Guthega	1648.05	28
-36.38358	148.3727	Guthega	1668.87	11
-36.37273	148.36449	Guthega	1688.4	11
-36.38343	148.37384	Guthega	1693.55	13
-36.37831	148.37944	Guthega	1699.1	4
-36.37262	148.36298	Guthega	1728.16	23
-36.38319	148.37537	Guthega	1731.58	8
-36.36901	148.36368	Guthega	1734.32	10
-36.36798	148.36267	Guthega	1766.99	21
-36.3828	148.37755	Guthega	1788.41	2
-36.3828	148.38217	Guthega	1797.15	2
-36.37821	148.38403	Guthega	1845.96	18
-36.38046	148.383	Guthega	1846.76	3
-36.04676	148.37598	Jagungal	1548.29	6

-36.03489	148.37399	Jagungal	1589.09	2
-36.15094	148.37041	Jagungal	1648.02	13
-36.14683	148.37373	Jagungal	1681.92	12
-36.14052	148.38283	Jagungal	1748.78	8
-36.14643	148.38187	Jagungal	1836.69	2

Table S2: Sample site information of *K. tristis* across three mountains.

Latitude	Longitude	Region	Elevation	Individuals collected
-36.50037	148.30493	Thredbo	1413.51	1
-36.5205	148.26352	Thredbo	1595.92	11
-36.5205	148.26712	Thredbo	1599.58	4
-36.52044	148.26405	Thredbo	1599.71	3
-36.51939	148.2636	Thredbo	1636.23	7
-36.51909	148.26413	Thredbo	1649.99	7
-36.51681	148.2645	Thredbo	1696.61	11
-36.51041	148.26857	Thredbo	1797.42	21
-36.49192	148.29214	Thredbo	1801.71	4
-36.50668	148.27151	Thredbo	1849.29	31
-36.49326	148.28958	Thredbo	1868.84	5
-36.50432	148.27248	Thredbo	1895.72	41
-36.50193	148.2729	Thredbo	1937.04	25
-36.37298	148.36568	Guthega	1648.05	11
-36.37273	148.36449	Guthega	1688.4	12
-36.37262	148.36298	Guthega	1728.16	18
-36.36901	148.36368	Guthega	1734.32	5
-36.37884	148.38058	Guthega	1743.92	1
-36.51912	148.25587	Guthega	1746.12	2
-36.36798	148.36267	Guthega	1766.99	12
-36.51498	148.25266	Guthega	1795.61	13
-36.37883	148.38217	Guthega	1797.15	1
-36.51372	148.25159	Guthega	1844.74	40
-36.51228	148.25078	Guthega	1889.57	8
-36.50948	148.25044	Guthega	1940.47	42
-36.50848	148.24933	Guthega	1989.78	26
-36.1439	148.38594	Jagungal	1891.22	2

Table S3: Sample site information of *K. cognatus* across three mountains

Latitude	Longitude	Region	Elevation	Individuals collected
-36.50037	148.30493	Thredbo	1413.51	5
-36.52415	148.26375	Thredbo	1550.96	19
-36.49474	148.29539	Thredbo	1671.56	7
-36.38229	148.37073	Guthega	1599.92	2
-36.38437	148.36865	Guthega	1636.44	4
-36.37757	148.3781	Guthega	1641.8	26
-36.37298	148.36568	Guthega	1648.05	4
36.38358	148.3727	Guthega	1668.87	7
-36.38343	148.37384	Guthega	1693.55	17
-36.37831	148.37944	Guthega	1699.1	26
-36.38319	148.37537	Guthega	1731.58	32
-36.37884	148.38058	Guthega	1743.92	17
-36.3828	148.37755	Guthega	1788.41	32
-36.37883	148.38217	Guthega	1797.15	17
-36.37821	148.38403	Guthega	1845.96	15
-36.38046	148.383	Guthega	1846.76	40
-36.04676	148.37598	Jagungal	1548.29	3
-36.03489	148.37399	Jagungal	1589.09	15
-36.15094	148.37041	Jagungal	1648.02	3
-36.14683	148.37373	Jagungal	1681.92	7
-36.14052	148.38283	Jagungal	1748.78	7
-36.14643	148.38187	Jagungal	1836.69	9
-36.1439	148.38594	Jagungal	1891.22	16

Table S4: Pearson correlation between tested variables. Precipitation = Annual rainfall; Temperature = Maximum summer temperature; FPC= Foliage Projective Cover.

	Precipitation	**Temperature**	**Elevation**	**FPC**
Precipitation	1.00			
Temperature	-0.93	1.00		
Elevation	0.91	-0.98	1.00	
FPC	-0.07	0.19	-0.25	1.00

Table S5: Correlations between the relative abundances (RA) of three species with Foliage Projective Cover (FPC %).

Species RA vs FPC	Estimate ± SE	Chisq	DF	P
K. usitatus	0.22±0.07	2.7	1	0.10
K. tristis	0.13±0.09	0.05	1	0.80
K. cognatus	0.27±0.09	4.7	1	0.03

Chapter 5 Supplementary material: Selection signatures associate with elevational niche in two co-occurring alpine grasshoppers (genus *Kosciuscola*)

Sonu Yadav, Adam J. Stow, Rachael Y. Dudaniec

Text S1. DArT extraction, SNP calling, filtering and quality check

To call the SNPs, DNA sequences were aligned via BLAST using the *Locusta migratoria* reference. To check for contamination, sequences were also aligned to bacterial and fungal genomes (NCBI). Furthermore, to call SNPs all tags from all libraries were included in DArTsoft14 proprietary software and were clustered using DArT PL's C++ algorithm at the threshold distance of 3. This was followed by parsing of the clusters into separate SNP loci using a range of technical parameters, especially the balance of read counts for the allelic pairs. Additional selection criteria were based on analysis of approximately 1,000 controlled cross populations. Technical parameters to call true allelic variants from paralogous sequences were facilitated by testing for Mendelian distribution of alleles in these populations. In addition multiple samples were processed from DNA to allelic calls as technical replicates and scoring consistency was used as the main selection criteria for high quality/low error rate markers

DNA extraction was performed using the automated DNA extraction protocol on a 'TECAN freedom evo 100' liquid handling robot with a commercially available magnetic bead-based DNA extraction kit (Macherey-Nagel). DNA digestion and ligation reactions were performed as per Kilian et al. (2012). Digested and ligated samples were amplified for 30 cycles using the following PCR conditions: 94°C for 1 min, 94°C for 20 sec, 58°C for 30 sec, 72°C for 45 sec, 72°C for 7 min.

Proprietary analytical pipelines (DArTsoft14 – DArT's proprietary software) were used to process sequences generated from each lane. After quality filtering, approximately 2,500,000 (± 7%) sequences per sample were retained. DArT uses a reproducibility score (i.e. the proportion

of technical replicate assay pairs for which the marker score is consistent) and polymorphism information content (PIC: an index ranging from zero to one and inform allele variations of SNP marker) to assess the quality and information content of SNP calls. The threshold for the reproducibility score is set to 97%. Further quality control was undertaken by removing any bacterial or viral contaminant sequences using alignment to matching sequences within the GenBank and DArT databases.

Table S1: Details of outliers detected under selection by BayeScan for both the species.

K. usitatus			
SNP number	Probability	qval	Fst
1848	0.98	0.01	0.16
3679	1.00	0.00	0.23
4517	1.00	0.00	0.21
4561	0.97	0.01	0.16
5373	0.82	0.05	0.15
K. tristis			
SNP number	Probability	qval	Fst
3616	0.99	0.01	0.28
4375	1.00	0.00	0.29

Table S2. Variance inflation factor (VIF) of predictor variables used to run RDA and pRDA analysis. FPC= Foliage Projective Cover, Precseas= Precipitation seasonality, Rough= Terrain roughness, FZ= Number of frost days, BD= soil bulk density, pH= soil pH, FL= femur length.

Predictor variables	VIF *K. usitatus*	VIF *K. tristis*	VIF *Common data*
Elevation	1.82	2.23	1.56
FPC	1.88	3.36	2.15
Precseas	2.10	4.28	2.61
Rough	2.45	7.14	3.21
FZ	2.09	1.91	1.83
BD	2.06	2.40	1.98
pH	3.00	2.42	2.45
FL	1.02	1.11	1.03

Table S3. Details of global RDA, significant axes and pRDA for *K. usitatus*

Global RDA					
	Df	Variance	F	Pr(>F)	R^2
Model	8	0.05	1.36	0.001	0.021
Residual	121	0.56			
	Three axes				
	Df	Variance	F	Pr(>F)	
	1	0.02	3.21	0.001	
	1	0.01	1.26	0.001	
	1	0.01	1.16	0.001	
Global pRDA					
	Df	Variance	F	Pr(>F)	0.005
Model	8	0.04	1.09	0.001	
Residual	115	0.57			

Table S4. Details of global RDA, axes significance and pRDA for *K. tristis*

Global RDA					
	Df	Variance	F	Pr(>F)	R^2
Model	8	0.06	1.65	0.001	0.037
Residual	126	0.57			
	Three axes				

	Df	Variance	F	Pr(>F)	
	1	0.03	5.68	0.001	
	1	0.01	1.15	0.001	
	1	0.01	1.13	0.001	

Global pRDA					
	Df	Variance	F	Pr(>F)	0.004
Model	8	0.04	1.05	0.048	
Residual	89	0.40			

Table S5. Details of global RDA, axes significance and pRDA for *common data*.

Global RDA					
	Df	Variance	F	Pr(>F)	R^2
Model	8	0.06	2.81	0.001	0.052
Residual	257	0.68			
	Two axes used for outlier detection				
	Df	Variance	F	Pr(>F)	
	1	0.04	14.45	0.001	
	1	0.01	3.54	0.198	

Global pRDA					
	Df	Variance	F	Pr(>F)	0.001
Model	8	0.01	1.05	0.12	
Residual	141	0.26			

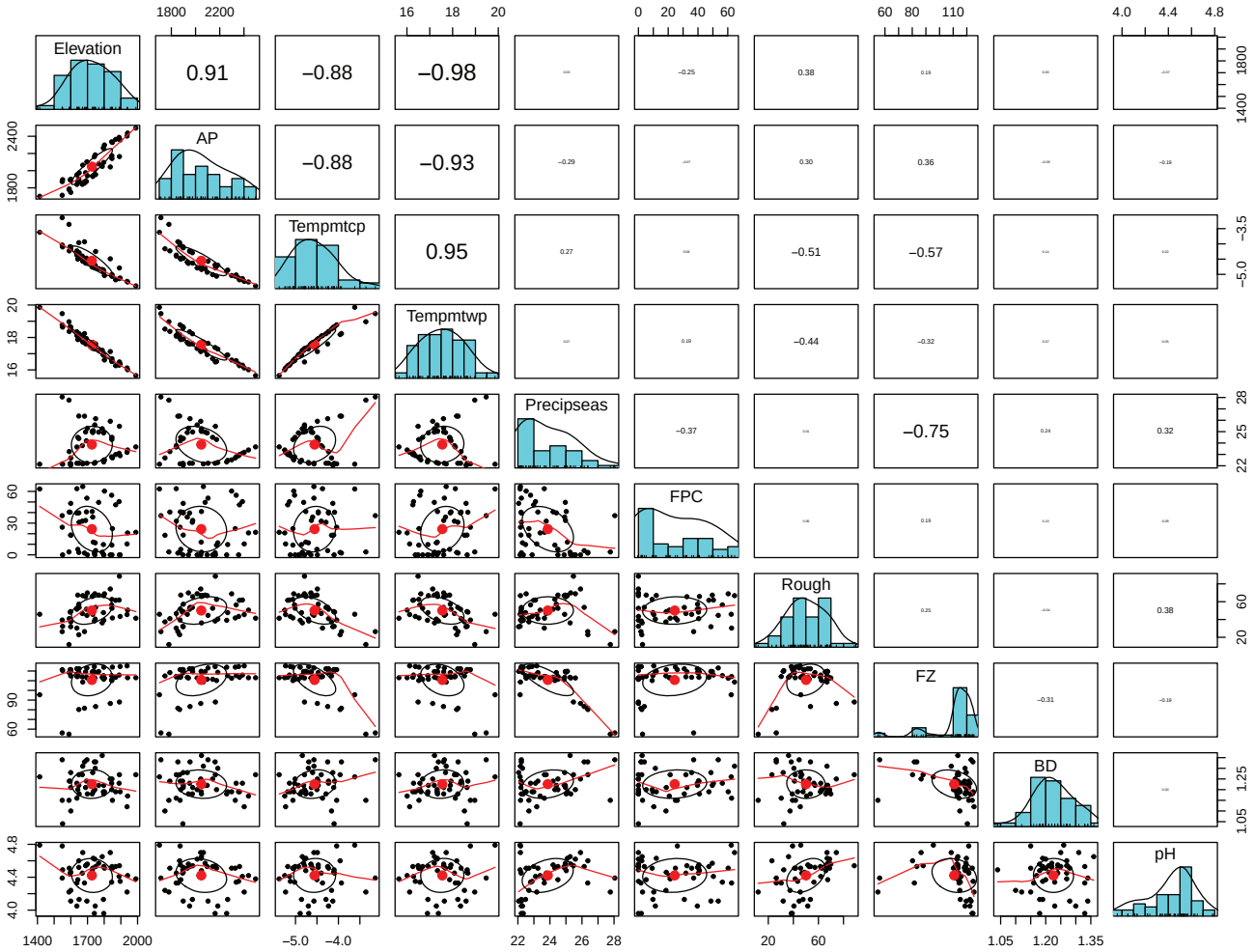


Figure S1: Panel of plots showing the extent of correlation between environmental variables. Values represent the Pearson correlation coefficient. Temperature and precipitation are highly correlated with elevation. AP = annual rainfall, Tempmtcp = mean temp in coldest period, Tempmtwp =mean temp in warmest period, Precipseas =seasonality of rainfall, FPC = Foliage Projective Cover, Rough = roughness with 500m kernel, FZ = number of frost (< -2 degree) days, BD = soil bulk density 0-200cm, pH = soil ph 0-200cm.

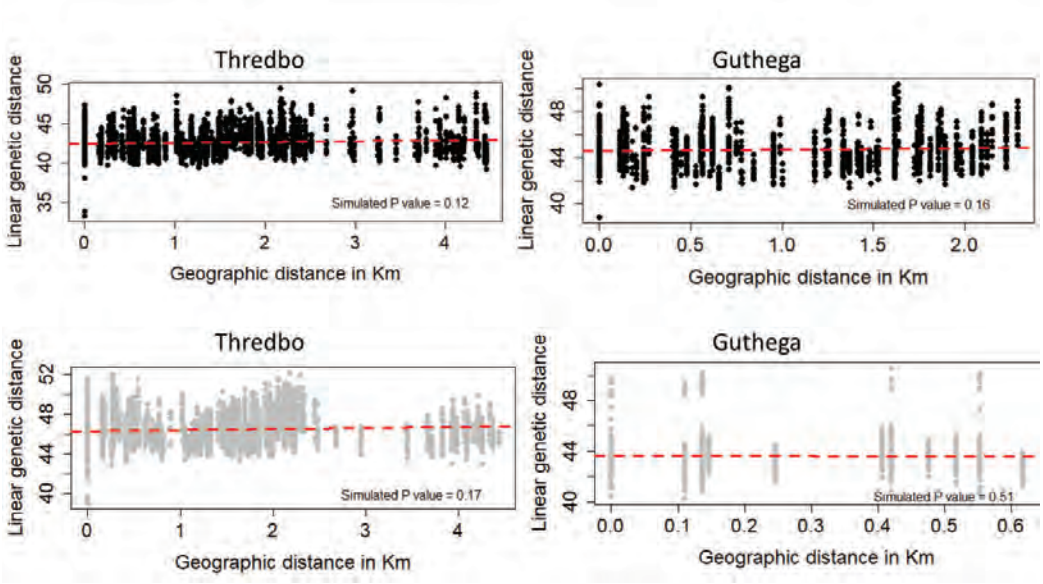
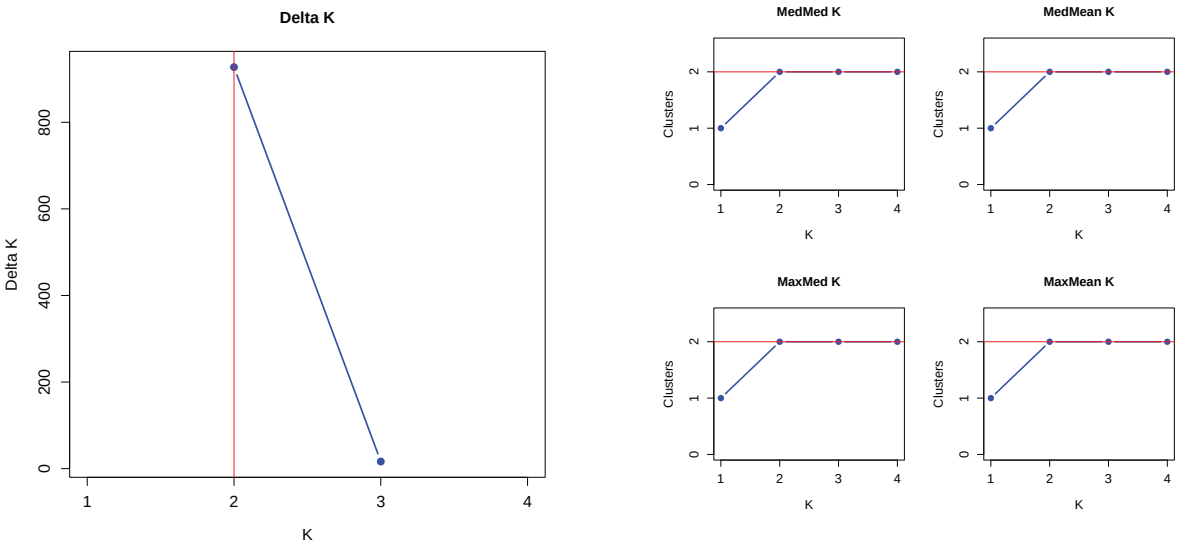
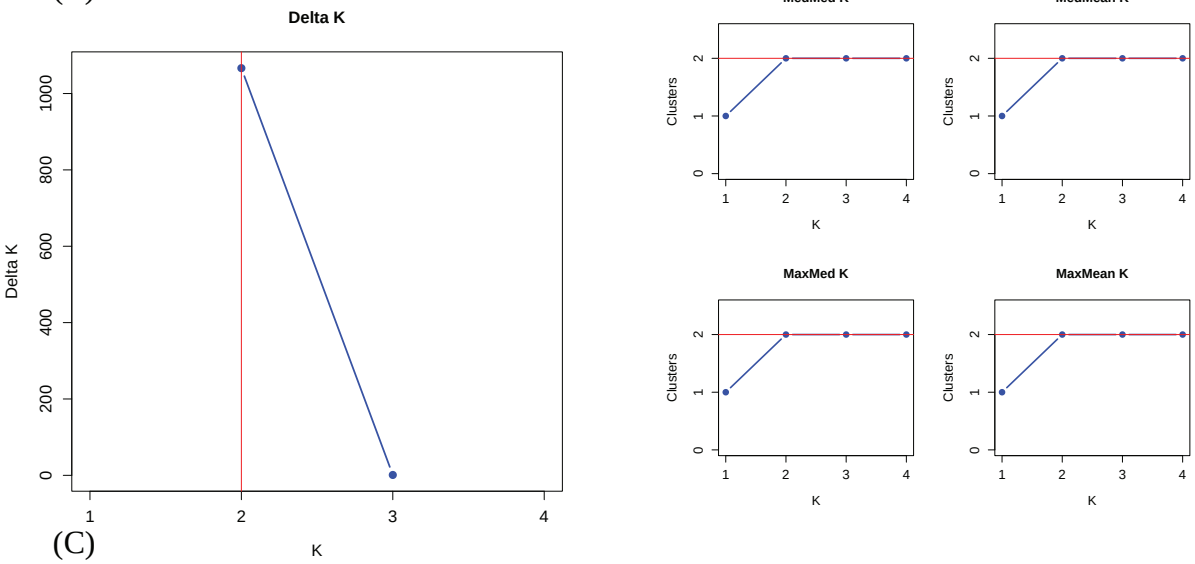


Figure S2: Graphs indicating no isolation by distance within regions in both the species. Top two graphs in black dots indicate *K. usitatus* and bottom two graphs in grey dots represent *K. tristis.*

(A)


Delta K
Delta K
K
MedMed K
MedMean K
MaxMed K
MaxMean K
Clusters
K

(B)


Delta K
Delta K
K
MedMed K
MedMean K
MaxMed K
MaxMean K
Clusters
K

(C)

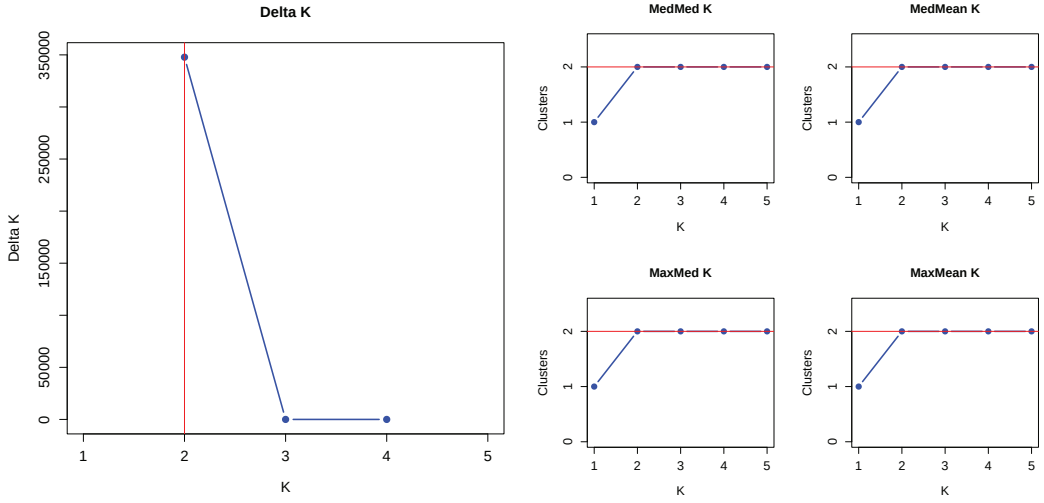
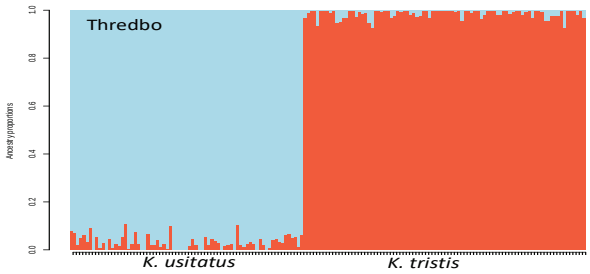

Delta K
Delta K
K
MedMed K
MedMean K
MaxMed K
MaxMean K
Clusters
K

Figure S3. STRUCTURESELECTOR plots showing genetic clustering between two mountain regions using Delta K method and four estimators of STRUCTURESELECTOR (A) *K. usitatus*; (B) *K. tristis* and (C) *common data* used to test hybridisation between species.

