Landscape genetic connectivity and sex-specific responses in a range expanding damselfly

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12th October 2018

This thesis is presented for the degree of Masters of Research

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This thesis is written in the form of a journal article from Molecular Ecology, with the following exceptions: continuous line numbers and running head are absent, the figures are integrated into the text, the addition of a table of contents, a background chapter is included before research chapter and the introduction, methods, results and discussion are extended.

Declaration

I wish to acknowledge the following assistance in the research detailed in this report:

Rachael Dudaniec for assistance with experimental design and manuscript preparation. Bengt Hansson and Erik Svensson for funding and discussion. Rachael Dudaniec, Lesley Lancaster, Hanna Bensch and Paul Caplat for collecting *Ischnura elegans* in Sweden. Rachael Dudaniec, Martin Andersson for DNA extraction, next-generation genotyping and SNP identification. Hanna Bensch and John Waller for conducting morphological measurements. Chuan Ji Yong for conducting the initial filtering of genetic markers and genetic structure analysis. Rachael Dudaniec and Sonu Yadav for assistance with analyses. Rachael Dudaniec for comments on drafts of this manuscript.

All other research described in this report is my own original work.

Alexander Robert Carey

12th October 2018

Acknowledgements

To the distinguished members of 43 Waring St (past, present and honouree), your shenanigans and exploits gave me the motivation to continue my education. Thank you for getting me through not only this year but throughout my entire time at university. It's been a lot of fun and I hope it continues for many years to come.

To the wonderful people who undertook this thesis journey alongside me, your support, both technical and emotional, from the office to the bar, was valued to no end. Your enthusiasm inspired me and your reality checks kept me grounded. Also, thank you to all the postgraduate students throughout the department who seem to come from every corner of the world and made me feel better about still being in Sydney. I loved getting to know so many of you and hope one day I'll see you on your home turf.

To the biology staff of Macquarie University, who trained me up over the last five years. This thesis is the culmination of the dedication and inspiration you distilled in me from my first day here. Hope it's any good!

To my lab mates of the conservation genetics lab, thank you very much for making day after day in a windowless office seem fun and enjoyable and for assisting me through countless technical glitches. Thank you to Sonu Yadav for her analytical support, many words of wisdom and frequent tomfoolery.

To my supervisor, Rachael Dudaniec, thank you very much for your constant support and guidance. Even when on the other side of the world you seem to always be on hand for assistance. I've learnt so much it terrifies me how little I knew at the start. I'm sure I'll be the first of your many happy masters students.

To my amazing family, it's a rare thing to have such strong support spread out along the extended branches of the family tree. Particularly, Mum and Dad, thanks for inspiring me from the time I was born and continuing to do so today. Your bottomless support and faith in me is actually quite ridiculous. Thanks.

And finally, to the blue-tailed damselfly, *Ischnura elegans,* I've never actually seen you in real life and I can't be certain you even exist but thanks for this interesting story and I'll come visit you one day soon.

Background: Factors affecting landscape genetic patterns in range expanding species

One of the most obvious effects of global climate change is the widespread shifts in species distributions. Species ranges are dynamic; continually expanding and contracting in response to the large changes in temperature, precipitation and CO^2 concentration that have been documented over the last 2.5 million years (Davis & Shaw, 2001; Excoffier et al., 2009). Range expansions due to contemporary anthropogenic climate change have been documented from the first half of the twentieth century (Ford, 1945; Uvarov, 1931) and have since been prolifically described in hundreds of species from around the globe (Bradshaw & Holzapfel, 2006; Buckley et al., 2012; Hoffmann & Sgrò, 2011; Lancaster et al., 2015; Lavergne et al., 2010; Parmesan, 2006; Parmesan & Yohe, 2003; Pateman et al., 2012). For example, treelines have shifted latitudes in Sweden (Kullman, 2001) and Canada (Lescop-Sinclair & Payette, 1995) and elevations in Russia (Meshinev et al., 2000; Moiseev & Shiyatov, 2003) and New Zealand (Wardle & Coleman, 1992), birds in the UK moved northward an average of 18.9km over a 20-year period (Thomas & Lennon, 1999) and some copepod species have shifted more than 1000km northward corresponding to changes in oceanic climate regimes (Beaugrand et al., 2002). Insects, being particularly responsive to changes in temperature, have been the focus of many studies with expansions being documented in 22 of 35 European butterfly species (Parmesan et al., 1999), 38 of 41 UK odonate species (Hickling et al., 2005), as well as in heteropterans, neuropterans and orthopterans (Hickling et al., 2006). These widespread range expansions have many ecological, evolutionary and genetic consequences.