(A)



(B)

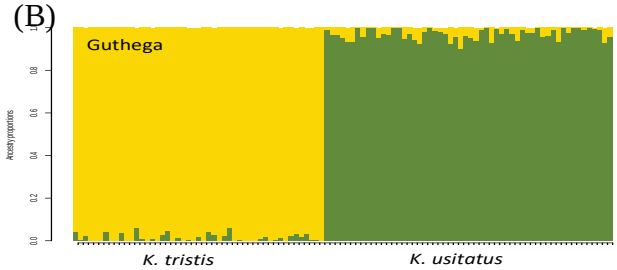
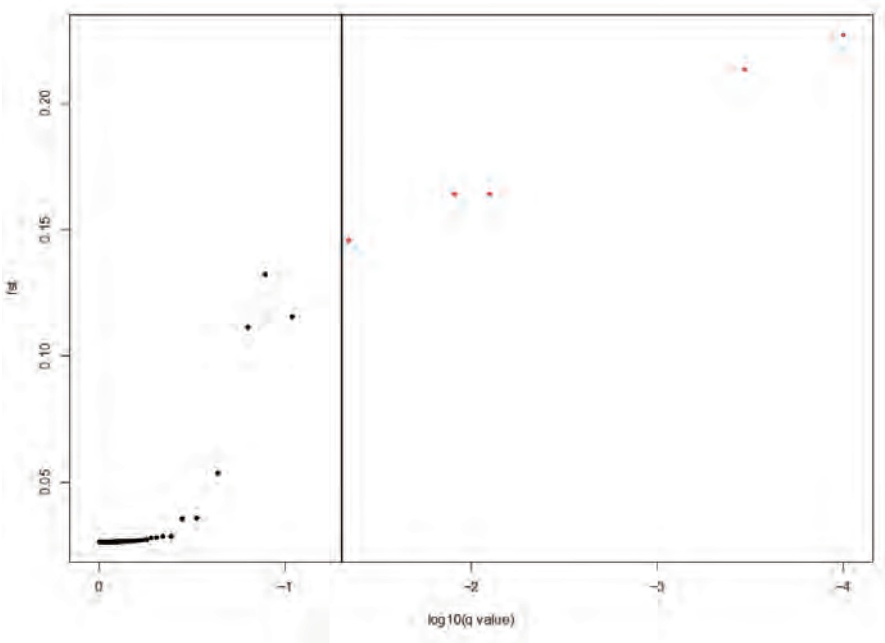


Figure S4: Within region admixture analysis on common data using *snmf* function in LEA R package. A) Thredbo and B) Guthega.

(A)



(B)

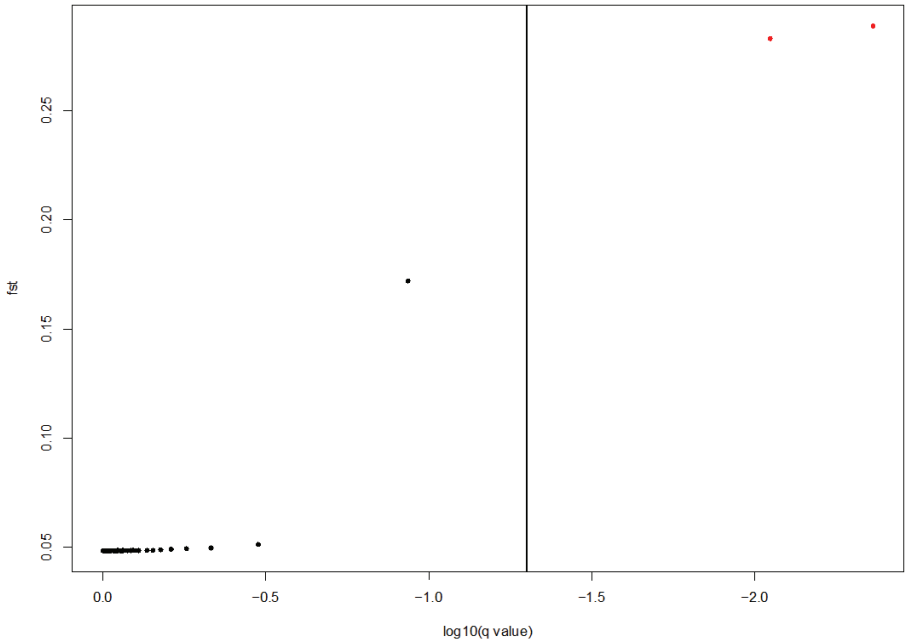


Figure S5. BayeScan plots showing detection of SNPs under selection (A) *K. usitatus* indicating five outliers under selection, (B) *K. tristis* showing two outliers under divergent selection.

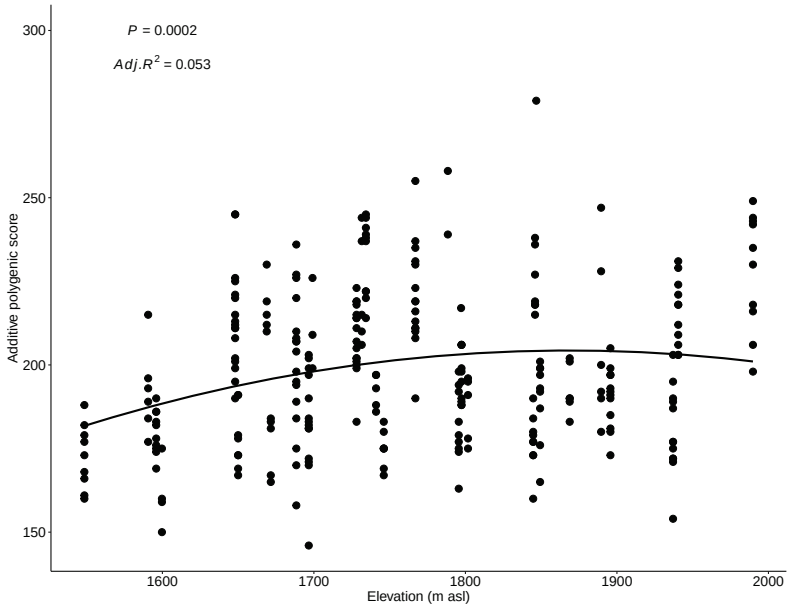


Figure S6: Correlation between individual additive polygenic scores and elevation in common dataset P=0.0002, Adj. R^2= 0.053. The solid line represents regression line from quadratic model. Dots represent an individual grasshopper.

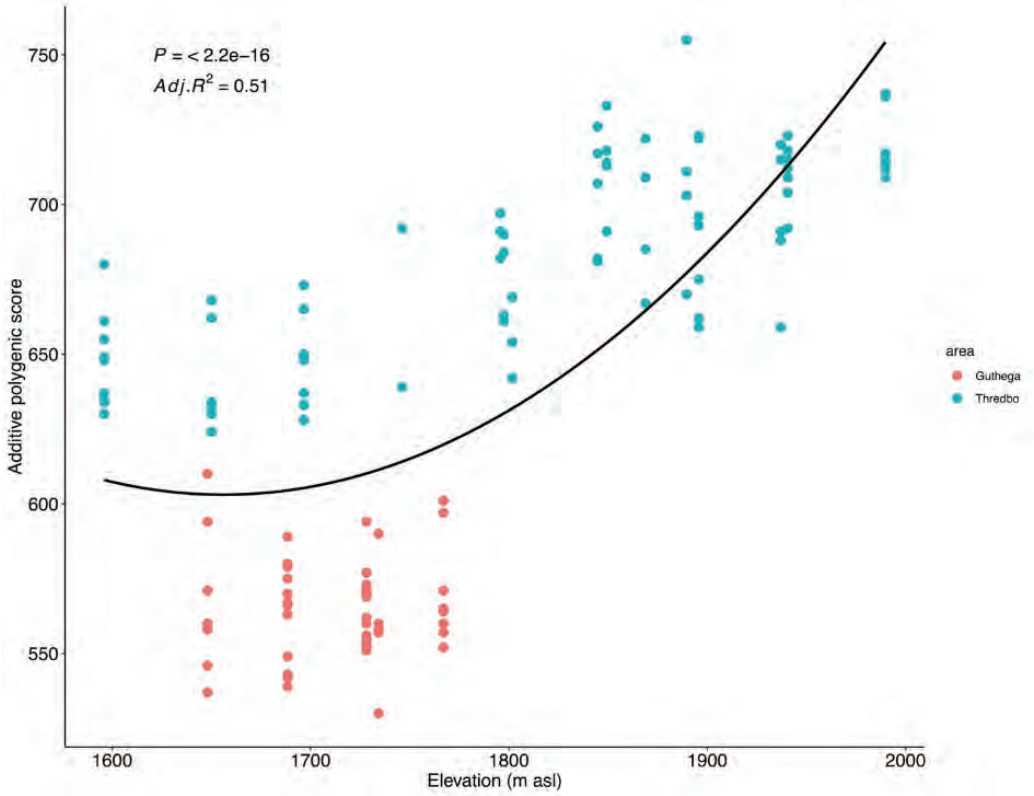


Figure S7: Correlation between individual additive polygenic scores and elevation in *K. tristis* showing that additive polygenic scores vary differently in the two regions.

APPENDIX 1

Phaulacridium vittatum mating pair photo got featured on the cover of Journal of insect Science

(Photo: Sonu Yadav)

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Research

Morphological Variation Tracks Environmental Gradients in an Agricultural Pest, *Phaulacridium vittatum* (Orthoptera: Acrididae)

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Abstract

Invertebrate pests often show high morphological variation and wide environmental tolerances. Knowledge of how phenotypic variation is associated with environmental heterogeneity can elucidate the processes underpinning these patterns. Here we examine morphological variation and relative abundance along environmental gradients in a widespread agricultural pest, native to Australia, the wingless grasshopper *Phaulacridium vittatum* (Sjöstedt). We test for correlations between body size, wing presence, and stripe polymorphism with environmental variables. Using multiple regression and mixed-effects modeling, body size and stripe polymorphism were positively associated with solar radiation, and wing presence was positively associated with foliage projective cover (FPC). There were no associations between body size or morphological traits with relative abundance. However, relative abundance was positively associated with latitude, soil moisture, and wind speed, but was negatively associated with FPC. Therefore, sites with low relative abundance and high forest cover were more likely to contain winged individuals. Overall, our results suggest that environmental and climatic conditions strongly influence the relative abundance and the distribution of morphotypes in *P. vittatum*, which is likely to affect dispersal and fitness in different landscapes. This knowledge is useful for informing how environmental change might influence the future spread and impact of this agricultural pest.

Key words: wingless grasshopper, *Phaulacridium vittatum*, agricultural pest, relative abundance, morphological variation

The distribution, abundance, and phenology of several insect pests have altered in response to changing climatic conditions (Olfert and Weiss 2006, Parmesan 2007, Trnka et al. 2007, Da Silva et al. 2011). Shifts in environmental variables, such as temperature, play a key role in the severity of phytophagous insect pest infestations (e.g., Baker et al. 2015). Major outbreaks of phytophagous insects are exacerbated by ongoing warming conditions and extreme climatic events such as drought and floods (Jepsen et al. 2008, Zhang et al. 2009). Furthermore, the negative impacts of phytophagous insect pests on crop production (Oerke 2006) and forest harvest (Aukema et al. 2011) are often economically significant (Bradshaw et al. 2016). Therefore, it is important to understand the environmental determinants and interactions that facilitate the occurrence and spread of insect pests under currently shifting climatic regimes.

Some insects show variation in genetic, physiological, and morphological traits across broad climatic gradients (Lancaster et al. 2015, 2016, reviewed in Hoffmann et al. 2017, Dudaniec et al. 2018). For insect pests, variations in morphological traits may confer benefits that enable wide environmental tolerances, survival, and colonization across large geographic areas (Gomez and Van Dyck 2012, Parsons and Joern 2014, Bai et al. 2016), or increased chances of spread through efficient use of local resources (e.g., as shown in pest aphids; Simon and Peccoud 2018). The relationship between environmental variables and the distribution of morphological variation can therefore be indicative of the capacity of insect pests to persist under shifting environmental conditions and may also provide evidence for local selection processes.

Polymorphism in insects can be maintained by various mechanisms such as balancing selection (Lindtke et al. 2017), a combination of multiple selection pressures (Takahashi et al. 2011), or adaptive or nonadaptive phenotypic plasticity (Ghalambor et al. 2007, Kelly et al. 2012, Sgrò et al. 2016). Morphological traits may also be regulated via frequency or density dependence. In this case, the fitness of different morphs shifts in response to their prevailing frequencies or in response to competitive interactions among conspecifics (Le Rouzic et al. 2015, Lancaster et al. 2017, Nosil et al. 2018). In addition, a clear role of climatic and ecological factors in shaping morphological variations in insects is reported in several

studies (Dearn 1990, Zera and Denno 1997, Chown and Gaston 2010). For example, different color morphs can be selected for according to local environmental variables (Tanaka 2004, Tanaka et al. 2012). Dispersal traits such as wing dimorphism may show variation under temporally and spatially diverse climatic gradients (Harrison 1980, Roff 1986) and in relation to photoperiod, temperature, and latitude (Sasaki et al. 2002, Zeng and Zhu 2014, Rosetti and Remis 2018). Variation in climate can also shape the distribution of insect body size along latitudinal gradients (Masaki 1967). A necessary step in understanding these relationships is tearing apart environment effects on morphological traits and their influence on fitness. For example, different morph frequencies may confer differential survival according to local climatic conditions (Forsman 1999). For insect pests, such information offers a starting point for characterizing species resilience under shifting climatic conditions.

Here, we examine how morphological traits vary along environmental gradients in a widespread agricultural pest, the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt), an endemic species to Australia. Adult grasshoppers exhibit morphological variation in three key traits: wing presence/absence, stripe polymorphism (Fig. 1; three distinct morphs; Dearn 1984, Key 1992), and body size (both within and between sexes; Key 1992). Body size of males (ranging from 6 to 9 mm) is smaller than females (average size of 6–13 mm; Key 1992). *Phaulacridium vittatum* has nonoverlapping generations, with eggs undergoing diapause in winter, and the first hatchlings emerging in spring between October and November (Clark 1967). Development involves five instar stages, and adults survive until late March and April. Prior to European colonization of Australia, *P. vittatum* was restricted to savannah woodlands or grasslands, but with forest clearing for pasture and agriculture, suitable breeding habitat increased dramatically, contributing to the species' current widespread distribution and high abundance that can reach pest proportions (Dearn 1984). Adult grasshoppers cause damage to pasture, gardens, economically important crops (Clark 1967, Roberts 1972), and juvenile eucalyptus trees (Loch and Floyd 2001) and can compete with livestock for pasture (Bailey et al. 1994). Severe outbreaks can be cyclic and have been documented every 4 or 5 yr in areas with suitable habitats and weather conditions (Milner et al. 1994).

Several selective forces may be responsible for maintaining morphological polymorphisms in *P. vittatum*. For example, stripe polymorphism in *P. vittatum* appears to be maintained by disassortative mating (i.e., increased frequency of matings between striped and unstriped individuals) as documented by Dearn (1980, 1984). Another potential selective force maintaining stripe morph in *P. vittatum* is frequency-dependent selection exerted by predators (Dearn 1984) as reported in other species (Losey et al. 1997, Gray and McKinnon 2007).

Fig. 1. Stripe polymorphism in *Phaulacridium vittatum*: (a) fully striped morph with two full longitudinal white stripes, (b) unstriped morph, (c) partially striped morph with two partial white stripes (photographs by S. Yadav).

Evidence also suggests that morphological variation in *P. vittatum* is influenced by latitude, climatic variables, and habitat type. Latitudinal cline in stripe pattern polymorphism has been suggested in previous studies (Dearn 1981). Radiation seasonality and rainfall best explained latitudinal clines in *P. vittatum* body size (Harris et al. 2012). Furthermore, thermoregulatory effects of melanism have been shown in this species, where darker colored individuals (without stripes) maintained a higher body temperature than lighter color individuals (Harris et al. 2013a,b). In terms of habitat type, unstriped morphs were present at a higher frequency in more forested habitats, perhaps a result of camouflage advantage (Dearn 1984). In addition, a higher frequency of winged individuals was observed in shrub-dominated areas, forest margins, and gardens than in open pastures (Clark 1967). Despite these early studies, little is known of the broader spatial distribution of morphological variation or abundance of *P. vittatum* in relation to local environmental variables, which is important for understanding the ecology and potential spread and persistence of this agricultural pest.

With morphological, environmental, and site relative abundance data collected for *P. vittatum* across 36 sites spanning an eight degree latitudinal gradient (from 29.18° S to 36.50° S) along the east coast of Australia, we examine 1) the effect of latitude and environmental variables (i.e., soil moisture, precipitation, wind speed, solar radiation, and foliage projective cover [FPC]) on site relative abundance and the frequency of morphological traits (body size, wing presence/absence, and stripe polymorphism) and 2) evidence for density-dependent selection on morphology by examining the interaction between site relative abundance and the frequency of morphological traits.

Materials and Methods

Sampling

Phaulacridium vittatum grasshoppers were sampled from late January to early February of 2017 when most individuals had reached maturity. In total, 36 sites were sampled across the east coast of New South Wales spanning ~900 km, covering eight degrees of latitude (from 29.18° S to 36.50° S), a mean annual temperature gradient of 4.6–17.4°C, and an elevation gradient of 133–1,966 m asl (Supp Table S1 [online only], Fig. 2). Sites were located approximately 50–60 km apart, and sample sizes ranged from 6 to 34 individuals per site (mean = 28.49 ± 0.17). The elevation, latitude, and longitude of each site were recorded using a Garmin e-Trex 20 handheld GPS. Most sites were situated adjacent to or within open pastures, along roadside edges, and some sites were situated inside national parks within eucalyptus woodlands and other native shrubs. A site was declared absent of *P. vittatum* after searching for a minimum of ~15 min by three people (total search time = 45 min) without any sightings or captures.

Samples were collected either using a sweep net or by hand using small plastic containers. Habitat type does not have a large effect on spotting and catching grasshoppers as captures are largely cued by the obvious hopping activity of the grasshoppers. Similarly, stripe pattern did not introduce sampling bias as stripes are not readily visible from afar and do not influence capture rates. All collected specimens were stored on ice until processed and preserved in 90% ethanol. The following details were recorded on live samples during sample processing: sex (male/female), morphology: (winged/wingless), (striped/partially striped/unstriped), and femur length (mm), the latter being measured with digital calipers to the nearest 0.01 mm as a proxy for body size in grasshoppers (Masaki 1967).

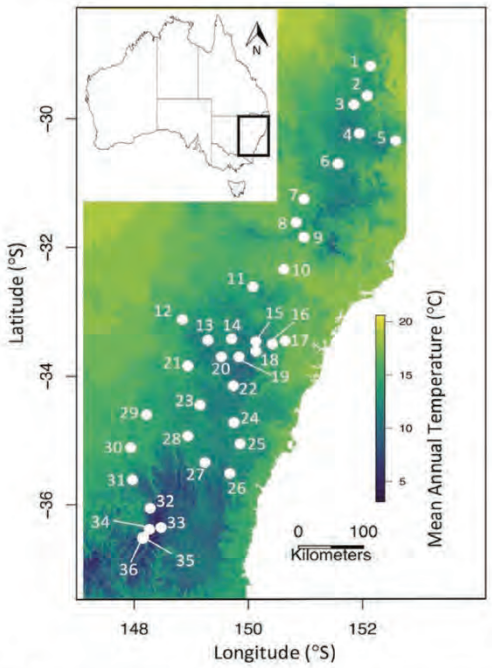

Fig. 2. Sample sites (n = 36) indicated by filled circles over a map of mean annual temperature (°C, BIO1) along the east coast of New South Wales, Australia.

The relative abundance of *P. vittatum* at each site was measured via capture rate per minute, calculated as the number of grasshoppers captured divided by the number of people searching multiplied by the number of minutes spent searching (Lancaster et al. 2015). All procedures were performed in accordance with the animal ethics guidelines of Macquarie University, Australia, and sampling permissions were obtained from local government authorities and national parks (license number: SL101832).

Environmental Data

Environmental variables were selected based on field observations and findings of previous studies on the ecology of *P. vittatum*. Latitude and environmental variables such as radiation seasonality and rainfall are known to influence body size variation in *P. vittatum* (Harris et al. 2012). Foliage cover is important for egg laying in this species, as eggs are laid in open areas with bare ground between vegetation patches (Clark 1967). In addition, soil properties such as soil moisture and soil type have been shown to influence density (Miao et al. 2018), oviposition, and therefore further offspring viability in grasshoppers (Herrmann et al. 2010). Wind speed was chosen because wind is beneficial for passive dispersal of insects (Drake 1994, Venette and Ragsdale 2004) and may aid in the colonization of new areas by facilitating long-distance dispersal (Anderson et al. 2010, Colombari and Battisti 2016).

Mean annual temperature (BIO1) and mean annual precipitation (BIO12) were extracted per sampling site from WorldClim v1.4 (Hijmans et al. 2005). Mean annual wind speed and mean annual solar radiation were extracted from WorldClim v2.0 (Fick and Hijmans 2017). All data from WorldClim were used at a spatial resolution of 1 km^2. FPC % was extracted from a buffered area of 100 m surrounding each of the sites from the TERN AusCover database (2011) with a spatial resolution of 5 m^2. Data for each of 36 sites were extracted (Supp Table S1 [online only]) using the *raster* package (Hijmans and Van Etten 2014) in R v3.3.2 (R Core Team 2014).

Soil type data were downloaded from the New South Wales Office of Environment and Heritage (OEH 2017) and obtained for each sample site using QGIS v2.18 (QGIS Development Team 2009). Soil type data were then used to classify soil moisture for each site and was categorized based on the clay content and water holding capacity of the soil, as defined in the Australian Soils Classification (ASC) and Great Soil Group guidelines (GSG; Raymond 2016). Soils with high to intermediate water holding capacity based on ASC and GSG classifications were scored as 1 (high moisture), whereas soils with low water holding capacity were scored as 0 (low moisture; Supp Table S4 [online only] for site-based information).

A Pearson's correlation matrix was obtained between all environmental variables measured at each site (Supp Table S2 [online only], Supp Text S1 [online only]). After removing variables with very high correlation (r > 0.80), a total of six variables were used for further analyses, i.e., 1) solar radiation, 2) wind speed, 3) soil moisture, 4) FPC, 5) BIO12, and 6) latitude. Despite the high correlation between latitude and solar radiation (r = 0.81), we retained latitude to examine its influence on site relative abundance. Nonetheless, both variables were not used together in the same model.

Statistical Approach

All statistical analyses were performed in R v3.3.2. We analyzed our data (n = 954 individuals) using multiple regression, and generalized linear mixed-effects model (GLMM) and linear mixed-effects model (LMM). The statistical significance of models was examined using likelihood ratio tests within ANOVA by comparing fitted models with the null model including only the response variable, random effects (in case of the mixed model), and intercept. Insignificant fixed effects were removed from the model by applying the "analysis of deviance test" using the *drop1()* command with a chi-square test. The model was refitted after dropping insignificant fixed effects. We performed a nonlinear transformation on the response variable wherever possible (relative abundance and femur length) by using log-transformation. Site ID was included as a random effect in mixed modeling to account for nonindependence between sampling sites. To examine the goodness of fit of the GLMM and LMM, conditional R^2 statistics were calculated using the MuMIn R package (Barton 2018).

Relative Abundance

Multiple linear regression modeling was used to examine the influence of latitude, FPC, soil moisture, and wind speed on the relative abundance of *P. vittatum* at each site. In addition, the relative importance of each fixed effect and confidence intervals for relative importance were determined using the *boot.relimp()* and *booteval.relimp()* functions of the relaimpo v2.2-2 R package (Grömping 2006) by implementing 1,000 bootstraps.

Wing Polymorphism

Wing polymorphism was divided into two categories: winged individuals were categorized as 1 and wingless individuals were categorized as 0. We tested for the association of solar radiation, wind speed, FPC, soil moisture, relative abundance, body size, and stripe pattern on wing presence using GLMM in the lme4 R package

(Bates et al. 2015) and the function *glmer()*. The final model was refitted after dropping insignificant fixed effects and using site relative abundance as the random effect to account for its influence on the relationship and to provide additional explanatory power to the model.

Stripe Polymorphism

Stripe polymorphism in *P. vittatum* appears to be genetically controlled by three alleles at an autosomal locus (C^S = full stripe, C^I = partial stripe, C^P = plain) following a hierarchical dominance pattern ($C^S > C^I > C^P$; Dearn 1983). As previously done by Dearn (1978, 1981), and due to a low number of partially striped individuals in our data set, partially striped morphs were combined with striped morphs into an "any striped" category, pooling C^S and C^I genotypes. Stripe polymorphism was classified into two categories: any striped individuals were coded as 1 and unstriped individuals were coded as 0. We constructed a GLMM, using stripe polymorphism as a response variable and solar radiation, wind speed, FPC, soil moisture, relative abundance, body size, and wing polymorphism as fixed effects. After dropping, insignificant fixed-effects model was refitted using site relative abundance as the random effect.

Body Size

We fitted an LMM to examine the influence of environmental variables on body size of males and females combinedly using the *lmer()* function within the lme4 R package (Bates et al. 2015) with the fixed-effects solar radiation, BIO12, FPC, soil moisture, wind speed, and relative abundance. The final model was refitted using significant fixed effect. We conducted type II Wald chi-square tests using the "car" R package (Fox and Weisberg 2011) to obtain *P* values for the fitted model. In addition, a linear model was fitted for body size of both males and females individually with significant fixed effect.

Results

Environmental Correlations with Relative Abundance

We found a significant positive association of relative abundance with latitude ($P = 0.001$), soil moisture ($P \leq 0.001$), and wind speed ($P \leq 0.001$), whereas FPC showed significant negative association ($P \leq 0.001$), with total variance explained by the model = 23% (Fig. 4, Table 1). FPC had the highest relative importance among other fixed effects followed by wind speed, soil moisture, and latitude (Fig. 3, Supp Table S3 [online only]). Several sites in our study with elevation above 1,000 m ($n = 12$ sites) had greater FPC (mean = 23.27 ± 5.92; Supp Fig. S4 [online only]), compared with sites of elevation below 1,000 m ($n = 24$ sites, mean = 10.01 ± 2.73). In the case of soil moisture, out of 36 sites, 17 were categorized as having intermediate to high soil moisture and had an average relative abundance of 0.36 (± 0.06), compared with 19 sites with low soil moisture that had an average relative abundance of 0.29 (± 0.05).

Environmental Correlations with Morphology

In total, 72 out of 954 (7.55%) individuals were winged, and 882 out of 954 (92.45%) were wingless. Within each of the 36 sample sites, the proportion of winged individuals ranged from 0 to 0.73 (mean = 0.10 ± 0.02) and wingless from 0.27 to 1.00 (mean = 0.90 ± 0.02). A positive correlation was found between FPC and wing presence (GLMM estimate = 0.03, $P = 0.03$ conditional $R^2 = 0.41$; Supp Fig. S2 [online only]). However, other variables did not show any significant correlation with wing presence or absence (Table 2). The addition of site relative abundance as a random effect increased the fit of the model (random effect variance: 1.94, SD 1.39).

In total, 15.6% of individuals were striped, 8.1% partially striped, and 76.2% unstriped. Within each of the 36 sample sites, the proportion of fully striped individuals ranged from 0 to 0.36 (mean = 0.15 ± 0.01), partially striped individuals ranged from 0 to 0.40 (mean = 0.09 ± 0.01), and unstriped individuals ranged from 0.58 to 0.93 (mean = 0.76 ± 0.01). The proportion of both fully and partially striped morphs in each site was positively associated with solar radiation (GLMM estimate = 0.31, $P \leq 0.001$, conditional $R^2 = 0.042$). No other significant associations were observed with stripe polymorphism (Table 2). Site relative abundance had no effect on the relationship and did not explain any of the variance.

Body size varied within males (range 5.36–10.63 mm; mean = 8.25 ± 0.03 mm) and females (range 7.41–12.28 mm; mean = 10.3 ± 0.03 mm) along the sampled gradient. A significant positive correlation was found between body size and solar radiation (LMM: $\chi^2 = 5.56$, df = 1, $P = 0.01$, conditional $R^2 = 0.079$; Supp Fig. S1 [online only]). However, we did not find a significant association with other fixed effects (Table 3). Males were shown to have a stronger relationship (linear model $P < 0.001$) than females (linear model $P = 0.03$). Furthermore, site relative abundance had no influence on the relationship (random effect variance = 0.0).

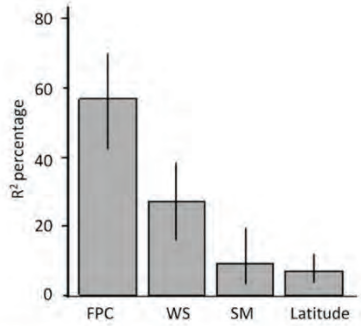

Fig. 3. Relative importance of four fixed effects on relative abundance in multiple regression analysis with bootstrapped 95% confidence intervals; FPC = foliage projective cover (%); WS = wind speed (m/s); SM = soil moisture.

Table 1. Summary of multiple linear regression analysis for site relative abundance with four fixed effects

Fixed effects	Estimate	SE	*t* value	Pr (>|*t*|)
Latitude	0.03	0.01	3.18	0.001
Soil moisture	0.22	0.03	5.99	<0.001
Wind speed	0.42	0.04	9.88	<0.001
Foliage projective cover (%)	−0.02	0.00	−14.78	<0.001

Table 2. Summary of the generalized linear mixed-effects model for wing polymorphism and stripe polymorphism

Fixed effects	Estimate	Pr (>z value)	Estimate	Pr (>z value)
	Wing polymorphism		Stripe polymorphism	
Solar radiation	0.58	0.06	**0.31**	**< 0.001**
Wind speed	−0.23	0.34	−0.08	0.66
Foliage projective cover (%)	**0.03**	**0.03**	−0.00	0.87
Soil moisture	−0.08	0.87	0.26	0.18
Relative abundance	−0.56	0.06	0.06	0.40
Body size	−0.00	0.96	−0.04	0.45
Stripe pattern	−0.07	0.82	—	—
Wing pattern	—	—	−0.05	0.85

Significant associations are shown in bold.

Table 3. Analysis of deviance table summary using type II Wald chi-square tests for linear mixed-effects model fit for body size

Fixed effects	Estimate	Df	χ^2	Pr (>χ^2)
Initial LMM				
Solar radiation	**0.03**	**1.00**	**6.66**	**0.01**
Mean annual precipitation	0.03	1.00	2.84	0.09
Foliage projective cover (%)	0.00	1.00	1.51	0.21
Soil moisture	0.02	1.00	2.42	0.11
Wind speed	−0.03	1.00	1.67	0.19
Relative abundance	0.02	1.00	0.40	0.52
Final model (after dropping insignificant fixed effects)				
Solar radiation	**0.01**	**1.00**	**5.56**	**0.01**

Significant results are indicated in bold. LMM (linear mixed-effects model).

Discussion

Our study reveals significant relationships between environmental variables, site abundance, and the distribution of morphological traits in an agricultural insect pest. We found site relative abundance was positively influenced by latitude, wind speed, and soil moisture and was negatively influenced by foliage cover. Solar radiation was the principal factor influencing stripe polymorphism and body size variation in *P. vittatum*. Furthermore, site relative abundance did not significantly associate with morphological traits, indicating no evidence for density-dependent processes governing morphological trait variation; however, this requires further investigation. Our study demonstrates how multiple environmental factors can influence the distribution of morphological variation and relative abundance of *P. vittatum*, and contributes knowledge toward our capacity to understand how widespread pests cope with heterogeneous environments via morphological variation.

Environmental Variables and Relative Abundance

Overall, higher numbers of *P. vittatum* were observed at southern latitudinal sites (Fig. 4a). Climatic variables that correlate with latitude can have strong effects on local abundance (Bale et al. 2002). For instance, in grasshoppers, climatic factors such as temperature and precipitation are critical in controlling population dynamics, both directly by influencing life-history traits and indirectly by controlling resource availability (Fielding and Brusven 1990, De Wysiecki et al. 2011, Jonas et al. 2015). However, identifying the influence of individual variables is challenging due to the complex interaction between them. For example, in our study sites, mean annual temperature is strongly correlated with mean annual precipitation ($r = -0.83$) and wind speed ($r = -0.90$; Supp Table S2 [online only]). Furthermore, some of our sites are located near or within the Australian Alps (latitudinal range of 35–36° S), which have higher elevation (Supp Table S1 [online only]) and receive greater solar radiation (Supp Fig. S3 [online only]). These strong interactions make it difficult to effectively tease apart which variable is most significant in driving the variation in relative abundance of *P. vittatum*. Given that our model explains a modest 23% of the variance, additional factors are likely to be involved in regulating the abundance.

A decrease in relative abundance was observed with increased FPC (Fig. 4d). A higher percentage of FPC at high elevation sites in our study (Supp Fig. S4 [online only]) may hamper dispersal and connectivity among individuals, which in turn may reduce site relative abundances. Furthermore, this observed a negative association between relative abundance, and FPC is likely to be affected by site preference and resource availability. Open pastures and grazing areas are among the primary habitats of *P. vittatum*, where low FPC provides suitable sites for egg laying (Clark 1967). Furthermore, high vegetation cover negatively affects early instar survival due to a scarcity of preferable food resources that grow in more disturbed areas (i.e., prostrate and rosette-forming plants growing under pasture; Clark 1967). Last, in grasshoppers, basking is a crucial factor to regulate body temperature, facilitated by shuttling between sun and shade (Chappell and Whitman 1990), which is also exhibited by *P. vittatum* (Harris et al. 2015). Compromised thermoregulation due to high vegetation cover and reduction in suitable basking sites could influence fitness and decrease relative abundance (Kearney et al. 2009).

Sites with greater wind speed had higher relative abundance (Fig. 4c) suggesting a role of wind-assisted dispersal in the population dynamics of *P. vittatum*. In insects, wind can aid long-distance migration (Drake and Farrow 1988) and drive selection processes (Dudaniec et al. 2018), and for pest species, wind-assisted flight can lead to accelerated invasion to new areas, outbreaks, and major

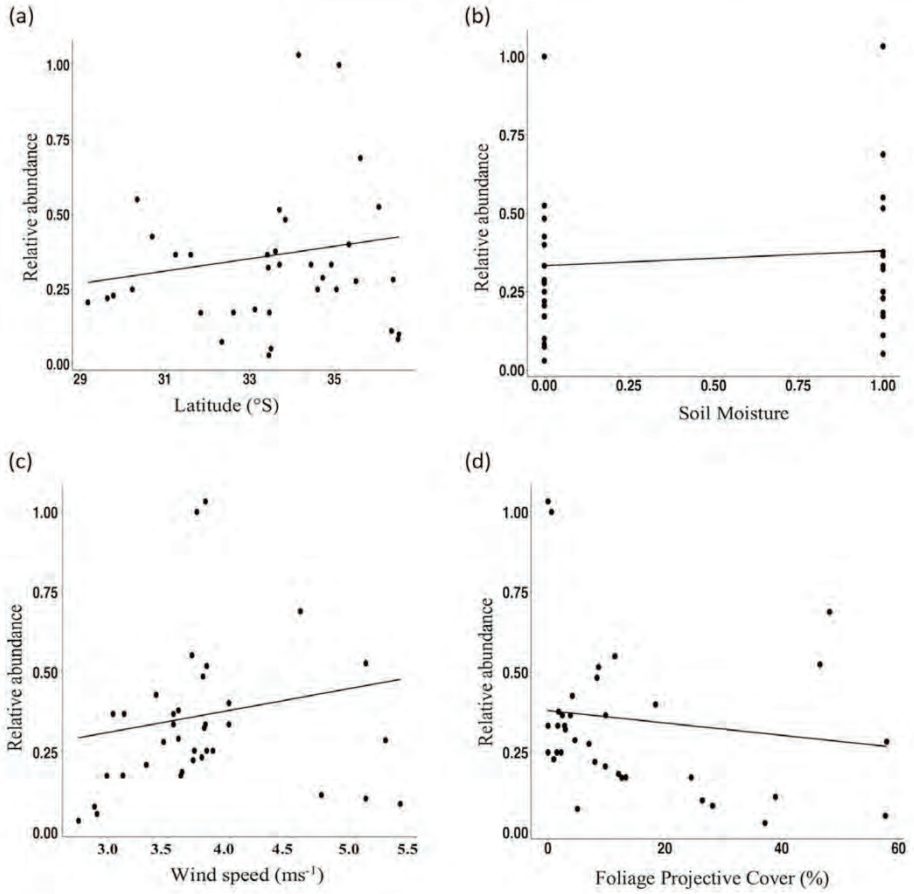

Fig. 4. The relationship between four significant fixed effects used in multiple regression analysis and relative abundance: (a) latitude: ($P = 0.001$); (b) soil moisture ($P < 0.001$); (c) wind speed ($P < 0.0001$); and (d) foliage projective cover (%; $P < 0.0001$).

crop losses (Drake 1994, Liebhold and Tobin 2008, Anderson et al. 2010). Wind speed and the presence of wings in *P. vittatum* are likely to facilitate both passive and active dispersal, respectively, facilitating its broad distribution. Wind speed can also regulate local ambient temperatures, and indeed, we found that wind speed was highly correlated with mean annual temperature ($r = -0.90$). Hence, a combined effect of wind speed and temperature is likely to be a key driver of relative abundance.

A significant effect of soil moisture on relative abundance is evident from our study (Fig. 4b). Soil can control insect abundances via influencing life-history traits of phytophagous insects, such as egg laying, nymphal development (as shown in the lubber grasshopper, *Romalea microptera* (Beauvois) (Orthoptera: Acrididae); Herrmann et al. 2010), or via regulation of habitat resources (McColloch and Hayes 1922) and controlling the growth of suitable food resources (Miao et al. 2018). Soil moisture content can support vegetation growth; this may compromise egg laying in *P. vittatum* because females preferentially lay egg pods in patches of bare ground (Clark 1967). However, high soil moisture content may facilitate food resource availability after emergence, supporting higher relative abundances, as observed in our study. Therefore, soil moisture may influence the life stages of *P. vittatum* differentially with greater benefits after emergence than during egg and early instar phases. Furthermore, the local distributions of *P. vittatum* have been found to be influenced by the interaction between soil moisture and the occurrence of parasitic nematodes and entomopathogenic fungi (Milner and Prior 1994, Clift and Baker 1998), indicating evidence for an indirect effect of soil moisture.

Wing Polymorphism and Foliage Projective Cover

Wing polymorphism is a common phenomenon in several insects with ecological and evolutionary implications (Harrison 1980, Roff 1986). Winged and wingless morphs may be retained in different frequencies depending on environmental conditions such as temperature, precipitation (Sasaki et al. 2002, Rosetti and Remis 2018), and habitat type (Denno 1994). For instance, planthoppers (Homoptera: Delphacidae) are more likely to have wingless morphs in more stable habitats and dispersive, winged morphs in less stable

habitats (Denno et al. 1991). Our study found a larger proportion of winged individuals at sites with high foliage cover (Supp Fig. S2 [online only]), which supports the observations of a previous study on *P. vittatum* by Clark (1967), who found that the wingless form was abundant in pastures (>75% wingless adults) and the winged form was abundant in forested areas (60–70% winged individuals). Our concurrent finding that relative abundance also decreases significantly with increased FPC (Fig. 4d) indicates that high FPC sites are not the preferred habitat of this species. This suggests that high foliage sites are of lower habitat suitability for *P. vittatum* and may select for higher frequencies of traits that facilitate dispersal into new areas, such as wings (Denno et al. 1996).

The development of wings in insects can be a trade-off between dispersal and reproduction (Guerra and Pollack 2007, Guerra 2011, Steenman et al. 2015). For example, an analysis of life-history traits of 22 insect species by Roff (1986) showed wingless forms have higher fecundity and reproduce earlier than winged forms. Although wingless forms can show high fecundity and reproductive rates in stable, good-quality habitats, in unfavorable environments, winged forms may be favored for their high dispersal ability (Harrison 1980, Denno et al. 1991, Zera and Harshman 2001). The winged form of *P. vittatum* may be advantageous for dispersal during outbreaks to avoid competition with conspecifics and locate new resources. For example, in an outbreak in 1980/1981, the winged form of *P. vittatum* was found to successfully undertake dispersal distances of 100 to >500 m from the center of infested areas to favorable peripheral pastures (Farrow et al. 1982). Therefore, the presence of wings can provide additional benefit to this pest species to disperse to new areas, increasing widespread infestation. Last, population densities can also influence the frequency of winged and wingless individuals (Poniatowski and Fartmann 2009, Ríos Martínez and Costamagna 2018). Our results also indicate a possible influence of relative abundance on the presence of wings in this species, however further study on density-dependent effects of relative abundance on the frequency of wings in *P. vittatum* is needed to clearly disentangle this effect.

Stripe Polymorphism and Solar Radiation

Solar radiation is an important factor for the body temperature regulation in grasshoppers (Pepper and Hastings 1952), while melanism is important for the efficient use of solar radiation (Trullas et al. 2007). However, under high solar radiation, dark coloration might impose negative effects on fitness causing overheating of melanics, whereas lighter color morphs may benefit in such conditions (Trullas et al. 2007). We found a positive correlation between solar radiation and individuals with white stripes in *P. vittatum* (Fig. 5, Table 2), which may be involved in reducing heat absorption in sites with higher solar radiation. Likewise, darker individuals without stripes in cooler sites may benefit from increased heat absorption efficiency (True 2003), but this remains to be tested. Effects of melanism on the thermoregulatory behavior of *P. vittatum* have been examined by Harris et al. (2013a,b), who found darker morphs (unstriped morph) warm up rapidly and also have preferred high temperature as compared to lighter morphs. The presence or absence of stripes may therefore be under selection due to its important role in thermoregulation (Harris et al. 2013b).

Several other factors such as predator avoidance via crypsis, disassortative mating, or density-dependent selection exerted by predators (Dearn 1984) may also influence the maintenance and distribution of stripe polymorphism. Disassortative mating is beneficial for maintaining alleles responsible for a rare phenotypic trait

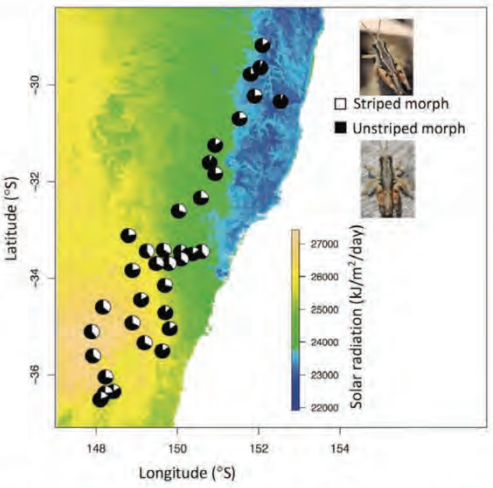

Fig. 5. The change in striped morph (striped and partially striped pattern) and unstriped morph frequency is shown along the solar radiation gradient. White corresponds to the proportion of striped morphs within a site, and black corresponds to the proportion of unstriped morph within a site.

(Follett et al. 2014). In *P. vittatum*, a strong selective constraint on striped individuals with homozygous C^S genotypes has been indicated previously via observations of their reduced viability (Dearn 1984). Strong viability selection on the homozygous genotype indicates a possible role of disassortative mating (observed in *P. vittatum* by Dearn 1980, 1984) in maintaining heterozygous genotypes for stripe pattern. The co-occurrence of striped and unstriped morphs within sites also suggests that balancing selection may be operating to maintain stripe polymorphism in this species, which may be driven by fitness advantages of different morphs under different environmental conditions (e.g., in relation to solar radiation). Further study is needed to identify, first, the role of disassortative mating and frequency dependence in maintaining stripe polymorphism and, second, whether genes associated with stripes are under environmental selection, which may help to unveil the traits' adaptive significance.

The relatively low frequency of striped and partially striped individuals observed in our study is consistent with observations made in previous studies (Dearn 1981, 1984; Harris et al. 2012). Furthermore, we did not find a significant association between foliage cover and stripe pattern as was observed by Dearn (1984). This is possibly due to our use of foliage cover as a continuous variable (%), as opposed to Dearn (1984), who applied a categorical approach (forest vs pasture) with a much smaller spatial scale with just two 50–100 km transects located approximately 100 km apart. This inconsistency with the observations made by Dearn (1984) suggests that the relationship between stripe polymorphism and forest habitat does not hold true at broader spatial scales. Furthermore, an increase in the frequency of striped individuals (including striped and partially striped) was found from north to south (ranging from 28° S to 38° S) in Dearn (1981). The significant positive correlation between stripe pattern and solar radiation we find in the current study suggests that solar radiation is likely to be one of the contributing variables shaping this latitudinal increase in stripe pattern morphs.

Body Size and Solar Radiation

Body size variation in insects is a vital life-history trait due to its relationship with physiology and fitness (Honěk 1993, Nylin and Gotthard 1998) and exhibits clinal variation along latitudinal gradients in many ectotherms. Bergmann's rule states that body size increases with latitude, but the opposite of this (body size decreases with increase in latitude) has been found to apply in insects (Blackburn et al. 1999, Chown and Gaston 2010, but see Cushman et al. 1993, Kaspari and Vargo 1995). Factors such as temperature, seasonality, humidity, diet, and interactions among these factors can cause linear or nonlinear clines in ectotherm body size (Roff 1980, Kennington et al. 2003, Ho et al. 2009, Çağlar et al. 2014).