The dynamics of range expansions are shaped by the novel environmental conditions beyond the range edge, both biotic and abiotic, and the species life history. Broadly speaking, small animals with short life cycles and large population sizes will have a better chance at adapting to new environmental conditions beyond their range limit, compared to large animals with long life cycles and small population sizes (Bradshaw & Holzapfel, 2006). Additional pressures are applied through novel species interactions and changes in ecological compositions (Fitt & Lancaster, 2017; Telwala et al., 2013; Therry et al., 2016). Some large-scale analyses have suggested that novel species combinations caused by asynchronous migrations will increase regional (gamma) and local (alpha) biodiversity (Menéndez et al., 2006; Thuiller et al., 2011), but this will depend on the area being colonised (Lebouvier et al.,

2011). For example, studies in high latitudes, which are typically more open to invasion, suggest that colonising species may outcompete and replace native species (Alexander et al., 2015). Simulations, however, have revealed that competitive abilities dramatically decline during a range expansion (Burton et al., 2010), suggesting the ability to successfully colonise new areas is dependent on presence of competing species. Abiotic landscape features also influence the dynamics of range expansions as reductions in habitat suitability are common at range edges (Balestrieri et al., 2010; Bennie et al., 2013; Hill et al., 2001). Despite having become habitable and, therefore, being colonised, climates at the leading edge of a range expansion often contain associated environmental pressures that differ from the core, pre-adapted climate (Lancaster et al., 2015). These novel environmental pressures, natural or anthropogenic, can act as barriers to dispersal or restrict gene flow, greatly decreasing the genetic diversity necessary for range edge populations to adapt and continue to expand (Dudaniec et al., 2018). Understanding the influence of landscape and environmental features on the genetic connectivity of range edge populations is crucial to predict future species distributions and range expansion consequences.

Bringing together the disciplines of landscape ecology and population genetics allows a quantitative assessment of how landscape and environmental features influence population structure, gene flow and local adaptation (Manel et al., 2003). In isolation, these disciplines have developed a number of analytical techniques to elucidate landscape effects, however, in combination they avoid some of the subjectivity of projecting population genetic patterns onto landscapes (Richardson et al., 2016). Landscape genetics is the result of the union between the two disciplines and is appealing due to its capacity to answer questions such as: (i) how has recent global change (i.e., land use, land cover and climate change) affected patterns of neutral and adaptive genetic variation; and (ii) are species likely to adapt to ongoing global change on an ecological timescale (Manel & Holderegger, 2013)? Furthermore, by evaluating explicit landscape effects (including human-modified landscapes) on genetic diversity, landscape genetics offers a powerful tool for conservation and management (Balkenhol et al., 2017; Richardson et al., 2016). The approach is straightforward: genetic data coupled with geographic data are analysed together to explicitly quantify the effects of the landscape on micro-evolutionary processes, such as gene flow, drift, and selection, using neutral and adaptive genetic data (Balkenhol et al., 2016; Richardson et al., 2016). This allows researchers to identify populations that are: panmictic with no impact from landscape features, isolated by geographic distance (IBD) or isolated by