In *P. vittatum*, body size variation was previously explained by rainfall and radiation seasonality (Harris et al. 2012). Our study suggests that solar radiation is significantly associated with increased body size in both males and females (Supp Fig. S1 [online only], Table 3). Our finding of an insignificant relationship between annual precipitation and body size (Table 3) contrasts with previous observations made by Harris et al. (2012), which found this association to be significant. The number of study sites analyzed (n = 61) in Harris et al. (2012) was larger than in the present study (n = 36) and spans a latitudinal gradient that is approximately eight degrees wider. However, in the present study, we analyze nearly five times the number of individuals as in Harris et al. (2012; i.e., 198 individuals vs 954 individuals), allowing us to gain meaningful averages per site and higher data resolution.

Furthermore, the strong association between solar radiation and latitude in our study suggests other factors associated with latitude may also influence body size variation in *P. vittatum*. For example, the availability of a longer growing season at higher latitudes may extend growth and development periods and result in larger adult body size (Blanckenhorn 2000, Blanckenhorn and Demont 2004). Larger body size, in turn, can have further fitness advantage such as increased chances of survival and mating (e.g., Waller and Svensson 2017). Our study suggests that climatic and topographical variables are interacting selection pressures acting on the latitudinal variation in body size in *P. vittatum*. Further study using genetic analyses that examine signatures of selection along the sampled environmental gradient would be beneficial to elucidate this.

Conclusion

Environmental changes can drive an increased frequency of invertebrate pest outbreaks that bring economic costs and threaten food security (Macfadyen et al. 2018). Knowledge of how environmental conditions and habitat variation are correlated with morphological traits in invertebrate pests is therefore a necessary step for understanding pest responses and persistence under variable climatic conditions. Our study identifies a significant role of environmental and climatic variables on the relative abundance and distribution of morphological variation in *P. vittatum*, indicating that this species exhibits high variability, and potential adaptive responses that assist survival in different environments. We find that variation in body size, and in two discrete morphological traits, wing presence and stripe polymorphism can be maintained at large spatial scales, probably as a result of spatially varying selective pressures that are at least partially explained by environmental gradients. Notably, our study uses a data set collected in just one season and provides a snapshot of correlations that may or may not be upheld over longer temporal scales, perhaps due to shifts in weather and resources. The results of this study are informative for investigation into the genetic basis of selection on morphology and environment, and ultimately be informative for understanding the future spread and persistence of this agricultural pest.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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References Cited

Anderson, K. L., T. E. Deveson, N. Sallam, and B. C. Congdon. 2010. Wind assisted migration potential of the island sugarcane planthopper *Eumetopina flavipes* (Hemiptera: Delphacidae): implications for managing incursions across an Australian quarantine frontline. J. Appl. Ecol. 47: 1310–1319.

Aukema, J. E., B. Leung, K. Kovacs, C. Chivers, K. O. Britton, J. Englin, S. J. Frankel, R. G. Haight, T. P. Holmes, A. M. Liebhold, et al. 2011. Economic impacts of non-native forest insects in the continental United States. PLoS One 6: e24587.

Bai, Y., J. J. Dong, D. L. Guan, J. Y. Xie, and S. Q. Xu. 2016. Geographic variation in wing size and shape of the grasshopper *Trilophidia annulata* (Orthoptera: Oedipodidae): morphological trait variations follow an eco-geographical rule. Sci. Rep. 6: 32680.

Bailey, P., A. Frensham, A. Hincks, and M. Newton. 1994. Competition for herbage by *Phaulacridium vittatum* (Sjöstedt) (Orthoptera: Acrididae) and sheep during summer drought. Aust. Entomol. 33: 175–179.

Baker, M. B., P. D. Venugopal, and W. O. Lamp. 2015. Climate change and phenology: *Empoasca fabae* (Hemiptera: Cicadellidae) migration and severity of impact. PLoS One 10: e0124915.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, and J. Farrar. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Global Change Biol. 8: 1–16.

Barton, K. 2018. MuMIn: multi-model inference. R package version 1.40.4. (https://CRAN.R-project.org/package=MuMIn).

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1-48.

Blackburn, T. M., K. J. Gaston, and N. Loder. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Divers. Distrib. 5: 165–174.

Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? Q. Rev. Biol. 75: 385–407.

Blanckenhorn, W. U., and M. Demont. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? Integr. Comp. Biol. 44: 413–424.

Bradshaw, C. J., B. Leroy, C. Bellard, D. Roiz, C. Albert, A. Fournier, M. Barbet-Massin, J. M. Salles, F. Simard, and F. Courchamp. 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7: 12986.

Çağlar, S. S., Ç. Karacaoğlu, A. C. Kuyucu, and İ. K. Sağlam. 2014. Humidity and seasonality drives body size patterns in males of the bush cricket *Isophya rizeensis* Sevgili, 2003 (Orthoptera: Tettigoniidae: Phaneropterinae). Insect Sci. 21: 213–226.

Chappell, M., and D. Whitman. 1990. Grasshopper thermoregulation, pp. 143–172. *In* A. J. Chapman (ed.), Biology of grasshoppers. John Wiley and Sons, New York.

Chown, S. L., and K. J. Gaston. 2010. Body size variation in insects: a macroecological perspective. Biol. Rev. Camb. Philos. Soc. 85: 139–169.

Clark, D. 1967. A population study of *Phaulacridium vittatum* Sjost (Acrididae). Aust. J. Zool. 15: 799–872.

Clift, A., and G. Baker. 1998. Quantification of the relationship between rainfall and parasitism of *Phaulacridium vittatum* (Sjöstedt) by mermithid nematodes in the Central Tablelands of South-Eastern Australia. J. Orthoptera Res. 7: 77–82.

Colombari, F., and A. Battisti. 2016. Spread of the introduced biocontrol agent *Torymus sinensis* in north-eastern Italy: dispersal through active flight or assisted by wind? BioControl 61: 127–139.

Cushman, J. H., J. H. Lawton, and B. F. Manly. 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. Oecologia 95: 30–37.

Da Silva, E. D., T. M. Kuhn, and L. B. Monteiro. 2011. Oviposition behavior of *Grapholita molesta* Busck (Lepidoptera: Tortricidae) at different temperatures. Neotrop. Entomol. 40: 415–420.

De Wysiecki, M. L., M. Arturi, S. Torrusio, and M. M. Cigliano. 2011. Influence of weather variables and plant communities on grasshopper density in the Southern Pampas, Argentina. J. Insect Sci. 11: 1–14.

Dearn, J. M. 1978. Polymorphisms for wing length and colour pattern in the grasshopper *Phaulacridium vittatum* (Sjost.). Aust. J. Entomol. 17: 135–137.

Dearn, J. M. 1980. Evidence of non-random mating for the colour pattern polymorphism in field populations of the grasshopper *Phaulacridium vittatum*. Aust. Entomol. 18: 241–243.

Dearn, J. M. 1981. Latitudinal cline in a colour pattern polymorphism in the Australian grasshopper *Phaulacridium vittatum*. Heredity 47: 111–119.

Dearn, J. M. 1983. Inheritance of the colour pattern polymorphism in the wingless grasshopper *Phaulacridium vittatum* Sjöstedt (Orthoptera: Acrididae). Aust. Entomol. 22: 217–218.

Dearn, J. M. 1984. Colour pattern polymorphism in the grasshopper *Phaulacridium vittatum*. I. Geographic variation in Victoria and evidence of habitat association. Aust. J. Zool. 32: 239–249.

Dearn, J. M. 1990. Color pattern polymorphism, pp. 517–549. *In* A. J. Chapman (ed.), Biology of grasshoppers. John Wiley and Sons, New York.

Denno, R. F. 1994. The evolution of dispersal polymorphisms in insects: the influence of habitats, host plants and mates. Res. Popul. Ecol. 36: 127.

Denno, R. F., G. K. Roderick, K. L. Olmstead, and H. G. Dobel. 1991. Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. Am. Nat. 138: 1513–1541.

Denno, R. F., G. K. Roderick, M. A. Peterson, A. F. Huberty, H. G. Dobel, M. D. Eubanks, J. E. Losey, and G. A. Langellotto. 1996. Habitat persistence underlies intraspecific variation in the dispersal strategies of planthoppers. Ecol. Monogr. 66: 389–408.

Drake, V. 1994. The influence of weather and climate on agriculturally important insects: an Australian perspective. Aust. J. Agric. Res. 45: 487–509.

Drake, V., and R. Farrow. 1988. The influence of atmospheric structure and motions on insect migration. Annu. Rev. Entomol. 33: 183–210.

Dudaniec, R. Y., C. J. Yong, L. T. Lancaster, E. I. Svensson, and B. Hansson. 2018. Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). Mol. Ecol. 27: 2576–2593.

Farrow, R., G. Nicolas, and J. Dowse. 1982. Migration in the macropterous form of the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt) during an outbreak. Aust. Entomol. 21: 307–308.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37: 4302–4315.

Fielding, D. J., and M. Brusven. 1990. Historical analysis of grasshopper (Orthoptera: Acrididae) population responses to climate in southern Idaho, 1950–1980. Environ. Entomol. 19: 1786–1791.

Follett, P. A., F. Calvert, and M. Golden. 2014. Genetic studies using the orange body color type of *Nezara viridula* (Hemiptera: Pentatomidae): inheritance, sperm precedence, and disassortative mating. Ann. Entomol. Soc. Am. 100: 433–438.

Forsman, A. 1999. Variation in thermal sensitivity of performance among colour morphs of a pygmy grasshopper. J. Evol. Biol. 12: 869–878.

Fox, J., and S. Weisberg. 2011. Multivariate linear models in R. An R companion to applied regression. Sage, Thousand Oaks, CA.

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21: 394–407.

Gray, S. M., and J. S. McKinnon. 2007. Linking color polymorphism maintenance and speciation. Trends Ecol. Evol. 22: 71–79.

Gomez, G. S. M., and H. Van Dyck. 2012. Ecotypic differentiation between urban and rural populations of the grasshopper *Chorthippus brunneus* relative to climate and habitat fragmentation. Oecologia 169: 125–133.

Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. J. Stat. Softw. 17: 1–27.

Guerra, P. A. 2011. Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: a meta-analysis. Biol. Rev. Camb. Philos. Soc. 86: 813–835.

Guerra, P. A., and G. S. Pollack. 2007. A life history trade-off between flight ability and reproductive behavior in male field crickets (*Gryllus texensis*). J. Insect Behav. 20: 377–387.

Harris, R., P. McQuillan, and L. Hughes. 2012. Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. J. Biogeogr. 39: 1450–1461.

Harris, R. M., P. McQuillan, and L. Hughes. 2013a. A test of the thermal melanism hypothesis in the wingless grasshopper *Phaulacridium vittatum*. J. Insect Sci. 13: 1–18.

Harris, R. M., P. McQuillan, and L. Hughes. 2013b. Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). PLoS One 8: e80243.

Harris, R. M., P. McQuillan, and L. Hughes. 2015. The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. J. Therm. Biol. 52: 75–83.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annu. Rev. Ecol. Syst. 11: 95–118.

Herrmann, D. L., A. E. Ko, S. Bhatt, J. E. Jannot, and S. A. Juliano. 2010. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. Ann. Entomol. Soc. Am. 103: 227–235.

Hijmans, R. J., and J. Van Etten. 2014. raster: Geographic data analysis and modeling. R package version, 2.2-31. (http://cran.r-project.org/package=raster).

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25: 1965–1978.

Ho, C. K., S. C. Pennings, and T. H. Carefoot. 2009. Is diet quality an overlooked mechanism for Bergmann's rule? Am. Nat. 175: 269–276.

Hoffmann, A. A. 2017. Rapid adaptation of invertebrate pests to climatic stress? Curr. Opin. Insect Sci. 21: 7–13.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66: 483–492.

Jepsen, J. U., S. B. Hagen, R. A. Ims, and N. G. Yoccoz. 2008. Climate change and outbreaks of the geometrids *Operophtera brumata* and *Epirrita autumnata* in subarctic birch forest: evidence of a recent outbreak range expansion. J. Anim. Ecol. 77: 257–264.

Jonas, J. L., W. Wolesensky, and A. Joern. 2015. Weather affects grasshopper population dynamics in continental grassland over annual and decadal periods. Rangeland Ecol. Manag. 68: 29–39.

Kaspari, M., and Vargo, E. L. 1995. Colony size as a buffer against seasonality: Bergmann's rule in social insects. Am. Nat. 145: 610–632.

Kearney, M., R. Shine, and W. P. Porter. 2009. The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. Proc. Natl. Acad. Sci. USA 106: 3835–3840.

Kelly, S. A., T. M. Panhuis, and A. M. Stoehr. 2012. Phenotypic plasticity: molecular mechanisms and adaptive significance. Compr. Physiol. 2: 1417–1439.

Kennington, W. J., J. R. Killeen, D. B. Goldstein, and L. Partridge. 2003. Rapid laboratory evolution of adult wing area in *Drosophila melanogaster* in response to humidity. Evolution 57: 932–936.

Key, K. L. 1992. Taxonomy of the genus *Phaulacridium* and a related new genus (Orthoptera: Acrididae). Invertebr. Syst. 6: 197–243.

Lancaster, L. T., R. Y. Dudaniec, B. Hansson, and E. I. Svensson. 2015. Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion. J. Biogeogr. 42: 1953–1963.

Lancaster, L. T., R. Y. Dudaniec, P. Chauhan, M. Wellenreuther, E. I. Svensson, and B. Hansson. 2016. Gene expression under thermal stress varies across a geographical range expansion front. Mol. Ecol. 25: 1141–1156.

Lancaster, L. T., R. Y. Dudaniec, B. Hansson, and E. I. Svensson. 2017. Do group dynamics affect colour morph clines during a range shift? J. Evol. Biol. 30: 728–737.

Le Rouzic, A., T. F. Hansen, T. P. Gosden, and E. I. Svensson. 2015. Evolutionary time-series analysis reveals the signature of frequency-dependent selection on a female mating polymorphism. Am. Nat. 185: E182–E196.

Liebhold, A. M., and P. C. Tobin. 2008. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53: 387–408.

Lindtke, D., K. Lucek, V. Soria-Carrasco, R. Villoutreix, T. E. Farkas, R. Riesch, S. R. Dennis, Z. Gompert, and P. Nosil. 2017. Long-term balancing selection on chromosomal variants associated with crypsis in a stick insect. Mol. Ecol. 26: 6189–6205.

Loch, A., and R. Floyd. 2001. Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in south-western Australia: history, current perspectives and future prospects. Austral Ecol. 26: 458–466.

Losey, J. E., J. Harmon, F. Ballantyne, and C. Brown. 1997. A polymorphism maintained by opposite patterns of parasitism and predation. Nature 388: 269.

Macfadyen, S., G. McDonald, and M. P. Hill. 2018. From species distributions to climate change adaptation: knowledge gaps in managing invertebrate pests in broad-acre grain crops. Agric. Ecosyst. Environ. 253: 208–219.

Masaki, S. 1967. Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). Evolution 21: 725–741.

McColloch, J., and W. P. Hayes. 1922. The reciprocal relation of soil and insects. Ecology 3: 288–301.

Miao, H. T., Y. Liu, L. Y. Shan, and G. L. Wu. 2018. Linkages of plant-soil interface habitat and grasshopper occurrence of typical grassland ecosystem. Ecol. Indic. 90: 324–333.

Milner, R., and C. Prior. 1994. Susceptibility of the Australian plague locust, *Chortoicetes terminifera*, and the wingless grasshopper, *Phaulacridium vittatum*, to the fungi Metarhizium spp. Biol. Control 4: 132–137.

Milner, R., T. Hartley, G. Lutton, and C. Prior. 1994. Control of *Phaulacridium vittatum* (Sjöstedt) (Orthoptera: Acrididae) in field cages using an oil-based spray of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycetina: Hyphomycetes). Aust. J. Entomol. 33: 165–167.

Nosil, P., R. Villoutreix, C. F. de Carvalho, T. E. Farkas, V. Soria-Carrasco, J. L. Feder, B. J. Crespi, and Z. Gompert. 2018. Natural selection and the predictability of evolution in Timema stick insects. Science 359: 765–770.

Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. Annu. Rev. Entomol. 43: 63–83.

Oerke, E. C. 2006. Crop losses to pests. J. Agric. Sci. 144: 31–43.

Olfert, O., and R. Weiss. 2006. Impact of climate change on potential distributions and relative abundances of *Oulema melanopus*, *Meligethes viridescens* and *Ceutorhynchus obstrictus* in Canada. Agric. Ecosyst. Environ. 113: 295–301.

(OEH) Office of Environment and Heritage. 2017. Great Soil Group (GSG) Soil Type map of NSW. NSW Office of Environment and Heritage, Sydney, Australia. (https://datasets.seed.nsw.gov.au/dataset/great-soil-group-gsg-soil-type-map-of-nsw1cf19) (accessed 17 November 2017).

Parmesan, C. 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biol. 13: 1860–1872.

Parsons, S. M., and A. Joern. 2014. Life history traits associated with body size covary along a latitudinal gradient in a generalist grasshopper. Oecologia 174: 379–391.

Pepper, J., and E. Hastings. 1952. The effects of solar radiation on grasshopper temperatures and activities. Ecology 33: 96–103.

Poniatowski, D., and T. Fartmann. 2009. Experimental evidence for density-determined wing dimorphism in two bush-crickets (Ensifera: Tettigoniidae). Eur. J. Entomol. 106: 599–605.

(QGIS) Quantum Geographic Information System Development Team. 2009. QGIS Geographic Information System. Open Source Geospatial Foundation. (https://www.qgis.org/en/site/).

(TERN) Terrestrial Ecosystems Research Network AusCover. 2011. Woody Extent and Foliage Projective Cover – SPOT, OEH algorithm, NSW coverage. Obtained from [http://www.auscover.org.au/purl/spot-woody-extent-fpc-nsw-2011 made available by the AusCover facility (http://www.auscover.org.au) of the Terrestrial Ecosystem Research Network (TERN, http://www.tern.org.au) (accessed 20 November 2017).

R Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, I. 2016. The Australian soil classification. CSIRO Publishing. (http://www.clw.csiro.au/aclep/asc_re_on_line/soilkey.htm).

Ríos Martínez, A., and A. Costamagna. 2018. Effects of crowding and host plant quality on morph determination in the soybean aphid, *Aphis glycines*. Entomol. Exp. Appl. 166: 53–62.

Roberts, R. 1972. A newly recognized from of pasture damage by *Phaulacridium vittatum* (Acrididae) on the northern tablelands of NSW. Aust. J. Entomol. 11: 257–258.

Roff, D. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. Oecologia 45: 202–208.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40: 1009–1020.

Rosetti, N., and M. I. Remis. 2018. Spatial variation in body size and wing dimorphism correlates with environmental conditions in the grasshopper *Dichroplus vittatus* (Orthoptera: Acrididae). Environ. Entomol. 47: 519–526.

Sasaki, R., F. Nakasuji, and K. Fujisaki. 2002. Environmental factors determining wing form in the lygaeid bug, *Dimorphopterus japonicus* (Heteroptera: Lygaeidae). Appl. Entomol. Zool. 37: 329–333.

Sgrò, C. M., J. S. Terblanche, and A. A. Hoffmann. 2016. What can plasticity contribute to insect responses to climate change? Annu. Rev. Entomol. 61: 433–451.

Simon, J. C., and J. Peccoud. 2018. Rapid evolution of aphid pests in agricultural environments. Curr. Opin. Insect Sci. 26: 17–24.

Steenman, A., A. Lehmann, and G. Lehmann. 2015. Life-history trade-off between macroptery and reproduction in the wing-dimorphic pygmy grasshopper *Tetrix subulata* (Orthoptera Tetrigidae). Ethol. Ecol. Evol. 27: 93–100.

Takahashi, Y., S. Morita, J. Yoshimura, and M. Watanabe. 2011. A geographic cline induced by negative frequency-dependent selection. BMC Evol. Biol. 11: 256.

Tanaka, S. 2004. Environmental control of body-color polyphenism in the American grasshopper, *Schistocerca americana*. Ann. Entomol. Soc. Am. 97: 293–301.

Tanaka, S., K. Harano, and Y. Nishide. 2012. Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity. J. Insect Physiol. 58: 89–101.

Trnka, M., F. Muška, D. Semerádová, M. Dubrovský, E. Kocmánková, and Z. Žalud. 2007. European corn borer life stage model: regional estimates of pest development and spatial distribution under present and future climate. Ecol. Model. 207: 61–84.

True, J. R. 2003. Insect melanism: the molecules matter. Trends Ecol. Evol. 18: 640–647.

Trullas, S. C., J. H. van Wyk, and J. R. Spotila. 2007. Thermal melanism in ectotherms. J. Therm. Biol. 32: 235–245.

Venette, R., and D. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97: 219–226.

Waller, J. T., and E. I. Svensson. 2017. Body size evolution in an old insect order: no evidence for Cope's rule in spite of fitness benefits of large size. Evolution 71: 2178–2193.

Zeng, Y., and D. H. Zhu. 2014. Geographical variation in body size, development time, and wing dimorphism in the cricket *Velarifictorus micado* (Orthoptera: Gryllidae). Ann. Entomol. Soc. Am. 107: 1066–1071.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42: 207–230.

Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 32: 95–126.

Zhang, Z., B. Cazelles, H. Tian, L. C. Stige, A. Bräuning, and N. C. Stenseth. 2009. Periodic temperature-associated drought/flood drives locust plagues in China. Proc. R. Soc. Lond. B Biol. Sci. 276: 823–831.

APPENDIX 2

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ORIGINAL ARTICLE

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Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (*Phaulacridium vittatum*)

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Abstract

Widespread species that exhibit both high gene flow and the capacity to occupy heterogeneous environments make excellent models for examining local selection processes along environmental gradients. Here we evaluate the influence of temperature and landscape variables on genetic connectivity and signatures of local adaptation in *Phaulacridium vittatum*, a widespread agricultural pest grasshopper, endemic to Australia. With sampling across a 900-km latitudinal gradient, we genotyped 185 *P. vittatum* from 19 sites at 11,408 single nucleotide polymorphisms (SNPs) using ddRAD sequencing. Despite high gene flow across sites (pairwise F_{ST} = 0.0003–0.08), landscape genetic resistance modelling identified a positive nonlinear effect of mean annual temperature on genetic connectivity. Urban areas and water bodies had a greater influence on genetic distance among sites than pasture, agricultural areas and forest. Together, F_{ST} outlier tests and environmental association analysis (EAA) detected 242 unique SNPs under putative selection, with the highest numbers associated with latitude, mean annual temperature and body size. A combination of landscape genetic connectivity analysis together with EAA identified mean annual temperature as a key driver of both neutral gene flow and environmental selection processes. Gene annotation of putatively adaptive SNPs matched with gene functions for olfaction, metabolic detoxification and ultraviolet light shielding. Our results imply that this widespread agricultural pest has the potential to spread and adapt under shifting temperature regimes and land cover change.

KEYWORDS
environmental association analysis, grasshopper, landscape genomics, landscape resistance, local adaptation, *Phaulacridium vittatum*

1 | INTRODUCTION

Differential local selection pressures can result in spatially structured patterns of adaptive genetic variation (Manel & Holderegger, 2013; Schoville et al., 2012) that are generally considered to arise when gene flow is limited (García-Ramos & Kirkpatrick, 1997). High gene flow between populations may interfere with local adaptation by homogenizing locally adapted genotypes and alleles under weak selection (Antonovics, 1976; Lenormand, 2002; Yeaman & Whitlock, 2011). However, the establishment and maintenance of local adaptation also depends on the introduction of novel genetic variation on which selection can act (Fitzpatrick, Gerberich, Kronenberger, Angeloni, & Funk, 2015; Kawecki & Ebert, 2004; Tigano & Friesen, 2016), and local adaptation may still occur under

the neutralizing effects of high gene flow (e.g., birds: Attard et al., 2018; trees: Gonzalo-Turpin & Hazard, 2009; sharks: Momigliano et al., 2017; frogs: Muir, Biek, Thomas, & Mable, 2014). Despite expectations that high genetic connectivity limits selection on adaptive loci within populations (Barton & Partridge, 2000; Kawecki & Ebert, 2004; Rasanen & Hendry, 2008), what we know about the interplay between gene flow and local adaptation remains varied and inconsistent (Savolainen, Lascoux, & Merila, 2013). Therefore, concurrent examination of both neutral genetic connectivity and signatures of selection are needed to understand their relative contribution towards shaping adaptive genetic processes.

The interaction between gene flow and local adaptation is of particular interest for species that occupy large geographical ranges and a broad ecological niche. Many insect pests fit this description and experience variable environmental conditions across heterogeneous landscapes. Studies have reported the influence of abiotic environmental variables (e.g., Llewellyn et al., 2003; Sandrock, Razmjou, & Vorburger, 2011; Zhang et al., 2018), geographical barriers (Wang et al., 2017) and landscape features (Keller et al., 2013; Lozier, Strange, & Koch, 2013) on neutral gene flow in widespread insects. Regarding local adaptation in insect pests, some studies identify loci under selection in relation to environmental gradients (Feng, Jiang, & Fan, 2015; Janes et al., 2014; Manel, Conord, & Després, 2009), and insecticide resistance (Crossley, Chen, Groves, & Schoville, 2017; Paris et al., 2010). However, selection processes relating to climate variables have not been well documented in insect pests (Hoffmann, 2017; Kirk, Dorn, & Mazzi, 2013; Pelissie, Crossley, Cohen, & Schoville, 2018), resulting in limited knowledge about their evolutionary response to shifting climate regimes. Despite their clear interaction, studies that simultaneously examine landscape or climate-mediated gene flow, as well as selection processes in the same study system are scarce.

Polymorphisms in physical characteristics are often observed across the distributions of some invasive or pest insects (e.g., Pener & Simpson, 2009), as well as developmental trait changes in response to temperature gradients, such as changes in hatching time (Falt-Nardmann, Klemola, Roth, Ruohomaki, & Saikkonen, 2016), diapause (Moraiti, Nakas, & Papadopoulos, 2014) and voltinism (Yamanaka, Tatsuki, & Shimada, 2008). In insects, morphological traits relevant for dispersal (e.g., wing presence, wing length), thermoregulation (e.g., melanism) and fecundity (e.g., body size) can offer fitness advantages across variable environments (Roff, 1986; Rosetti & Remis, 2018; Tanaka, Harano, & Nishide, 2012; Trullas, van Wyk, & Spotila, 2007), and may lead to selection at the genomic level (Bai, Dong, Guan, Xie, & Xu, 2016; Brisson, 2010; Maeno & Tanaka, 2010; Zhang, Brisson, & Xu, 2019). The success of widespread generalist insects appears to be largely determined by interactions between morphology, environment and behaviour, which are affected by both neutral and adaptive genetic processes. Teasing apart these processes for economically important insect species is a contemporary challenge under current climate and land use change.

Landscape genomics approaches allow for complementary assessment of how environmental and landscape features influence genetic connectivity (Manel & Holderegger, 2013), and environmental selection processes (Ahrens et al., 2018; Balkenhol et al., 2017). Recent advancements in statistical approaches such as environmental association analyses (EAAs) allow for the correlation between allele frequency and environmental variables to be tested (Coop, Witonsky, Di Rienzo, & Pritchard, 2010; Frichot, Schoville, Bouchard, & Francois, 2013; Gunther & Coop, 2013), and may identify selection on many genes of small effect (Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015). Identifying key drivers of neutral and adaptive landscape genomic relationships requires concurrent analyses of genetic connectivity and environmental selection using the same data set. For widespread species in particular, such a two-tiered analytical approach allows for a more complete understanding of how neutral genetic connectivity interacts with adaptive genetic divergence.

Commonly known as the wingless grasshopper, *Phaulacridium vittatum* is endemic to Australia and considered a pest of agricultural pasture and crops (Key, 1992). Despite existing historically in low numbers within native forests and grasslands, increasing pastural and agricultural land use is thought to have triggered population expansions and outbreaks (Dearn, 1984; Key, 1992). Adult *P. vittatum* can cause serious damage to pasture and compete with livestock for green clover when green pasture is at a shortage during the summer (Bailey, Frensham, Hincks, & Newton, 1994). The species also causes damage to tree nurseries (including native eucalyptus; Loch & Floyd, 2001), and economically important crops such as grapes, vegetables and fruit trees (Clark, 1967; Roberts, 1972). Cyclical outbreaks of *P. vittatum* have been recorded every 4 or 5 years and are often observed during unusually dry weather conditions (Roberts, 1972).

Morphological variation in *P. vittatum* has been described in four distinct traits: wing presence/absence, dorsal stripe polymorphism, colour polymorphism and body size (Harris, McQuillan, & Hughes, 2012; Yadav, Stow, Harris, & Dudaniec, 2018). Previous studies on *P. vittatum* have shown significant associations between wing presence, stripe polymorphism and body size with local habitat (i.e., foliage cover), climatic conditions (i.e., solar radiation, precipitation) and latitude (Clark, 1967; Dearn, 1984; Harris et al., 2012; Harris, McQuillan, & Hughes, 2013a, 2015; Yadav et al., 2018). Furthermore, melanism determined by the presence or absence of dorsal stripes provides a thermoregulatory advantage such that darker individuals with no stripes maintain higher body temperatures (Harris, McQuillan, & Hughes, 2013a, 2013b). These previous findings provide evidence for morphological tracking of environmental gradients and suggest a fitness advantage of different morphotypes in particular environments. However, the genetic basis or selection processes acting on this morphological variation are unknown.

Given that *P. vittatum* is common, with a large population size, broad distribution and a capacity to move long distances (Key, 1992), high levels of gene flow are expected. Nonetheless we predict that landscape and climatic features will affect genetic connectivity and adaptive genetic variation given the diverse environmental conditions the species occupies. With a sampling design spanning an ~900-km latitudinal gradient of the *P. vittatum* distribution along the east coast of Australia, we conduct a landscape genomic analysis to

examine: (a) how temperature and land cover affect neutral genetic connectivity; and (b) whether patterns of adaptive genetic variation are associated with environmental variables and/or variation in morphological traits (via F_{ST} outlier tests and EAA). Our approach demonstrates a simultaneous assessment of landscape and climate effects on neutral gene flow and adaptive genetic variation, with implications for informing spatially explicit management strategies for pest species across environmental gradients.

2 | MATERIALS AND METHODS

2.1 | Study species and sampling

The wingless grasshopper (*Phaulacridium vittatum*; Acrididae) is endemic to Australia with a widespread distribution in the eastern parts of the continent (23–43°S; 0–1,500 m; Key, 1992), largely concentrated towards the coastline where agricultural areas and grazed pastures are dominant. This species breeds annually and has nonoverlapping generations. Hatching takes place in late spring (October–December) with adults emerging in summer (December–February). Development involves five instar stages and adults survive until late March and sometimes into April or May (Clark, 1967).

Collection of *P. vittatum* was undertaken in late January to early February in 2017, for 2 weeks, from 19 sites that spanned ~900 km between 29.18 and 36.52°S (Table S1 in Appendix S1; Figure 1a). Spatial coordinates (latitude, longitude) and elevation were recorded for each site using a handheld GPS device (Garmin International). We implemented a paired-gradient sampling design where possible, with at least two sites per latitudinal range (see Dudaniec, Yong, Lancaster, Svensson, & Hansson, 2018). This design is recommended to ensure representation of environmental heterogeneity with spatial replication (Rellstab et al., 2015), and is found to be more effective at detecting weak spatial selection in comparison with random or transect designs (Lotterhos & Whitlock, 2015). Grasshoppers were collected from semibare grassy patches along roadsides, borders of national parks, dry and green pastures, and from shrubland in the Australian Kosciuszko alpine region, with a total elevational range spanning 133–1,573 m above sea level (a.s.l.) and mean annual temperature gradient ranging from 6.0 to 17.4°C (Table 1). Insect sweep nets or small plastic jars were used to capture *P. vittatum*, and approximately equal proportions of males and females were collected from each site. All samples were stored in 95% ethanol for DNA preservation.

2.2 | Morphological data

Phaulacridium vittatum exhibits variation in wing presence or absence; the majority of individuals have short nonfunctional wings whereas others have long functional wings. Studies have demonstrated an increased frequency of winged individuals in areas of high vegetation cover and near forested habitats (Clark, 1967; Yadav et al., 2018). This species also exhibits a stripe pattern polymorphism with three morphotypes (Dearn, 1984): (a) two complete white dorsal longitudinal stripes, (b) partial white stripes from head to pronotum and (c) complete absence of stripes (Key, 1992). Stripe polymorphism appears to be controlled by a tri-allelic autosomal locus (C^S, full stripe; C^I, partial stripe; C^P, plain) with a dominance hierarchy, represented as, $C^S > C^I > C^P$ (Dearn, 1983). The function of stripes is not fully understood but may have implications for predator avoidance, mate choice or thermoregulation (Dearn, 1980, 1984; Harris et al., 2013a, 2013b; Yadav et al., 2018). We examined morphological characters for each captured live individual and recorded data in the following categories: (a) stripe polymorphism: fully striped, partially striped and unstriped; and (b) wing dimorphism: winged and wingless. We measured femur length (in mm) using vernier calipers (accurate to 0.02 mm), which is a commonly used proxy for body size in grasshoppers (Lehmann & Lehmann, 2008; Masaki, 1967; Mousseau & Roff, 1989; Yadav et al., 2018).

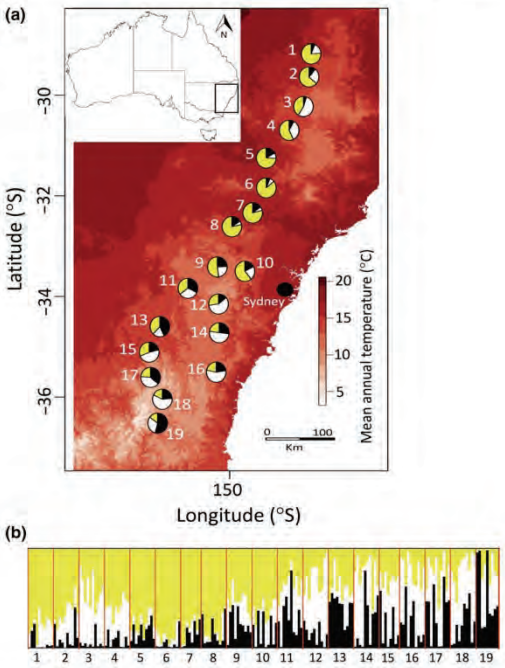

FIGURE 1 (a) Map of study area and sample sites (n = 19) displayed against Mean Annual Temperature (BIO1). STRUCTURE analysis indicated three genetic clusters (K = 3) with the proportion of individuals assigned to each cluster per site depicted in coloured pie charts (yellow = cluster 1; white = cluster 2; black = cluster 3). (b) Bar plot of assignment probabilities to each cluster for each individual using STRUCTURE. Dividing lines separate the 19 sites [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | DNA extraction, sequencing and filtering

DNA extraction was undertaken using the head tissue of 188 individuals of *P. vittatum* (from 19 sites), performed at the Diversity Arrays

TABLE 1 Sample size for each site (Site ID), numbers of males and females, elevation (m) and mean annual temperature (°C)

Site ID	No. of samples	Females	Males	Elevation (m)	Mean annual temperature (°C)
1	10	5	5	802	15
2	10	5	5	1,103	13.2
3	10	5	5	1,309	11.6
4	10	5	5	1,045	12.8
5	10	5	5	483	16
6	10	5	5	413	15.9
7	8	4	4	133	17.4
8	10	5	5	421	15.5
9	10	5	5	816	12.5
10	10	5	5	984	11.8
11	10	3	7	738	12.5
12	10	5	5	982	11.1
13	10	5	5	400	14.3
14	10	5	5	633	13.1
15	8	4	4	226	15.4
16	10	6	4	665	12.5
17	10	5	5	1,004	10.4
18	10	5	5	1,573	6.7
19	9	4	5	1,548	6

Technology sequencing (DArTseq) facility (Canberra, Australia; more detail in Appendix S1). A modified double-digest restriction-site associated DNA (ddRAD) sequencing protocol described in Kilian et al. (2012) was performed using a combination of *Pst*I–*Hpa*II restriction enzymes in library preparation. The *Pst*I enzyme adaptor was modified to contain an Illumina adaptor sequence, primer sequence and a variable-length barcode as described by Elshire et al. (2011), whereas the *Hpa*II adaptor contained an Illumina flow cell attachment and overhang sequence. Digested and ligated fragments with *Pst*I–*Hpa*II adaptors were amplified and sequenced on an Illumina HiSeq2500 and after trimming, reads of 65 bp in length were obtained, each with a unique identifier. DNA sequences were aligned via BLAST using the *Locusta migratoria* reference genome with an *E*-value of 5e−3 and minimum sequence identity of >70%. To check for contamination, sequences were also aligned to bacterial and fungal genomes (NCBI). Further information on single nucleotide polymorphism (SNP) calling, quality checking and filtering is provided in Text S1: Appendix S1).

DNA sequences were subject to further filtering using the *filter_dart()* function of the RADIATOR package (Gosselin, 2017) in R (R Core Team, 2017). Monomorphic SNPs were excluded followed by removal of SNPs with a reproducibility of <90%, a call rate of <60% (i.e., the genotype is called in a minimum of 60% of the samples) and a depth of coverage of <8×. A minor allele frequency (MAF) threshold of ≥0.02 was applied to filter alleles using an SNP-based approach to exclude alleles potentially resulting from sequencing error. A minimum of two populations (out of 19) was required to pass the MAF threshold in order to retain the SNP marker.

2.4 | F_{ST} outlier detection

Analyses to detect selection were conducted on the full SNP data set, which included all SNPs per RAD tag but excluded tightly linked loci identified using PLINK version 1.9 (Purcell et al., 2007; with one marker from each pair excluded with $R^2 > 0.8$; following Xuereb, Benestan, et al., 2018). We used two F_{ST} outlier detection methods to assess congruence across approaches: (a) OUTFLANK (Whitlock & Lotterhos, 2015) and (b) BAYESCAN 2.1 (Foll & Gaggiotti, 2008). OUTFLANK is based on the Lewontin and Krakauer (1973) method, while accounting for sampling error and nonindependence between sampled populations. OUTFLANK detects outliers under divergent selection by initially inferring the F_{ST} distribution from multiple loci and then fits a χ^2 model to the centre of the distribution, resulting in a null distribution. This null distribution is then used to detect outlier loci. We used a left and right trim value of 0.05 as suggested by Whitlock and Lotterhos (2015). Two runs of OUTFLANK were performed, initially setting the total number of putative genetic clusters (*K*) to 19 (total number of sites) and a second run was performed using *K* based on the outcome of the STRUCTURE analysis (see below). Loci with an expected heterozygosity of <10% were excluded as recommended by Whitlock and Lotterhos (2015), and the false discovery rate (FDR) was set to ≤5%.

BAYESCAN works on a Dirichlet multinomial distribution and accounts for different rates of immigration across subpopulations. Outlier loci under selection are detected by estimating Bayesian posterior probability using a reversible jump Markov chain Monte Carlo (MCMC) algorithm. BAYESCAN identifies putative SNPs under positive (i.e., leads to increasing F_{ST}) or negative (i.e., leads to decreasing F_{ST}) selection, the latter having much lower certainty and a higher FDR. In BAYESCAN, we implemented a 50,000 burn-in, 100,000 total number of iterations, prior odds set to 100 and FDR of ≤5%.

2.5 | Genetic structure analysis

To obtain a putatively neutral SNP data set for genetic structure analyses, an "unlinked" data set with a single SNP per locus (chosen randomly per RAD tag) was generated to reduce the effects of physical linkage. We tested for loci deviating from Hardy–Weinberg equilibrium (HWE) using the *pegas* R package (Paradis, 2010) and excluded loci from the "unlinked" data set that deviated from HWE at a 0.05 (simulations = 1,000) significance threshold in at least 10 of our 19 sites. Finally, we removed F_{ST} outliers identified above and this filtered data set was used for genetic structure and connectivity analyses.

We calculated pairwise F_{ST} between 19 sites (Reynolds, Weir, & Cockerham, 1983) in ARLEQUIN 3.5 (Excoffier & Lischer, 2010) with a 0.05 significance level and 1,000 permutations. Isolation by distance (IBD) was assessed using a Mantel test of pairwise F_{ST} and

geographical distance (km) between sites (number of permutations = 100,000) in the VEGAN version 2.5-2 package in R (Oksanen et al., 2013). Genetic structure was further investigated using STRUCTURE 2.3.2 (Pritchard, Stephens, & Donnelly, 2000), which uses a Bayesian algorithm to assign individuals with shared ancestry into groups. Genetic structure analysis was carried out without providing prior location information, using the admixture model with correlated allele frequencies. To reduce computational time, we ran STRUCTURE in parallel on multiple cores using STRAUTO (Chhatre & Emerson, 2017). We used 50,000 burn-in, 500,000 MCMC repeats with a range of *K* from one to 12 and 10 iterations per *K*. The optimal *K* was selected using STRUCTURESELECTOR (Li & Liu, 2018), which assigns a subpopulation to a cluster if the membership coefficient mean MedMeaK (median of means), MaxMeaK (maximum of means) or median MedMedK (median of medians) and "MaxMedK" (maximum of medians) to a particular cluster is greater than the threshold value of 0.5. In addition, STRUCTURESELECTOR also uses the Δ*K* method (Evanno, Regnaut, & Goudet, 2005; Puechmaille, 2016), which compares the rate of change in log likelihood between each *K* and selects *K* with the maximum value of Δ*K*.

2.6 | Temperature and landscape data

To test the effects of temperature and landscape variables on genetic connectivity in *P. vittatum*, two geographical data sets were used in raster format: (a) mean annual temperature (BIO1, hereafter referred to as "Annual Temp"); and (b) land cover. Annual temperature data for the study extent were downloaded from the WORLDCLIM version 1.4 database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) at ~1-km^2 resolution. Temperature was predicted to be a major factor influencing genetic connectivity due to its important role in dispersal (Whitman, 1987), the population dynamics of grasshoppers (Chapman, 1990) and the distribution of herbivorous ectotherms (Bale et al., 2002; Pepper & Hastings, 1952). The preferred temperature range of *P. vittatum* is 27.5–30°C, and it avoids temperatures below 15°C (Harris, McQuillan, & Hughes, 2015). Thus, we hypothesized warmer areas would provide less resistance to dispersal for *P. vittatum*. The values for Annual Temp were therefore inverted to test for increasing resistance as temperature decreases.

Land cover data were downloaded from the Department of Agriculture and Water Resources, The Australian Bureau of Agricultural and Resource Economics and Sciences, Land use management (ABARES, 2017) and converted to a cell resolution of 150 × 150 m. Based on prior ecological knowledge of *P. vittatum*'s distribution and abundance in different habitats and locations (Clark, 1967), we collapsed land cover data into five categories from least to most resistance by recoding the corresponding categories within the raster from 1 to 5 using the *r.reclass* module in GRASS GIS (Neteler, Bowman, Landa, & Metz, 2012). The categories were as follows: (a) pastures and agricultural areas, (b) rural infrastructure, (c) forest and conservation areas, (d) urban area and (e) water bodies (Figure S1 in Appendix S1).

The lowest resistance to movement was assigned to pasture and agricultural areas. Our previous study supports this classification because sites with the highest relative abundance of *P. vittatum* had low vegetation cover and occurred in agricultural areas (Yadav et al., 2018), while such areas are also suitable for egg laying in *P. vittatum* (Clark, 1967). The second least-resistance land cover was assigned to rural areas consisting of animal and plant production facilities and rural residential areas with and without agriculture, which are often present close to pastoral areas. The third ranking was assigned to forest and conservation areas. Forested areas were hypothesized to provide more resistance to movement than rural areas because *P. vittatum* is absent from rainforest and dense sclerophyll forested areas (Key, 1992) but can be found near the boundary of pasture and forested areas (Dearn, 1984), and grasslands within forested national parks. Urban areas were assigned with fourth ranking (i.e., manufacturing, urban residential, transport and other service infrastructure). The final and most resistant land cover was assigned to water bodies (i.e., lake, reservoir, river, marsh and estuary water), which are considered a significant barrier to movement.

2.7 | Landscape resistance surface generation

To assess the influence of temperature and landscape variables on genetic connectivity, we used an isolation by resistance approach applying circuit theory (McRae & Beier, 2007) and sample size-corrected Akaike information criterion (AICc) model selection. This approach has been shown to effectively identify isolation by resistance including linear and nonlinear relationships (Dudaniec et al., 2013, 2016; Shirk, Wallin, Cushman, Rice, & Warheit, 2010). We generated multiple resistance surfaces from our parameterized Annual Temp and Land Cover data to test for multiple hypotheses about their effects on genetic distance. To generate alternative resistance surfaces with varying resistance, we applied a similar method as implemented by Dudaniec et al. (2013, 2016), which is a modified approach of Shirk et al. (2010), whereby different values of intercept (α) and slope (γ) parameters are used to create linear and nonlinear resistance surfaces. We applied the following formulas to generate resistance relationships:

$$r_i = 1 + \alpha\,(T_i - 1/\max - 1)^{\gamma} \quad (1)$$

$$r_i = 1 + \alpha\,(L_i - 1/\max - 1)^{\gamma} \quad (2)$$

where r_i is the resistance of cell *i*, T_i is the Annual Temp of cell *i* (Equation 1), L_i is the rank of Land Cover type of cell *i* (Equation 2) and *max* is the maximum value of the raster surface. Furthermore, α determines the maximum possible resistance value and γ determines the shape of the relationship (slope) between tested variables and resistance. As outlined above, resistance was assumed to be negatively associated with Annual Temp and positively associated with Land Cover rank.