environmental features (IBE). Researchers, land managers and policymakers can then make targeted conservation decisions based on these findings (Manel & Holderegger, 2013). Landscape genetic techniques have also become broadly accessible in recent years with the availability of precise spatial data (Anderson & Gaston, 2013; Pettorelli et al., 2005), high quality genetic markers and next-generation sequencing (Anderson & Gaston, 2013; Pettorelli et al., 2005), statistical methodologies (Dudaniec et al., 2016; Peterman, 2014), increased computational power (Kidd & Ritchie, 2006; Paul & Song, 2012) and an array of analytical programs such as CIRCUITSCAPE (Shah & McRae, 2008) and STRUCTURE (Pritchard et al., 2000). Applying the landscape genetic approach to range expanding systems is promising due to the effects of declining habitat suitability and novel environmental conditions at species' range edges on micro-evolutionary processes (Balkenhol et al., 2017; Höglund, 2009; Keyghobadi, 2007; Sagarin et al., 2006). However, several range expansion processes can lead to a pattern of reduced gene flow and genetic diversity and it can be challenging to tease these apart from landscape effects (Cushman, 2015). Below, I identify seven processes associated with range expansions and outline how they can interfere with landscape or climatic drivers of neutral genetic structure.

Founder events

The colonisation of new environments by a restricted number of individuals often results in a population that is genetically non-representative of the broader gene pool (Austerlitz et al., 1997; Excoffier et al., 2009; Wereszczuk et al., 2017). Along with this is the steady reduction in heterozygosity with increasing distance from the ancestral population (DeGiorgio et al., 2011; Slatkin & Excoffier, 2012). The strength of founder events on demographic and genetic structure will be increased in species with restricted life-history traits (i.e. more specialised) and will inflate landscape effects during range expansions (Landguth et al., 2012; Wereszczuk et al., 2017). For example, species with the most specialised habitat requirements show reduced genetic diversity and stronger population bottlenecks at the range edge compared to generalist species (Hill et al., 2006). Organisms forced to move due to ongoing environmental change are often faced with fragmented and lower quality habitat towards the range edge; potentially reducing their ability to adapt and persist into the future (Hill et al., 2006; Schmitt & Hewitt, 2004). Adaptations to, or increased resistance from, conditions at the range edge can create patterns of genetic structure that follow environmental

or distance gradients and make it difficult to tease apart the influence of the environment from founder effects (Orsini et al., 2013).

Allele surfing

The reduction of allelic richness and heterozygosity that may be observed along the range expansion axis due to past and continual founder events can result in increased genetic drift (Balkenhol et al., 2017; White et al., 2013). This can lead to the fixation of allele frequencies that become more differentiated towards the range limit as rare alleles are able to 'surf' and become established (Peischl et al., 2013). The likelihood of alleles being surfed depends on their proportional presence in the founding populations and, therefore, both beneficial alleles (i.e. that can promote adaptation) and deleterious alleles can be surfed. Although frequent alleles have an increased chance of surfing, it is the rare surfed alleles that have the biggest impact on spatial genetic structure (Hofer et al., 2009; Klopfstein et al., 2006), and can, therefore, pose a problem for determining the influence of landscape and climatic variables. Our understanding of genetic patterns generated by range expansions have, for the most part, been the result of models, simulations (e.g. through programs such as SPLATCHE2 (Ray et al., 2010) and CDPOP (Landguth & Cushman, 2010) and laboratory experimentation; with very few studies observing allelic surfing empirically. Graciá et al. (2013) examined the neutral genetic structure of range expanding tortoises and found that rare alleles at the range centre became common at the range edge; following the predictions of allelic surfing theory. Similar patterns were found in species with completely different distributions and life histories, including strawberry poison-arrow frogs (Gehara et al., 2013) and humans (Sousa et al., 2014). More empirical studies are required; however, simulations continue to elucidate the genetic mechanisms underlying the evolution of traits at expanding range margins (Chuang & Peterson, 2016). These include increases in dispersal ability (Travis et al., 2010) and fecundity (Burton et al., 2010) and the loss of competitive ability (Burton et al., 2010) that are associated with surfed alleles. These processes will influence neutral genetic connectivity and need to be separated from landscape effects.