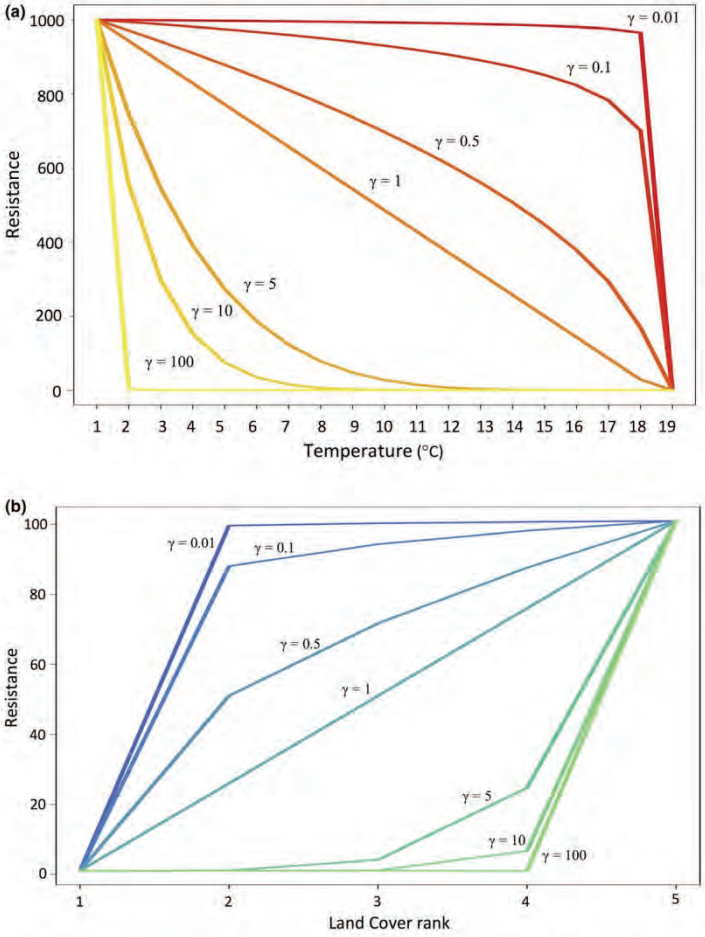

FIGURE 2 Line plots of the landscape resistance relationships tested for (a) Mean Annual Temperature (BIO1) and (b) Land Cover. The gamma (γ) value represents the slope of the relationship tested (i.e., linear vs. nonlinear relationships between resistance distance and genetic distance). Alpha (α) represents the maximum resistance value. In (b), Land Cover rankings are as follows: 1 = pasture and agricultural areas, 2 = rural development, 3 = forest and conservation areas, 4 = urban areas, 5 = water bodies [Colour figure can be viewed at wileyonlinelibrary.com]

We created resistance surfaces for all combinations by varying the values of parameters α and γ. We used five α values and seven γ values: α = 0, 5, 10, 100, 1,000 and γ = 0.01, 0.1, 0.5, 1, 5, 10, 100 for each of the Annual Temp and Land Cover data sets, resulting in 29 different resistance surfaces for each data set with subtle differences in the degree of resistance. An α = 0 equated to a null IBD model where all raster cells were assigned a value equal to 1, indicating no influence of resistance on genetic connectivity. Values of γ < 1 indicate resistance surfaces with increased sensitivity, γ = 1 a linear resistance relationship and γ> 1 resistance surfaces with reduced resistance sensitivity (Figure 2).

Pairwise resistance distance matrices were calculated for all 29 transformed rasters for both variables (i.e., Annual Temp and Land Cover) using CIRCUITSCAPE version 4.0.5 (McRae & Beier, 2007). CIRCUITSCAPE applies electrical circuit theory to identify all possible pathways of movement between focal points across a given raster data set and calculates average cumulative resistance between each pair of the sampling site. The pairwise mode was used to obtain pairwise resistance matrices between 19 sample sites with a four-cell connection scheme such that each sampling site was connected to its neighbouring four cells.

2.8 | Landscape genetic resistance modelling

Pairwise resistance distances between sites created in CIRCUITSCAPE for both Annual Temp and Land Cover were input as predictors of genetic distance (F_{ST}) between sites using a mixed-effects model with maximum likelihood population-effects (MLPE; Row, Knick, Oyler-McCance, Lougheed, & Fedy, 2017) as implemented within the *MLPE_rga()* function of the "ResistanceGA" R package (Peterman, 2018). The MLPE approach was found to have the highest accuracy in predicting landscape resistance relationships out of seven regression-based model selection methods assessed in a recent review (Shirk, Landguth, & Cushman, 2018).

To identify which model(s) best described genetic distance between sites, multimodel inference was used to obtain a candidate list of models based on AICc scores for both Annual Temp and Land Cover in the MUMIN version 1.40.4 R package (Barton, 2018). As AIC model selection was found to outperform other model selection approaches within MLPE (Row et al., 2017) we used AICc to evaluate the goodness of fit (Burnham & Anderson, 2003). The model with the lowest change in AICc score (ΔAICc = 0) and largest AIC weight (wAICc) was considered as most parsimonious. The inference was made from the relative importance of multiple models, which is more informative than a single best-selected model in the case of model uncertainty (Burnham & Anderson, 2003). We followed the ΔAICc criteria classification mentioned in Burnham and Anderson (2003) to obtain the models with the highest support.

2.9 | Environmental data for EAA

We chose environmental variables deemed relevant for local adaptation in *P. vittatum* based on existing ecological and biological knowledge of the species (Harris et al., 2012, 2015; Yadav et al., 2018). Justification of the environmental variables used in EAA is presented in Text S2: Appendix S1. Annual Temp, annual precipitation (BIO12), mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter (BIO11), precipitation of the warmest quarter (BIO18) and precipitation of the coldest quarter (BIO19) were extracted from WORLDCLIM version 1.4 (Hijmans et al., 2005). Data for annual wind speed and annual solar radiation were extracted from WORDCLIM version 2.0 (Fick & Hijmans, 2017). All data were extracted at a resolution of ~1 km^2 (Table S1 in Appendix S1 per site information). Foliage Projective Cover (% FPC) was extracted from the Terrestrial Ecosystems Research Network AusCover database (TERNAusCover, 2011) at a resolution of 5 × 5 m. To extract FPC values, a buffer of 100 m was used around each sampling site from which cell values were averaged using the *raster* R package (Hijmans & van Etten, 2016).

To determine soil moisture and soil acidity per site, soil type data for each site were obtained from the Office of Environment and Heritage, New South Wales (OEH, 2017) and raster values were extracted for each site using QGIS version 2.18 (QGIS, 2016). Soil moisture per site was classified based on clay content and water-holding capacity of the soil as described by The Australian Soils Classification guidelines (ASC) and "Great Soil Group" (GSG; Raymond, 2016). Likewise, pH was used to assess soil acidity: soils with pH < 5.5 were classified as "acidic" and coded as 1, whereas soils with pH > 5.5 were classified as "non-acidic" and were coded as 0 (Table S1 in Appendix S1), which is consistent with the soil acidity classification criteria of ASC and GSG.

To assess collinearity between the 11 environmental variables, latitude, longitude and elevation for use in EAA, we performed pairwise Pearson's correlation analyses of the site-based environmental data in R (Table S2 in Appendix S1) and discarded highly correlated variables (|*r*|> 0.80). The removal of highly correlated environmental variables is recommended for EAA as high collinearity may limit the ability to tease apart effects of individual variables and may lead to inconclusive results (Rellstab et al., 2015). After removing highly correlated variables, latitude plus six environmental variables were used in the Latent Factor Mixed Model (LFMM) program: (a) Annual Temp, (b) BIO18, (c) Wind Speed, (d) % FPC, (e) soil moisture and (f) soil acidity. We chose to retain Wind Speed despite having a high correlation with Annual Temp ($r = -0.86$) due to its significant role in determining the relative abundance of *P. vittatum* (Yadav et al., 2018) and potential role in selection in insects (e.g., Dudaniec et al., 2018).

2.10 | Morphological association analysis

Three discrete morphological traits were examined for significant SNP × morphology associations: wing dimorphism, stripe polymorphism and body size. Collinearity among the three morphological predictors and between the morphological and environmental predictors was assessed (Pearson |*r*|> 0.80) before including them in the analysis (Table S2 in Appendix S1). Winged individuals were coded as 1 and wingless individuals as 0. Stripe pattern was divided into four categories: (a) fully striped, (b) partially striped, (c) both fully and partially striped ("Striped"), and (d) unstriped (described in Yadav et al., 2018). Finally, body size (based on femur length) was tested for SNP associations as a continuous variable. Body size is sex biased in this species, whereby females have larger body size than males, and therefore to confirm outliers obtained from EAA are associated with body size and not biased by sex, we performed a *t*-test on allele frequencies of each outlier SNP and sex (coded as a binary variable).

2.11 | Detecting environmental and morphological signatures of selection: EAA

Using the full data set, we conducted SNP-association analyses for both environmental variables and morphological traits using the LFMM program (Frichot et al., 2013) within the LEA R package (Frichot & Francois, 2015). LFMM examines the association between environmental factors and allele frequencies using the MCMC algorithm while accounting for neutral genetic structure by introducing it as a latent factor and can effectively detect loci under weak selection (Frichot et al., 2013). We performed LFMM with 100,000 iterations after a 10,000 burn-in, five repetitions for each variable and the number of latent factors was based on STRUCTURE analysis outcome. Median *z* scores were combined across five runs, and adjusted *p* values were calculated as recommended by Frichot and Francois (2015). The Benjamini–Hochberg procedure was applied to obtain candidate loci with an expected FDR of 5%. To examine inflation in the test statistics, a genomic inflation factor (GIF) was calculated using the formula λ = median (z^2)/0.456 as described by Devlin and Roeder (1999) for each variable individually. GIF scores of each variable were then assessed for its closeness to 1.0 as suggested by Frichot and Francois (2015). When LFMM detected more than one SNP marker on the same RAD tag, we randomly retained

one of the markers for analyses of common and unique associations among variables.

2.12 | Gene annotation

Gene annotations were sought for all unique RAD tags that contained SNPs under putative selection (identified via F_{ST} outlier tests and EAA) using the NCBI BLAST platform (Johnson et al., 2008). Due to the unavailability of a *P. vittatum* genome, RAD tags were aligned to the *Locusta migratoria* genome (Wang et al., 2014) using the BLASTN mode, with an *E*-value threshold of 0.0001. Sequences were also annotated to genes classified as "insects" (Taxid:6960), using BLAST with an *E*-value threshold of 0.0001.

3 | RESULTS

3.1 | Data filtering

The sample size of sequenced individuals ranged from eight to 10 individuals per site (mean = 9.72 ± 0.16; Table 1). A total of 37,644 SNP markers (including polymorphic and monomorphic) were initially obtained from DArTseq with an average polymorphic information content (PIC; an index ranging from zero to one that indicates allelic variation per SNP marker) of 0.11. After filtering SNPs using *filter_dart()*, we identified 56 tightly linked SNPs (i.e., SNPs in linkage disequilibrium with $R^2 > 0.8$) using PLINK version 1.9 that were then excluded, resulting in a final full data set of 11,408 SNPs. For genetic structure analysis, a total of 3,257 SNPs were retained, which included only one SNP per RAD tag (i.e., "unlinked") and excluded F_{ST} outliers (see below), and loci deviating from HWE (n = 203). Three samples were excluded from the data set due to poor sample quality or a high proportion of missing data, resulting in a final sample size of 185 individuals.

3.2 | F_{ST} outlier detection

Out of 11,408 total SNPs, OUTFLANK identified three outlier SNPs under divergent selection that showed no evidence of linkage as they were located on separate DNA fragments and were not identified to be in linkage disequilibrium using PLINK version 1.9 (Table S3, Figure S2A in Appendix S1). Both runs of OUTFLANK (i.e., with K = 19 and K = 3; identified by STRUCTURE, below) identified the same three outlier SNPs. BAYESCAN identified 16 outlier SNPs under divergent selection, also located on distinct DNA fragments (Table S4, Figure S2B in Appendix S1) and a single outlier under balancing selection. All three outliers detected by OUTFLANK were in common with the outliers detected by BAYESCAN.

3.3 | Genetic structure

Pairwise F_{ST} using 3,257 SNPs (filtered as described above) varied between 0.0003 and 0.08 across sites with mean F_{ST} = 0.030 (Table S5 in Appendix S1). A Mantel test indicated no IBD (p = 0.79, Mantel statistic r = −0.080; Figure S3 in Appendix S1). STRUCTURE (Pritchard et al., 2000) analysis indicated the most likely number of genetic clusters to be three (K = 3), based on the agreement between ΔK method and estimators of STRUCTURESELECTOR (Figure S4 in Appendix S1). Out of the four estimators of STRUCTURESELECTOR i.e., MedMed, MedMean, MaxMed and MaxMean, all except MaxMed indicated optimal K to be 3 (Figure S4 in Appendix S1). Based on these results we used K = 3 as the optimal K for further analyses. However, individuals were weakly assigned to each cluster with high genetic admixture (Figure 1b). Based on a ≥0.50 assignment probability cut-off, mean (±*SE*) assignment probabilities to each cluster were as follows: cluster 1 = 0.70 (±0.01), cluster 2 = 0.68 (±0.05) and cluster 3 = 0.67 (±0.02). Individuals from more northern and midlatitudinal ranges (sites 1–11) were predominantly assigned to cluster 1 (Figure 1b), whereas individuals from southern latitudes (sites 12–19) were chiefly assigned to cluster 2. Some northern latitude sites (sites 1–4) also showed a greater assignment to cluster 2 than midlatitudinal sites (sites 5–8). Cluster 3 has a low frequency overall but was represented more in southern sites (sites 15–19).

3.4 | Landscape genetic resistance

For Annual Temp, models with ΔAICc > 1 were excluded from the confidence set of models (Table 2), which resulted in a candidate set of eight resistance surfaces (out of 29). The best supported resistance surface for Annual Temp was α = 1,000 and γ = 0.5 (ΔAICc = 0.00, w_i = 0.07), which indicates that maximum resistance is 1,000 times greater than zero (Figure 3a). The value of γ = 0.5 indicates temperature influenced genetic connectivity nonlinearly, with upper mean annual temperatures of around 19°C providing close to zero resistance to genetic connectivity (Figure 2a). Together, the eight-confidence set of models covered a weight of 0.46 (Table 2). All confidence sets of resistance surfaces had γ values < 1, indicating resistance surfaces with increased resistance sensitivity were most well supported.

For Land Cover, models with ΔAICc > 2 were excluded from the candidate set of models, which provided a set of 10 candidate resistance surfaces (Table 2). The best supported resistance surface had α = 100 and γ = 10 (ΔAICc = 0.00, w_i = 0.18; Table 2, Figure 3b). This suggests that the landscape resistance to gene flow is 100 times more resistant than the null model (i.e., zero) and shows a nonlinear relationship across sites. Resistance due to land cover rank was close to zero until rank three (i.e., pasture/agricultural areas, rural infrastructure, and forest/conservation areas), but started to increase up to rank five (i.e., water bodies; Figure 2b). Together, a confidence set of 10 models covered a total weight of 1.00 (Table 2). The confidence set of models had γ values ranging from 5, 10 and 100 (i.e., above γ = 1), indicating resistance surfaces with lower land cover resistance are favoured in the model selection. Furthermore, our best-selected resistance surface for Land Cover was 10 times less resistant to genetic connectivity (α = 100) compared to the best-selected Annual Temp resistance surface (α = 1,000; Figure 3b), indicating a greater impact of temperature on genetic connectivity than land cover.

TABLE 2 The candidate set of landscape resistance models shown in relation to the null model (no resistance, all cells = 0) for mean annual temperature (BIO1) and Land Cover

Alpha (α)	Gamma (γ)	logLik	AICc	Delta	Weight (*wi*)
Mean annual temperature					
1,000	0.5	473.58	−938.92	0.00	0.07
100	0.5	473.57	−938.91	0.01	0.07
10	0.5	473.52	−938.79	0.13	0.07
1,000	0.1	473.23	−938.21	0.71	0.05
5	0.5	473.23	−938.21	0.71	0.05
100	0.1	473.22	−938.21	0.71	0.05
10	0.1	473.21	−938.17	0.75	0.05
5	0.1	473.19	−938.14	0.78	0.05
Null model	—	473.02	−937.81	1.12	0.04
Land Cover					
100	10	473.73	−939.22	0.00	0.18
10	10	473.25	−938.25	0.98	0.11
10	5	473.17	−938.09	1.13	0.10
5	5	473.16	−938.08	1.15	0.10
1,000	100	473.12	−938.00	1.23	0.10
5	10	473.09	−937.94	1.28	0.09
100	100	473.06	−937.89	1.34	0.09
10	100	472.97	−937.69	1.53	0.08
5	100	472.94	−937.64	1.59	0.08
Null model	—	472.87	−937.50	1.72	0.07

Note: Alpha = maximum resistance value; Gamma = slope of the relationship.

3.5 | Environmental and morphological association analysis

LFMM analysis identified a total of 262 SNPs under putative selection out of 11,408 total SNPs (2.3%) of which 231 (mean F_{ST} = 0.17 ± 0.01; Figure S5 in Appendix S1) were located on unique DNA fragments and these were used in further analysis. Of these 231 SNPs, 177 were associated with environmental variables (unique and overlapping) and 54 were associated with morphological variables (Table 3). For geographical and environmental variables, the highest number of SNPs detected as being under putative selection were associated with latitude (n = 100, with n = 84 uniquely associated) and Annual Temp (n = 37, with n = 22 uniquely associated; Table 3). Fourteen SNPs were associated with Wind Speed (Figure S6 in Appendix S1), but these were also associated with other variables, including Annual Temperature (n = 7 in common), providing limited evidence for an independent effect of Wind Speed on local adaptation.

For morphological associations, the highest number of SNPs (n = 39) were associated with body size (Table 3, Figure S7A in Appendix S1). Of these associations, 36 were unique to body size. Only three outliers that associated with body size were also significantly associated with sex (p < 0.05), indicating that body size outliers were not biased by differences between the sexes. For signatures of selection in relation to stripe polymorphism, the highest number of SNP associations were with the partially striped morph (n = 12, all unique SNP associations). Only a few associations were found with the fully striped morph (n = 1) and unstriped morph (n = 1; Table 3, Figure S7B in Appendix S1). No associations were found in relation to wing dimorphism. GIF values (mean = 0.84 ± 0.03) ranged from a minimum of 0.63 for partially striped to a maximum of 1.06 for Wind Speed, while other variables had GIF scores close to 1.0. GIF scores and p-value distributions for each tested variable indicated that confounding genetic structure was well-accounted for in the model and our chosen FDR threshold was appropriate (Figure S8 in Appendix S1).

3.6 | Gene annotation

Gene annotation of DNA fragments that contained SNPs identified via F_{ST} outlier tests or EAA resulted in successful annotation of four SNPs, using the *Locusta migratoria* genome on the BLAST database (Table S6 in Appendix S1). All four annotated SNPs were identified only using EAA and showed significant associations with latitude (n = 1), Annual Temp (n = 1) and body size (n = 2) in LFMM. The SNPs associated with latitude and body size were annotated to cell membrane receptors (glutamate receptor 3 and ionotropic glutamate receptor gene), which are involved in neurotransmission, chemical signalling and olfaction (Dingledine, Borges, Bowie, & Traynelis, 1999; Usherwood, 1994). The SNP associated with Annual Temp was annotated to the gene glucosyl glucuronosyl transferase (mRNA

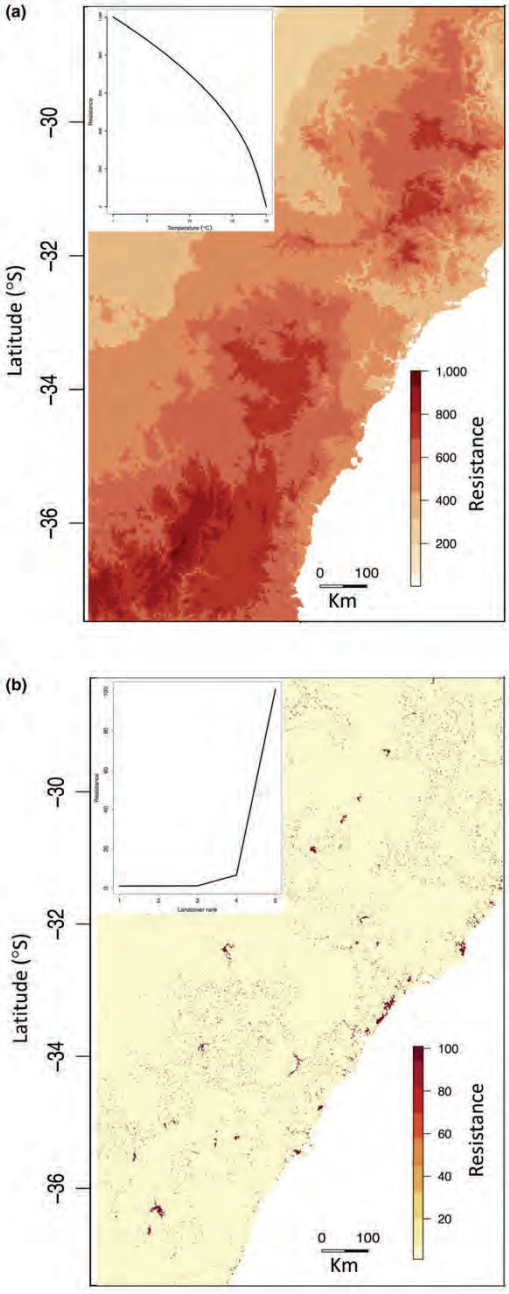

FIGURE 3 (a) The best supported landscape resistance model for Mean Annual Temperature (BIO1; α = 1,000; γ = 0.5). (b) The best supported landscape resistance model for Land Cover (α = 100; γ = 10). The darker regions represent higher resistance. Land Cover was 10 times less resistant to gene flow than BIO1. Land cover rankings are as follows: 1 = pasture and agricultural areas, 2 = rural development, 3 = forest and conservation areas, 4 = urban areas, 5 = water bodies. Insets show line plots of the most well-supported resistance relationships for each of BIO1 and Land Cover [Colour figure can be viewed at wileyonlinelibrary.com]

gene), which transcribes an enzyme involved in the transfer of a glucuronoyl group to other molecules and has a role in metabolism (Ahn, Vogel, & Heckel, 2012). Furthermore, annotation to "insects" as an organism using BLAST did not result in any additional annotations.

4 | DISCUSSION

Using complementary analyses of neutral landscape genetic connectivity and adaptive genetic variation, we find evidence for local adaptation in the presence of high gene flow along an environmental gradient for the wingless grasshopper, *P. vittatum*. Using a landscape genetic resistance approach, we were able to detect subtle barriers to genetic connectivity, mainly due to low mean annual temperatures. Cleared, agricultural habitat was associated with increased genetic connectivity, although resistance of this land cover was 10 times less than for temperature. Furthermore, mean annual temperature was associated with the greatest number of SNPs under putative selection compared with other variables. Our independent analyses of landscape resistance to gene flow and detection of selection signatures show that temperature is a dominant variable influencing evolutionary and dispersal processes in this agricultural pest. With respect to morphological variables, selection on body size was most evident based on the number of positive SNP associations. SNP annotations indicated the involvement of genes that may be relevant for local environmental adaptation across latitudinal gradients. By applying this two-tiered approach to a widespread pest species, we provide insights into the spatial drivers of genetic connectivity and the capacity of this agricultural pest to adapt to diverse local environments.