Competition

The exclusion of ecologically similar species from occupying the same geographic space has been explained by the interaction between competition and niche variation (Chesson, 2000; MacArthur & Levins, 1967). Extending from this, a recent hypothesis posits that if competitive abilities (e.g. body size, aggression and boldness) are more strongly

phylogenetically conserved than niche traits (e.g. thermal tolerance), a species will be more likely to competitively exclude distantly related species and co-exist with closely related species (Mayfield & Levine, 2010). This hypothesis has been supported in a range expanding damselfly in Scotland (*Ischnura elegans*), which was found to competitively displace native damselfly species that were more distantly related and was able to coexist with closely related species due to a similarity in competitive abilities (Fitt & Lancaster, 2017). These interspecific interactions may be more significant in reducing dispersal, gene flow and genetic diversity between range edge populations than environmental variables and, therefore, need to be partialled out of a landscape genetic analysis. For example, Medley et al. (2015) predicted that forest habitat would aid the genetic dispersal of an invasive mosquito (*Aedes albopictus*), but found that genetic connectivity was reduced, likely due to competition from other mosquito species present within the forest. Empirical evidence on the impact of interspecific interactions on species' range expansions is dominated by studies of plants (Svenning et al., 2014). Key results include: reduced growth rates in late-arriving species due to competition for resources (Van der Knaap et al., 2005), poor competitive ability of species that are dependent on fire disturbance outside their natural range (McLeod & MacDonald, 1997), and the spread of species after human disturbances leading to a loss of competitors (Bradshaw et al., 2010). Many species distribution and landscape genetic models omit the influence of interspecific competition and only consider landscape and environmental variables (Urban et al., 2012), likely due to a lack of information on biotic interactions. Just as landscape features can reduce genetic diversity and connectivity between range edge populations, so too can competition; slowing, halting or changing the course of the expansion wave.

Introgression and hybridisation

Following on from the competition-relatedness hypothesis (Mayfield & Levine, 2010), which predicts that closely related species are more likely to share distributions, is the often-observed occurrence of introgression and hybridisation, which can be promoted by range expansion processes. Hybridisation is broadly defined as the successful mating between individuals from two genetically differentiated lineages, and introgression is defined as the permanent infiltration of genes from one genetic lineage into the genome of another through repeated interbreeding (Hamilton & Miller, 2016; Stebbins, 1959; Wheeler & Guries, 1987). Hybridisation, as a result of range expansions, has been documented in brown argus butterflies (Mallet et al., 2011), grizzly and polar bears (Kelly et al., 2010), flying squirrels

(Melo-Ferreira et al., 2007) and westslope cutthroat trout (Rubidge & Taylor, 2004), to name a few (reviewed in Sánchez-Guillén et al., 2013). Although hybridisation has many negative connotations due to the risk of genetic assimilation, reducing diversity and causing outbreeding depression (Kleindorfer et al., 2014; Muhlfeld et al., 2009), there is also a strong argument for the genetic benefits that accompany hybridisation (Hamilton & Miller, 2016; Hoffmann & Sgrò, 2011; Sánchez-Guillén et al., 2016). The boost of genetic diversity and the potential for acquiring adaptive genes in hybridised individuals may provide a fitness benefit in new or changing environments (Kleindorfer et al., 2014; Peters et al., 2017). In this way, hybridisation and introgression may provide a faster response to changing environmental conditions than natural selection and a more stable response than phenotypic plasticity (Hamilton & Miller, 2016; Peters et al., 2017). Thus, hybridised individuals may have increased fitness within novel environmental conditions, offering an increased capacity for range expansion or local adaptation (Sánchez-Guillén et al., 2016; Wellenreuther et al., 2018). This has been observed in hybrid fruit flies adapting to extreme temperatures, enabling them to expand their range (Lewontin & Birch, 1966) and in hybrid ants adapting to cold temperatures following the hybridisation between an invasive species and native species (James et al., 2002). Just as with competition, hybridisation is an important interspecific interaction that should be considered along with landscape and environmental variables in predictions of future species distributions. Hybridisation and introgression can lead to rapid adaptation and divergence between range edge populations and can generate similar genetic patterns to that of isolation by environment. The potential of hybridisation should, where possible and relevant, be considered when attempting to determine landscape effects on range expanding species.

Temporal variations in gene flow

Range expansions are continual phenomena, as distributions shift over time leaving genetic signatures of both historical and contemporary movement patterns (Excoffier et al., 2009). Therefore, challenges in separating the drivers of neutral genetic structure in range expanding species arise when the range expansion occurs across multiple spatial or temporal scales. One of the main historical drivers of genetic structure for many species is post-glacial range expansion events, which work in combination with contemporary range expansion to shape current genetic patterns (Dudaniec et al., 2012; Swaegers, Mergeay, et al., 2014). Separating the genetic signature of multiple temporal processes becomes especially difficult as modern range expansions, resulting from climate change, often share the same latitudinal axis as post-

glacial range expansions (Hewitt, 2000; Swaegers, Mergeay, et al., 2014). Following fragmentation it can take tens to thousands of generations for an equilibrium to be reached between gene flow and genetic drift, resulting in time lags to detect landscape genetic effects (Landguth et al., 2010; Mona et al., 2014; Zellmer & Knowles, 2009). Therefore, the influence of contemporary landscape structure on genetic patterns can become confounded by historical demographic processes (Dudaniec et al., 2012). For instance, Pavlacky Jr et al. (2009) found that the contemporary landscape explained only slightly more genetic differentiation than did the reconstructed historical landscape in the rainforest bird *Orthonyx temmii*. However, it is not only historical and contemporary landscape structures that influence genetic patterns. The strength, rate and pattern of past landscape change can also determine the degree to which current landscapes influence genetic dispersal (Dudaniec et al., 2013). This can be accounted for by including the rate of landscape change in the analysis. For example, Dudaniec et al. (2013) included change in the proportion of landscape that was forest or urban over a 21-year period using satellite imagery to account for landscape change. Rapid rates of landscape change can lead to the detection of incorrect relationships between current landscapes and genetic structure if there has not been enough time for populations to achieve equilibrium (Anderson et al., 2010). As such, temporal variations in gene flow need to be considered in landscape genetic analyses of range expanding species subjected to varying rates of climate change.

Spatial variations in gene flow

Similarly, the spatial scale of sampling and analysis needs to be carefully considered so that it accounts for the species population size, dispersal capacity, life history and the geographic area under study (Dudaniec et al., 2013; Dudaniec et al., 2012; Landguth et al., 2010). Mismatches between the scale of observation and the underlying species biology can lead to flawed conclusions about a species sensitivity to landscape features (Anderson et al., 2010). This can be difficult as researchers often do not have a priori information about a species behaviour and ecology with which to plan their sampling design and analysis (Anderson et al., 2010). Ideally, the study area should be larger than the area occupied by the population of interest and larger than expected dispersal distances (Anderson et al., 2010). If this is not known, the creation of a buffer zone surrounding the study area can help to reduce confounding effects from individuals outside the extent of sampling (Cushman & Landguth, 2010; Dale & Fortin, 2014). Understanding species spatial extents is particularly difficult in range expansion studies. Additionally, dispersal during a range expansion can occur across