4.1 | Temperature and land cover effects on genetic connectivity

Widespread species with high dispersal capacity often show less pronounced spatial genetic structure with lower F_{ST} distributions (e.g., Llewellyn et al., 2003; Vorburger, Lancaster, & Sunnucks, 2003; Yin et al., 2017). Minimal genetic structuring in *P. vittatum* was anticipated due to the species' high abundance and mobility across pastures and agricultural areas of Australia (Farrow, Nicolas, & Dowse, 1982; Yadav et al., 2018). The lower genetic differentiation in wide-ranging species is often coupled with an absence of IBD (e.g., in cotton aphids: Gholamian, Razmjou, Bani Hashemian, & Sabouri, 2018) as found in the current study for *P. vittatum*. However, in species with broad tolerances in terms of habitat and climatic conditions, environmental factors may still facilitate or restrict gene flow in more subtle ways that are not detectable using standard, nonspatially explicit analyses of genetic structure.

In ectotherms, temperature predominanty controls locomotion (Whitman, 1987), developmental rates (Clissold & Simpson, 2015)

TABLE 3 SNP outliers detected using BAYESCAN, OUTFLANK and EAA (LFMM)

Approach	Variable	Total outliers	Unique outliers	GIF	Latitude	BIO1	BIO18	Wind speed	FPC	Soil moisture	Soil acidity	Fully striped	Partial striped	Striped	Unstriped	Body size	BayeScan
LFMM	Latitude	100	84	1.01	—												
Environment	BIO1	37	22	1.02	10	—											
	BIO18	6	4	0.79	1	1	—										
	Wind speed	14	4	1.06	5	7	1	—									
	FPC	6	4	0.78	2	2	0	0	—								
	Soil moisture	10	10	0.76	0	0	0	0	0	—							
	Soil acidity	4	4	0.80	0	0	0	0	0	0	—						
LFMM	Fully striped	1	0	0.77	0	0	0	0	0	0	0	—					
Morphology	Partial striped	12	12	0.63	0	0	0	0	0	0	0	0	—				
	Striped	1	0	0.76	0	0	0	0	0	0	0	1	0	—			
	Unstriped	1	0	0.76	0	0	0	0	0	0	0	1	0	1	—		
	Body size	39	36	0.97	2	0	0	0	0	0	0	1	0	1	1	—	
F_{ST} outlier	BAYESCAN	17		—	1	0	0	0	0	0	1	0	0	0	0	1	—
	OUTFLANK	3		—	1	0	0	0	0	0	0	0	0	0	0	1	3
All		251															

Note: A total of 251 SNPs were identified as F_{ST} outliers or were associated with environmental or morphological variables across all methods. Numbers of common outliers (i.e., those overlapping among analyses) are shown for each variable. The genomic inflation factor (GIF) is shown for variables tested using LFMM. BIO1 = Mean annual temperature (°C); BIO18 = Precipitation of warmest quarter (mm); FPC = Foliage Projective Cover (%). "Striped" includes both fully striped and partially striped individuals.

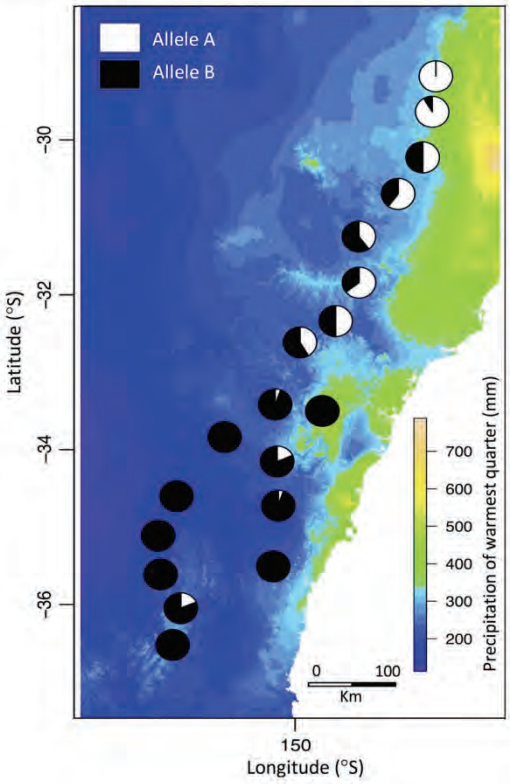

FIGURE 4 Allele frequency change across sites is shown for one SNP that was correlated with body size using all outlier detection methods (i.e., two F_{ST} outlier approaches and LFMM). This SNP shows a distinct north–south divide in allele frequency. The frequency of each SNP allele is displayed as the proportion of individuals with the allele per site (white, "Allele A"; black, "Allele B"), shown over BIO18 = Precipitation of the warmest quarter (mm) [Colour figure can be viewed at wileyonlinelibrary.com]

and habitat selection (Gillis & Possai, 1983) with important consequences for population size and dispersal (Cannon, 1998; Fielding & Brusven, 1990). Our analysis found that resistance due to mean annual temperature had an effect on genetic connectivity that was 10 times greater than land cover, providing evidence for the crucial role of temperature in regulating neutral genetic connectivity in *P. vittatum*. Studies have found that *P. vittatum* maintains its body temperature via behavioural thermoregulation, and this is facilitated by body melanism (Harris et al., 2013a, 2013b). Lower temperatures may therefore prevent preferred body temperatures from being maintained, which in turn may significantly restrict the dispersal and distribution of individuals with consequences for genetic connectivity.

The best resistance model for land cover indicated that pasture, agricultural areas, rural areas and forested areas facilitate movement and do not limit genetic connectivity of *P. vittatum* (Figure 2b). These land cover types provide preferred temperatures and offer food resources such as pasture and clover (Roberts, 1972). Furthermore, previous findings of high abundance of the winged form (i.e., the morph relevant for increased dispersal) within shrub-dominated areas and forest margins (Clark, 1967; Yadav et al., 2018) are consistent with our finding of low resistance to gene flow across partially forested areas. Although less of an effect than temperature, water bodies and urban areas were found to be significant barriers to gene flow and may restrict the movement of *P. vittatum*. This is possibly due to the difficulty in crossing large water bodies, and a limited availability of food resources in urban areas. Hence, our landscape genetic connectivity analysis suggests that the future spread of *P. vittatum* will be facilitated by the expansion of pastoral and agricultural land use, in combination with increasing temperatures under climate change. In Australia, a 0.4–2.0°C increase in annual average temperature is predicted by 2030 (Hughes, 2003). Additionally, Australia is undergoing rapid land clearing for pasture and agricultural purposes (Bradshaw, 2012; Evans, 2016). These rapid changes in landscape and temperature may therefore facilitate more frequent outbreaks of this species by supporting high population sizes and increasing economic damage.

4.2 | Evidence for local environmental adaptation

From an evolutionary perspective, gene flow can either facilitate or impede local adaptation, depending partially on the strength of local selection pressures (Garant, Forde, & Hendry, 2007). We find evidence for selection along our sampled environmental gradient, indicating local adaptive processes in *P. vittatum*. Similar to our finding of a dominating effect of mean annual temperature on landscape genetic connectivity, temperature was associated with the highest number of SNPs under putative selection in *P. vittatum*. Our results are consistent with other studies that have reported (via EAA and outlier tests) a key role of temperature in driving adaptive genetic differentiation in insects (e.g., Dudaniec et al., 2018; Feng et al., 2015; Waldvogel et al., 2018), and in other systems (e.g., trees: Jordan, Hoffmann, Dillon, & Prober, 2017; vertebrates: Manthey & Moyle, 2015; marine invertebrates: Xuereb, Kimber, Curtis, Bernatchez, & Fortin, 2018).

Although there was a small number of SNPs associated with soil moisture, all were unique to this variable, indicating that soil moisture may be a selection pressure for *P. vittatum*. This possibility is supported by our recent study on *P. vittatum* that reported a positive correlation between soil moisture and relative abundance along the same gradient (Yadav et al., 2018). Soil moisture significantly influences the viability of offspring in grasshoppers (Herrmann, Ko, Bhatt, Jannot, & Juliano, 2010) and may affect local population distributions via the presence of parasitic nematodes and entomopathogenic fungi of *P. vittatum* (Clift & Baker, 1998; Milner & Prior, 1994) and in other Orthoptera (Barbercheck, 1992). Notably, of all tested variables, latitude had the highest number of SNP associations (n = 100, unique = 84), which probably reflects a combination of

environmental changes along the sampled latitudinal gradient. Given the latitudinal pattern of genetic admixture we found (Figure 1b), the unique SNPs associating with latitude may be false positive signatures of selection affected by neutral genetic structure or may be related to untested environmental variables.

4.3 | Morphological variations and signature of selection

Of all the morphological variables tested, body size had the highest number of SNP associations in *P. vittatum*. Notably, the latitudinal cline in body size and the correlation of latitude with other environmental variables makes it difficult to tease apart selection signatures on body size alone. However, 92% (36 out of 39) of the SNPs under putative selection for body size were not significantly associated with any other environmental variable, and all except three outlier SNPs (92%) were unbiased by sex, indicating that these associations are not likely to be confounded by the effects of latitudinal genetic structure and sex. Notably, one of the SNP outliers relating to body size only was detected by both the F_{ST} outlier approaches as well as EAA and exhibits a striking change in allele frequency along the sampled gradient (Figure 4). Previously we found larger body size in sites of higher solar radiation towards southern latitudes, suggesting a possible underlying thermoregulatory advantage (Yadav et al., 2018). Our current results indicate that the latitudinal cline in body size of *P. vittatum* may have an adaptive genetic basis.

The lack of selection signatures in relation to wing dimorphism may suggest more complex or subtle genetic processes governing this trait, such as polygenic and additive gene interactions (e.g., Babin, Gagnaire, Pavey, & Bernatchez, 2017; Lotterhos, Yeaman, Degner, Aitken, & Hodgins, 2018) or polyphenism (phenotypic plasticity where a single genotype produces different phenotypes), as reported in several insect groups including Orthoptera (Simpson, Sword, & Lo, 2011; Zera, 2003; Zhang et al., 2019). The small number of SNP associations with stripe polymorphism indicated some evidence for selection on this trait, and associations were largely detected for the partially striped morph. Notably, the sensitivity of the EAA analysis as indicated by the GIF value within the LFMM (Table 3) and conservative *p* values for this variable (Figure S8 in Appendix S1) indicates higher confidence in these outliers (Frichot & Francois, 2015).

4.4 | Gene annotation of candidate SNPs

Our annotation of candidate SNPs provided some evidence that functional genes (i.e., glutamate and ionotropic receptors) may be under selection and, thus, involved in local adaptation of *P. vittatum* across environmental gradients. SNPs associated with these gene functions were also associated with body size and latitude (Table S6 in Appendix S1). Glutamate receptors (GluRs) mediate neurotransmission in the brain and are expressed largely in the central nervous system (Dingledine et al., 1999). Notably, the ionotropic receptors (IRs), a subfamily of ionotropic GluRs, have been shown to be involved in olfaction in migratory locusts (Wang et al., 2015) as well as chemical signalling and environmental cue detection in other insects (Benton, Vannice, Gomez-Diaz, & Vosshall, 2009; Croset et al., 2010; Rytz, Croset, & Benton, 2013). This involvement of GluRs and IRs in key pathways of detecting environmental cues suggests that genes coding for these membrane proteins are putatively under differential selective pressures across variable environments and may provide a fitness advantage in *P. vittatum* via improved perception of the immediate environment.

Another annotation identified glucosyl glucuronosyltransferases among our candidate SNPs. Uridine diphosphate-glucuronosyl transferase (UGT) is an important enzyme of the detoxification pathway and catalyses the addition of sugars to lipophilic compounds (Ahn et al., 2012). The detoxification role of UGT has also been well demonstrated in herbivorous insects (Ahn et al., 2011; Heidel-Fischer & Vogel, 2015) and pest insects (e.g., Krempl et al., 2016). Additionally, UGT is involved in UV-shielding (Daimon et al., 2010) and in pigmentation (Bock, 2016; Krempl et al., 2016). Along our sampled gradient, the annotated SNP relating to UGT was uniquely associated with mean annual temperature, a variable where selection pressures relating to UV light and the development of melanism are relevant. However, further experiments are required to establish this relationship between temperature and expression of UGT.

5 | CONCLUSION

Using a landscape genomic approach, we identify the relative contribution of common environmental drivers of both neutral and adaptive genetic processes, which are otherwise challenging to detect under conditions of high gene flow. In a widely distributed pest, *Phaulacridium vittatum*, we identify subtle landscape and climatic effects on genetic connectivity and environmental selection processes, despite weak genetic structure. Regarding the future impacts of *P. vittatum* as an agricultural pest, increased future temperatures under climate warming and land clearing are likely to facilitate a wider distribution and spread of this species to other areas. Increased availability of suitable habitat and temperatures may support higher population sizes leading to greater chances of outbreak and higher economic costs due to reduced agricultural production. Our study highlights the value of considering neutral and adaptive genetic processes together to understand the roles of gene flow and local adaptation in driving evolutionary responses in widely distributed species.

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AUTHOR CONTRIBUTIONS

All authors conceptualized and designed the study. S.Y. conducted fieldwork, labwork and performed analyses. S.Y. wrote the manuscript with editing and comments from R.Y.D. and A.J.S.

DATA AVAILABILITY STATEMENT

Data for SNP genotypes, morphological data, R code for resistance modelling and raster surfaces for Annual Temp and Land Cover are available at the Dryad repository at https://doi.org/10.5061/dryad.8823vb0.

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REFERENCES

ABARES (2017). *Catchment scale land use of Australia*. Retrieved from http://www.agriculture.gov.au/abares/aclump/

Ahn, S. J., Badenes-Perez, F. R., Reichelt, M., Svatos, A., Schneider, B., Gershenzon, J., & Heckel, D. G. (2011). Metabolic detoxification of capsaicin by UDP-glycosyltransferase in three *Helicoverpa* species. *Archives of Insect Biochemistry and Physiology*, *78*(2), 104–118. https://doi.org/10.1002/arch.20444

Ahn, S. J., Vogel, H., & Heckel, D. G. (2012). Comparative analysis of the UDP-glycosyltransferase multigene family in insects. *Insect Biochemistry and Molecular Biology*, *42*(2), 133–147. https://doi.org/10.1016/j.ibmb.2011.11.006

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J., Dillon, S., Umbers, K. D. L., & Dudaniec, R. Y. (2018). The search for loci under selection: Trends, biases and progress. *Molecular Ecology*, *27*(6), 1342–1356. https://doi.org/10.1111/mec.14549

Antonovics, J. (1976). The nature of limits to natural selection. *Annals of the Missouri Botanical Garden*, *63*(2), 224–247. https://doi.org/10.2307/2395303

Attard, C. R. M., Brauer, C. J., Sandoval-Castillo, J., Faulks, L. K., Unmack, P. J., Gilligan, D. M., & Beheregaray, L. B. (2018). Ecological disturbance influences adaptive divergence despite high gene flow in golden perch (*Macquaria ambigua*): Implications for management and resilience to climate change. *Molecular Ecology*, *27*(1), 196–215. https://doi.org/10.1111/mec.14438

Babin, C., Gagnaire, P. A., Pavey, S. A., & Bernatchez, L. (2017). RAD-Seq reveals patterns of additive polygenic variation caused by spatially-varying selection in the American eel (*Anguilla rostrata*). *Genome Biology and Evolution*, *9*(11), 2974–2986. https://doi.org/10.1093/gbe/evx226

Bai, Y., Dong, J. J., Guan, D. L., Xie, J. Y., & Xu, S. Q. (2016). Geographic variation in wing size and shape of the grasshopper *Trilophidia annulata* (Orthoptera: Oedipodidae): Morphological trait variations follow an ecogeographical rule. *Scientific Reports*, *6*, 32680. https://doi.org/10.1038/srep32680

Bailey, P., Frensham, A., Hincks, A., & Newton, M. (1994). Competition for herbage by *Phaulacridium vittatum* (Sjöstedt)(Orthoptera: Acrididae) and sheep during summer drought. *Australian Journal of Entomology*, *33*(2), 175–179.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., … Whittaker, J. B. (2002). Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x

Balkenhol, N., Dudaniec, R. Y., Krutovsky, K. V., Johnson, J. S., Cairns, D. M., Segelbacher, G., … Selmoni, O. (2017). *Landscape Genomics: Understanding relationships between environmental heterogeneity and genomic characteristics of populations*. Berlin, Germany: Springer.

Barbercheck, M. E. (1992). Effect of soil physical factors on biological-control agents of soil insect pests. *Florida Entomologist*, *75*(4), 539–548. https://doi.org/10.2307/3496136

Barton, K. (2018). *Package 'MuMIn'*. R package version 1.40. 4. Retrieved from https://CRAN.R-project.org/package=MuMIn

Barton, N., & Partridge, L. (2000). Limits to natural selection. *BioEssays*, *22*(12), 1075–1084. https://doi.org/10.1002/1521-1878(200012)22:12<1075:AID-BIES5>3.0.CO;2-M

Benton, R., Vannice, K. S., Gomez-Diaz, C., & Vosshall, L. B. (2009). Variant ionotropic glutamate receptors as chemosensory receptors in *Drosophila*. *Cell*, *136*(1), 149–162. https://doi.org/10.1016/j.cell.2008.12.001

Bock, K. W. (2016). The UDP-glycosyltransferase (UGT) superfamily expressed in humans, insects and plants: Animal–plant arms-race and co-evolution. *Biochemical Pharmacology*, *99*, 11–17. https://doi.org/10.1016/j.bcp.2015.10.001

Bradshaw, C. J. A. (2012). Little left to lose: Deforestation and forest degradation in Australia since European colonization. *Journal of Plant Ecology*, *5*(1), 109–120. https://doi.org/10.1093/jpe/rtr038

Brisson, J. A. (2010). Aphid wing dimorphisms: Linking environmental and genetic control of trait variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1540), 605–616. https://doi.org/10.1098/rstb.2009.0255

Burnham, K. P., & Anderson, D. R. (2003). *Model selection and multimodel inference: A practical information-theoretic approach*. Berlin, Germany: Springer Science & Business Media.

Cannon, R. J. C. (1998). The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Global Change Biology*, *4*(7), 785–796. https://doi.org/10.1046/j.1365-2486.1998.00190.x

Chapman, A. J. (1990). *Biology of grasshoppers*. Chichester, UK: John Wiley & Sons.

Chhatre, V. E., & Emerson, K. J. (2017). STRAUTO: Automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics*, *18*(1), 192. https://doi.org/10.1186/s12859-017-1593-0

Clark, D. P. (1967). A population study of *Phaulacridium vittatum* Sjost (Acrididae). *Australian Journal of Zoology*, *15*, 799–872. https://doi.org/10.1071/ZO9670799

Clift, A., & Baker, G. L. (1998). Quantification of the relationship between rainfall and parasitism of *Phaulacridium vittatum* (Sjöstedt) by mermithid nematodes in the Central Tablelands of South-Eastern Australia. *Journal of Orthoptera Research*, *7*, 77–82. https://doi.org/10.2307/3503497

Clissold, F. J., & Simpson, S. J. (2015). Temperature, food quality and life history traits of herbivorous insects. *Current Opinion in Insect Science*, *11*, 63–70. https://doi.org/10.1016/j.cois.2015.10.011

Coop, G., Witonsky, D., Di Rienzo, A., & Pritchard, J. K. (2010). Using environmental correlations to identify loci underlying local adaptation. *Genetics*, *185*(4), 1411–1423. https://doi.org/10.1534/genetics.110.114819

Croset, V., Rytz, R., Cummins, S. F., Budd, A., Brawand, D., Kaessmann, H., … Benton, R. (2010). Ancient protostome origin of chemosensory

ionotropic glutamate receptors and the evolution of insect taste and olfaction. *PLoS Genetics*, *6*(8), e1001064. https://doi.org/10.1371/journal.pgen.1001064

Crossley, M. S., Chen, Y. H., Groves, R. L., & Schoville, S. D. (2017). Landscape genomics of Colorado potato beetle provides evidence of polygenic adaptation to insecticides. *Molecular Ecology*, *26*(22), 6284–6300. https://doi.org/10.1111/mec.14339

Daimon, T., Hirayama, C., Kanai, M., Ruike, Y., Meng, Y., Kosegawa, E., … Shimada, T. (2010). The silkworm Green b locus encodes a quercetin 5-O-glucosyltransferase that produces green cocoons with UV-shielding properties. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(25), 11471–11476. https://doi.org/10.1073/pnas.1000479107

Dearn, J. M. (1980). Evidence of non-random mating for the colour pattern polymorphism in field populations of the grasshopper *Phaulacridium vittatum*. *Australian Journal of Entomology*, *18*(3), 241–243. https://doi.org/10.1111/j.1440-6055.1979.tb00847.x

Dearn, J. M. (1983). Inheritance of the colour pattern polymorphism in the wingless grasshopper *Phaulacridium vittatum* Sjöstedt (Orthoptera: Acrididae). *Australian Journal of Entomology*, *22*(3), 217–218.

Dearn, J. M. (1984). Colour pattern polymorphism in the grasshopper *Phaulacridium Vittatum*. I. Geographic variation in Victoria and evidence of habitat association. *Australian Journal of Zoology*, *32*(2), 239–249.

Devlin, B., & Roeder, K. (1999). Genomic control for association studies. *Biometrics*, *55*(4), 997–1004. https://doi.org/10.1111/j.0006-341X.1999.00997.x

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. F. (1999). The glutamate receptor ion channels. *Pharmacological Reviews*, *51*(1), 7–61.

Dudaniec, R. Y., Rhodes, J. R., Worthington Wilmer, J., Lyons, M., Lee, K. E., McAlpine, C. A., & Carrick, F. N. (2013). Using multilevel models to identify drivers of landscape-genetic structure among management areas. *Molecular Ecology*, *22*(14), 3752–3765. https://doi.org/10.1111/mec.12359

Dudaniec, R. Y., Worthington Wilmer, J., Hanson, J. O., Warren, M., Bell, S., & Rhodes, J. R. (2016). Dealing with uncertainty in landscape genetic resistance models: A case of three co-occurring marsupials. *Molecular Ecology*, *25*(2), 470–486. https://doi.org/10.1111/mec.13482

Dudaniec, R. Y., Yong, C. J., Lancaster, L. T., Svensson, E. I., & Hansson, B. (2018). Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Molecular Ecology*, *27*(11), 2576–2593. https://doi.org/10.1111/mec.14709

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*(5), e19379. https://doi.org/10.1371/journal.pone.0019379

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Evans, M. C. (2016). Deforestation in Australia: Drivers, trends and policy responses. *Pacific Conservation Biology*, *22*(2), 130–150. https://doi.org/10.1071/PC15052

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Falt-Nardmann, J., Klemola, T., Roth, M., Ruohomaki, K., & Saikkonen, K. (2016). Northern geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures than their southern conspecifics: Implications of climate change. *European Journal of Entomology*, *113*, 337–343. https://doi.org/10.14411/eje.2016.043

Farrow, R., Nicolas, G., & Dowse, J. (1982). Migration in the macropterous form of the wingless grasshopper, *Phaulacridium vittatum* (Sjöstedt) during an outbreak. *Australian Journal of Entomology*, *21*(4), 307–308.

Feng, X. J., Jiang, G. F., & Fan, Z. (2015). Identification of outliers in a genomic scan for selection along environmental gradients in the bamboo locust, Ceracris kiangsu. *Scientific Reports*, *5*, 13758. https://doi.org/10.1038/srep13758

Fick, S. E., & Hijmans, R. J. (2017). WORLDCLIM 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Fielding, D. J., & Brusven, M. (1990). Historical analysis of grasshopper (Orthoptera: Acrididae) population responses to climate in southern Idaho, 1950–1980. *Environmental Entomology*, *19*(6), 1786–1791. https://doi.org/10.1093/ee/19.6.1786

Fitzpatrick, S., Gerberich, J., Kronenberger, J., Angeloni, L., & Funk, W. (2015). Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, *18*(1), 37–47. https://doi.org/10.1111/ele.12388

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*(2), 977–993. https://doi.org/10.1534/genetics.108.092221

Frichot, E., & Francois, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, *6*(8), 925–929. https://doi.org/10.1111/2041-210x.12382

Frichot, E., Schoville, S. D., Bouchard, G., & Francois, O. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and Evolution*, *30*(7), 1687–1699. https://doi.org/10.1093/molbev/mst063

Garant, D., Forde, S. E., & Hendry, A. P. (2007). The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, *21*(3), 434–443. https://doi.org/10.1111/j.1365-2435.2006.01228.x

García-Ramos, G., & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, *51*(1), 21–28. https://doi.org/10.1111/j.1558-5646.1997.tb02384.x

Gholamian, E., Razmjou, J., Bani Hashemian, S. M., & Sabouri, A. (2018). Genetic structure of populations of *Aphis gossypii* (Hemiptera: Aphididae) on citrus trees in Northern Iran. *European Journal of Entomology*, *115*, 7–14. https://doi.org/10.14411/eje.2018.002

Gillis, J. E., & Possai, K. W. (1983). Thermal niche partitioning in the grasshoppers *Arphia conspersa* and *Trimerotropis suffusa* from a montane habitat in Central Colorado. *Ecological Entomology*, *8*(2), 155–161. https://doi.org/10.1111/j.1365-2311.1983.tb00494.x

Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*, *97*(4), 742–751. https://doi.org/10.1111/j.1365-2745.2009.01509.x

Gosselin, T. (2017). RADIATOR: *RADseq data exploration, manipulation and visualization using R*. R package version 0.0.5. Retrieved from https://github.com/thierrygosselin/radiator

Gunther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, *195*(1), 205–220. https://doi.org/10.1534/genetics.113.152462

Harris, R., McQuillan, P., & Hughes, L. (2012). Patterns in body size and melanism along a latitudinal cline in the wingless grasshopper, *Phaulacridium vittatum*. *Journal of Biogeography*, *39*(8), 1450–1461. https://doi.org/10.1111/j.1365-2699.2012.02710.x

Harris, R. M., McQuillan, P., & Hughes, L. (2013a). Experimental manipulation of melanism demonstrates the plasticity of preferred temperature in an agricultural pest (*Phaulacridium vittatum*). *PLoS ONE*, *8*(11), e80243. https://doi.org/10.1371/journal.pone.0080243

Harris, R. M. B., McQuillan, P., & Hughes, L. (2013b). A test of the thermal melanism hypothesis in the wingless grass-hopper *Phaulacridium vittatum*. *Journal of Insect Science*, *13*(1), 51.

Harris, R. M. B., McQuillan, P., & Hughes, L. (2015). The effectiveness of common thermo-regulatory behaviours in a cool temperate grasshopper. *Journal of Thermal Biology*, *52*, 75–83. https://doi.org/10.1016/j.jtherbio.2015.06.001

Heidel-Fischer, H. M., & Vogel, H. (2015). Molecular mechanisms of insect adaptation to plant secondary compounds. *Current Opinion in Insect Science*, *8*, 8–14. https://doi.org/10.1016/j.cois.2015.02.004

Herrmann, D. L., Ko, A. E., Bhatt, S., Jannot, J. E., & Juliano, S. A. (2010). Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. *Annals of the Entomological Society of America*, *103*(2), 227–235. https://doi.org/10.1603/An09131

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*(15), 1965–1978. https://doi.org/10.1002/joc.1276

Hijmans, R. J., & van Etten, J. (2016). *raster: Geographic data analysis and modeling*. R package version 2.5-8. Vienna, Austria: The R Foundation. Retrieved from https://CRAN.R-project.org/package=raster

Hoffmann, A. A. (2017). Rapid adaptation of invertebrate pests to climatic stress? *Current Opinion in Insect Science*, *21*, 7–13. https://doi.org/10.1016/j.cois.2017.04.009

Hughes, L. (2003). Climate change and Australia: Trends, projections and impacts. *Austral Ecology*, *28*(4), 423–443. https://doi.org/10.1046/j.1442-9993.2003.01300.x

Janes, J. K., Li, Y., Keeling, C. I., Yuen, M. M. S., Boone, C. K., Cooke, J. E. K., … Sperling, F. A. H. (2014). How the mountain pine beetle (*Dendroctonus ponderosae*) breached the Canadian rocky mountains. *Molecular Biology and Evolution*, *31*(7), 1803–1815. https://doi.org/10.1093/molbev/msu135

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: A better web interface. *Nucleic Acids Research*, *36*, W5–W9. https://doi.org/10.1093/nar/gkn201

Jordan, R., Hoffmann, A. A., Dillon, S. K., & Prober, S. M. (2017). Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: Implications for adaptive potential to projected climate change. *Molecular Ecology*, *26*(21), 6002–6020. https://doi.org/10.1111/mec.14341

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Keller, D., van Strien, M. J., Herrmann, M., Bolliger, J., Edwards, P. J., Ghazoul, J., & Holderegger, R. (2013). Is functional connectivity in common grasshopper species affected by fragmentation in an agricultural landscape? *Agriculture, Ecosystems & Environment*, *175*, 39–46. https://doi.org/10.1016/j.agee.2013.05.006

Key, K. (1992). Taxonomy of the genus *Phaulacridium* and a related new genus (Orthoptera: Acrididae). *Invertebrate Taxonomy*, *6*(1), 197–243. https://doi.org/10.1071/IT9920197

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., … Hopper, C. (2012). Diversity arrays technology: A generic genome profiling technology on open platforms. In F. Pompanon, & A. Bonin (Eds.), *Data production and analysis in population genomics* (pp. 67–89). Berlin, Germany: Springer.

Kirk, H., Dorn, S., & Mazzi, D. (2013). Molecular genetics and genomics generate new insights into invertebrate pest invasions. *Evolutionary Applications*, *6*(5), 842–856. https://doi.org/10.1111/eva.12071

Krempl, C., Sporer, T., Reichelt, M., Ahn, S.-J., Heidel-Fischer, H., Vogel, H., … Joußen, N. (2016). Potential detoxification of gossypol by UDP-glycosyltransferases in the two Heliothine moth species *Helicoverpa armigera* and *Heliothis virescens*. *Insect Biochemistry and Molecular Biology*, *71*, 49–57. https://doi.org/10.1016/j.ibmb.2016.02.005

Lehmann, G. U., & Lehmann, A. W. (2008). Variation in body size among populations of the bushcricket *Poecilimon thessalicus* (Orthoptera: Phaneropteridae): An ecological adaptation. *Journal of Orthoptera Research*, *17*(2), 165–170. https://doi.org/10.1665/1082-6467-17.2.165

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, *17*(4), 183–189. https://doi.org/10.1016/S0169-5347(02)02497-7

Lewontin, R., & Krakauer, J. (1973). Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics*, *74*(1), 175–195.

Li, Y. L., & Liu, J. X. (2018). StructureSelector: A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources*, *18*(1), 176–177.

Llewellyn, K. S., Loxdale, H. D., Harrington, R., Brookes, C. P., Clark, S. J., & Sunnucks, P. (2003). Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. *Molecular Ecology*, *12*(1), 21–34. https://doi.org/10.1046/j.1365-294X.2003.01703.x

Loch, A., & Floyd, R. (2001). Insect pests of Tasmanian blue gum, *Eucalyptus globulus globulus*, in south-western Australia: History, current perspectives and future prospects. *Austral Ecology*, *26*(5), 458–466. https://doi.org/10.1046/j.1442-9993.2001.01145.x

Lotterhos, K. E., & Whitlock, M. C. (2015). The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology*, *24*(5), 1031–1046. https://doi.org/10.1111/mec.13100

Lotterhos, K. E., Yeaman, S., Degner, J., Aitken, S., & Hodgins, K. A. (2018). Modularity of genes involved in local adaptation to climate despite physical linkage. *Genome Biology*, *19*(1), 157. https://doi.org/10.1186/s13059-018-1545-7

Lozier, J. D., Strange, J. P., & Koch, J. B. (2013). Landscape heterogeneity predicts gene flow in a widespread polymorphic bumble bee, *Bombus bifarius* (Hymenoptera: Apidae). *Conservation Genetics*, *14*(5), 1099–1110. https://doi.org/10.1007/s10592-013-0498-3

Maeno, K., & Tanaka, S. (2010). Genetic and hormonal control of melanization in reddish–brown and albino mutants in the desert locust *Schistocerca gregaria*. *Physiological Entomology*, *35*(1), 2–8.

Manel, S., Conord, C., & Després, L. (2009). Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread insect. *BMC Evolutionary Biology*, *9*(1), 288. https://doi.org/10.1186/1471-2148-9-288

Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology & Evolution*, *28*(10), 614–621. https://doi.org/10.1016/j.tree.2013.05.012

Manthey, J. D., & Moyle, R. G. (2015). Isolation by environment in White-breasted Nuthatches (Sitta carolinensis) of the Madrean Archipelago sky islands: A landscape genomics approach. *Molecular Ecology*, *24*(14), 3628–3638. https://doi.org/10.1111/mec.13258

Masaki, S. (1967). Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution*, *21*(4), 725–741. https://doi.org/10.1111/j.1558-5646.1967.tb03430.x

McRae, B. H., & Beier, P. (2007). Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 19885–19890. https://doi.org/10.1073/pnas.0706568104

Milner, R. J., & Prior, C. (1994). Susceptibility of the Australian plague locust, *Chortoicetes terminifera*, and the wingless grasshopper, *Phaulacridium vittatum*, to the Fungi *Metarhizium* Spp. *Biological Control*, *4*(2), 132–137. https://doi.org/10.1006/bcon.1994.1021

Momigliano, P., Harcourt, R., Robbins, W. D., Jaiteh, V., Mahardika, G. N., Sembiring, A., & Stow, A. (2017). Genetic structure and signatures of selection in grey reef sharks (*Carcharhinus amblyrhynchos*). *Heredity*, *119*(3), 142–153. https://doi.org/10.1038/hdy.2017.21

Moraiti, C., Nakas, C., & Papadopoulos, N. (2014). Diapause termination of *Rhagoletis cerasi* pupae is regulated by local adaptation and phenotypic plasticity: Escape in time through bet-hedging strategies. *Journal of Evolutionary Biology*, *27*(1), 43–54.

Mousseau, T. A., & Roff, D. A. (1989). Adaptation to seasonality in a cricket: Patterns of phenotypic and genotypic variation in body size and diapause expression along a cline in season length. *Evolution*, *43*(7), 1483–1496. https://doi.org/10.1111/j.1558-5646.1989.tb02598.x

Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: Temperature parameters drive adaptation to altitude in the common frog (*Rana temporaria*). *Molecular Ecology*, *23*(3), 561–574.

Neteler, M., Bowman, M. H., Landa, M., & Metz, M. (2012). GRASS GIS: A multi-purpose open source GIS. *Environmental Modelling & Software*, *31*, 124–130. https://doi.org/10.1016/j.envsoft.2011.11.014

OEH (2017). *Great Soil Group (GSG) Soil Type map of NSW, NSW Office of Environment and Heritage, Sydney*. Retrieved from https://datasets.seed.nsw.gov.au/dataset/great-soil-group-gsgsoil-type-map-of-nsw1cf19

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., & O'hara, R., … Wagner, H. (2013). *Package 'vegan'*. Community ecology package, version, *2*(9). Retrieved from http://vegan.r-forge.r-project.org/

Paradis, E. J. B. (2010). pegas: An R package for population genetics with an integrated–modular approach. *Bioinformatics*, *26*(3), 419–420. https://doi.org/10.1093/bioinformatics/btp696

Paris, M., Boyer, S., Bonin, A., Collado, A., David, J. P., & Despres, L. (2010). Genome scan in the mosquito *Aedes rusticus*: Population structure and detection of positive selection after insecticide treatment. *Molecular Ecology*, *19*(2), 325–337. https://doi.org/10.1111/j.1365-294X.2009.04437.x

Pelissie, B., Crossley, M. S., Cohen, Z. P., & Schoville, S. D. (2018). Rapid evolution in insect pests: The importance of space and time in population genomics studies. *Current Opinion in Insect Science*, *26*, 8–16. https://doi.org/10.1016/j.cois.2017.12.008

Pener, M. P., & Simpson, S. J. (2009). Locust phase polyphenism: An update. *Advances in Insect Physiology*, *36*(36), 1–272. https://doi.org/10.1016/S0065-2806(08)36001-9

Pepper, J., & Hastings, E. (1952). The effects of solar radiation on grasshopper temperatures and activities. *Ecology*, *33*(1), 96–103. https://doi.org/10.2307/1931255

Peterman, W. E. (2018). ResistanceGA: An R package for the optimization of resistance surfaces using genetic algorithms. *Methods in Ecology and Evolution*, *9*(6), 1638–1647. https://doi.org/10.1111/2041-210x.12984

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Puechmaille, S. J. J. M. E. R. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., … Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, *81*(3), 559–575. https://doi.org/10.1086/519795

QGIS. (2016). *QGIS geographic information system V.2.18. 10*. Chicago, IL: Open source geospatial foundation project. http://qgis.osgeo.org

R Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rasanen, K., & Hendry, A. P. (2008). Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters*, *11*(6), 624–636. https://doi.org/10.1111/j.1461-0248.2008.01176.x

Raymond, I. (2016). *The Australian soil classification*. Clayton, CA: CSIRO publishing.

Rellstab, C., Gugerli, F., Eckert, A. J., Hancock, A. M., & Holderegger, R. (2015). A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology*, *24*(17), 4348–4370. https://doi.org/10.1111/mec.13322

Reynolds, J., Weir, B. S., & Cockerham, C. C. J. G. (1983). Estimation of the coancestry coefficient: Basis for a short-term genetic distance. *Genetics*, *105*(3), 767–779.

Roberts, R. J. (1972). A newly recognized from of pasture damage by *Phaulacridium vittatum* (Acididae) on the northern tablelands of NSW. *Australian Journal of Entomology*, *11*(3), 257–258.

Roff, D. A. (1986). The evolution of wing dimorphism in insects. *Evolution*, *40*(5), 1009–1020. https://doi.org/10.1111/j.1558-5646.1986.tb00568.x

Rosetti, N., & Remis, M. I. (2018). Spatial variation in body size and wing dimorphism correlates with environmental conditions in the grasshopper *Dichroplus vittatus* (Orthoptera: Acrididae). *Environmental Entomology*, *47*(3), 519–526. https://doi.org/10.1093/ee/nvy025

Row, J. R., Knick, S. T., Oyler-McCance, S. J., Lougheed, S. C., & Fedy, B. C. (2017). Developing approaches for linear mixed modeling in landscape genetics through landscape-directed dispersal simulations. *Ecology Evolution*, *7*(11), 3751–3761. https://doi.org/10.1002/ece3.2825

Rytz, R., Croset, V., & Benton, R. (2013). Ionotropic receptors (IRs): Chemosensory ionotropic glutamate receptors in Drosophila and beyond. *Insect Biochemistry and Molecular Biology*, *43*(9), 888–897. https://doi.org/10.1016/j.ibmb.2013.02.007

Sandrock, C., Razmjou, J., & Vorburger, C. (2011). Climate effects on life cycle variation and population genetic architecture of the black bean aphid, *Aphis fabae*. *Molecular Ecology*, *20*(19), 4165–4181. https://doi.org/10.1111/j.1365-294X.2011.05242.x

Savolainen, O., Lascoux, M., & Merila, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, *14*(11), 807–820. https://doi.org/10.1038/nrg3522

Schoville, S. D., Bonin, A., Francois, O., Lobreaux, S., Melodelima, C., & Manel, S. (2012). Adaptive genetic variation on the landscape: Methods and Cases. *Annual Review of Ecology, Evolution, and Systematics*, *43*(43), 23–43. https://doi.org/10.1146/annurev-ecolsys-110411-160248

Shirk, A. J., Landguth, E. L., & Cushman, S. A. (2018). A comparison of regression methods for model selection in individual-based landscape genetic analysis. *Molecular Ecology Resources*, *18*(1), 55–67. https://doi.org/10.1111/1755-0998.12709

Shirk, A. J., Wallin, D. O., Cushman, S. A., Rice, C. G., & Warheit, K. I. (2010). Inferring landscape effects on gene flow: A new model selection framework. *Molecular Ecology*, *19*(17), 3603–3619. https://doi.org/10.1111/j.1365-294X.2010.04745.x

Simpson, S. J., Sword, G. A., & Lo, N. (2011). Polyphenism in insects. *Current Biology*, *21*(18), R738–R749. https://doi.org/10.1016/j.cub.2011.06.006

Tanaka, S., Harano, K., & Nishide, Y. (2012). Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity. *Journal of Insect Physiology*, *58*(1), 89–101. https://doi.org/10.1016/j.jinsphys.2011.10.002

TERNAusCover (2011). *Woody extent and Foliage Projective Cover - SPOT, OEH algorithm, NSW coverage*. Retrieved from http://www.auscover.org.au/purl/spot-woody-extent-fpc-nsw-2011

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Trullas, S. C., van Wyk, J. H., & Spotila, J. R. (2007). Thermal melanism in ectotherms. *Journal of Thermal Biology*, *32*(5), 235–245. https://doi.org/10.1016/j.jtherbio.2007.01.013

Usherwood, P. (1994). Insect glutamate receptors. In P. D. Evans (Ed.), *Advances in insect physiology* (vol. 24, pp. 309–341). Amsterdam, The Netherlands: Elsevier.

Vorburger, C., Lancaster, M., & Sunnucks, P. (2003). Environmentally related patterns of reproductive modes in the aphid Myzus persicae and the predominance of two 'superclones' in Victoria, Australia. *Molecular Ecology*, *12*(12), 3493–3504. https://doi.org/10.1046/j.1365-294X.2003.01998.x

Waldvogel, A. M., Wieser, A., Schell, T., Patel, S., Schmidt, H., Hankeln, T., … Pfenninger, M. (2018). The genomic footprint of climate adaptation in *Chironomus riparius*. *Molecular Ecology*, *27*(6), 1439–1456. https://doi.org/10.1111/mec.14543

Wang, X., Fang, X., Yang, P., Jiang, X., Jiang, F., Zhao, D., … Kang, L. E. (2014). The locust genome provides insight into swarm formation and long-distance flight. *Nature Communications*, *5*, 1–9. https://doi.org/10.1038/ncomms3957

Wang, Y.-Z., Li, B.-Y., Hoffmann, A. A., Cao, L.-J., Gong, Y.-J., Song, W., … Wei, S.-J. (2017). Patterns of genetic variation among geographic and host-plant associated populations of the peach fruit moth *Carposina sasakii* (Lepidoptera: Carposinidae). *BMC Evolutionary Biology*, *17*(1), 265. https://doi.org/10.1186/s12862-017-1116-7

Wang, Z., Yang, P., Chen, D., Jiang, F., Li, Y., Wang, X., & Kang, L. (2015). Identification and functional analysis of olfactory receptor family reveal unusual characteristics of the olfactory system in the migratory locust. *Cellular and Molecular Life Sciences*, *72*(22), 4429–4443. https://doi.org/10.1007/s00018-015-2009-9

Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of FST. *American Naturalist*, *186*(S1), S24–S36. https://doi.org/10.1086/682949

Whitman, D. W. (1987). Thermoregulation and daily activity patterns in a black desert rrasshopper, *Taeniopoda eques*. *Animal Behaviour*, *35*(6), 1814–1826. https://doi.org/10.1016/S0003-3472(87)80074-X

Xuereb, A., Benestan, L., Normandeau, E., Daigle, R. M., Curtis, J. M., Bernatchez, L., & Fortin, M. J. (2018). Asymmetric oceanographic processes mediate connectivity and population genetic structure, as revealed by RAD seq, in a highly dispersive marine invertebrate (*Parastichopus californicus*). *Molecular Ecology*, *27*(10), 2347–2364.

Xuereb, A., Kimber, C. M., Curtis, J. M., Bernatchez, L., & Fortin, M. J. (2018). Putatively adaptive genetic variation in the giant California sea cucumber (*Parastichopus californicus*) as revealed by environmental association analysis of restriction-site associated DNA sequencing data. *Molecular Ecology*, *27*(24), 5035–5048.

Yadav, S., Stow, A. J., Harris, R. M. B., & Dudaniec, R. Y. (2018). Morphological variation tracks environmental gradients in an agricultural pest, *Phaulacridium vittatum* (Orthoptera: Acrididae). *Journal of Insect Science*, *18*(6), 13. https://doi.org/10.1093/jisesa/iey121

Yamanaka, T., Tatsuki, S., & Shimada, M. (2008). Adaptation to the new land or effect of global warming? An age-structured model for rapid voltinism change in an alien lepidopteran pest. *Journal of Animal Ecology*, *77*(3), 585–596. https://doi.org/10.1111/j.1365-2656.2008.01367.x

Yeaman, S., & Whitlock, M. C. (2011). The genetic architecture of adaptation under migration–selection balance. *Evolution: International Journal of Organic Evolution*, *65*(7), 1897–1911. https://doi.org/10.1111/j.1558-5646.2011.01269.x

Yin, Y., Li, X., Chu, D., Zhao, X., Sathya, K., Douangboupha, B., … Chen, A. (2017). Extensive gene flow of white-backed planthopper in the Greater Mekong Subregion as revealed by microsatellite markers. *Scientific Reports*, *7*(1), 15905. https://doi.org/10.1038/s41598-017-16164-0

Zera, A. J. (2003). The endocrine regulation of wing polymorphism in insects: State of the art, recent surprises, and future directions. *Integrative and Comparative Biology*, *43*(5), 607–616. https://doi.org/10.1093/icb/43.5.607

Zhang, C. X., Brisson, J. A., & Xu, H. J. (2019). Molecular mechanisms of wing polymorphism in insects. *Annual Review of Entomology*, *64*, 297–314. https://doi.org/10.1146/annurev-ento-011118-112448

Zhang, L., Cai, W., Luo, J., Zhang, S., Li, W., Wang, C., … Cui, J. (2018). Population genetic structure and expansion patterns of the cotton pest *Adelphocoris fasciaticollis*. *Journal of Pest Science*, *91*(2), 539–550. https://doi.org/10.1007/s10340-017-0939-8

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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