multiple spatial scales (i.e. short and long-distance dispersal) which can lead to changes to genetic structure so that patterns no longer reflect the influence of landscape features. Mona et al. (2014) used simulation studies to quantify the effect of habitat fragmentation on the genetic diversity and differentiation between populations of a range expanding species. They found, as expected, the influence of long-distance dispersal counteracts the structure generated by landscape barriers and reduces population differentiation. Medley et al. (2015) studied an invasive mosquito that was dispersing through both long-distance dispersal (via highways from core populations to the range edge) and short distance dispersal (contagious spread). To account for the potential for long-distance dispersal to counteract the influence of the landscape on contagious spread, they used both least-cost (the path offering the least resistance) and circuit distance (cumulative cost of random dispersal through the surrounding landscape). They found that least-cost distance was more effective to describe broad-scale patterns of dispersal from core to range edge (reflecting highways) whereas circuit distance was better for predicting genetic structure at the fine scale (discussed in Cushman, 2015). The admixture of genes from multiple locations during a range expansion can mask the influence of landscape and environmental features on genetic connectivity and may require the examination of multiple spatial scales to untangle complex population genetic dynamics.

Increases in dispersal capacity

Morphological distinctions between range edge populations and those closer to the range centre are well documented (Krause et al., 2016; Phillips et al., 2006; Shine et al., 2011; Travis & Dytham, 2002). For species that are actively dispersing through the landscape, particularly invasive species, dispersal ability tends to increase toward the range edge (Chuang & Peterson, 2016; Shine et al., 2011). Dispersal ability is predicted to continue to increase at the expansion front, provided the dispersal-related traits are heritable, as the best dispersers will be near each other and will likely mate with each other; known as the Olympic village effect (Phillips et al., 2008). Increases in wing muscle mass, a dispersal-related trait, was observed within a range expansion gradient in the dainty damselfly, *Coenagrion scitulum,* and this trait was found to positively covary with flight endurance (Therry, Gyulavári, et al., 2014; Therry, Nilsson-Örtman, et al., 2014; Therry, Zawal, et al., 2014). These morphological changes along the range expansion gradient were also associated with thermal gradients (Therry, Gyulavári, et al., 2014; Therry, Nilsson-Örtman, et al., 2014). Furthermore, Swaegers et al. (2015) disentangled the neutral range expansion processes from non-neutral, temperature-driven selection processes to identify genes under selection

associated with these morphological changes. Such studies demonstrate the interwoven nature of selection on dispersal traits with landscape and environmental features. Increases in dispersal ability at the range front can result in decreased landscape effects on genetic differentiation between populations and is an additional factor to consider when interpreting landscape genetic relationships in range expanding species.

Conclusion and implications

From study design to experimentation to simulations, there are many ways to tease apart range expansion processes and landscape effects on genetic structure (Cushman, 2015). The best ways to account for these interacting processes will be specific to the species, study system and questions of interest. A well-designed study can sample data (1) in different study areas where the factor of interest is either present or not, (2) along environmental gradients, or (3) in various environmental strata (Balkenhol et al., 2017). These three designs would be particularly useful in range expanding species if they correspond with the axis of expansion. Experimentation on the effects of confounding variables, such as interspecific interactions, will strengthen biological inferences and can help achieve a general understanding of the relative influence of different landscape features on gene flow (Short Bull et al., 2011). Simulations can help generalise the expectations of landscape genetic structure along an expansion gradient and be used to explain the patterns being observed in real-world studies (Cushman, 2015). Accounting for all complexities is beyond the feasibility and resources of most, if not all, studies, however, careful consideration of these processes is vital to increase confidence in the inferences drawn about landscape and climatic effects on the genetic connectivity of range expanding species.

Landscape genetic connectivity and sex-specific responses in a range expanding damselfly (*Ischnura elegans*)

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Manuscript in preparation for Molecular Ecology

Abstract

Range shifts induced by climate change have been documented in many insect taxa. The damselfly, *Ischnura elegans,* is undergoing a poleward range expansion in northern Europe showing local adaptation to environmental conditions at the range edge in Sweden. However, the role of neutral genetic connectivity and sex-specific responses in the range expansion process is unknown. We examine relative abundance, sex-specific landscape genetic relationships and morphological variation in *I. elegans* along a ~600km range expansion axis. We analysed 29 landscape resistance surfaces against genetic distances (F_{ST} and $G`_{ST}$) calculated from 3,554 RAD seq-derived neutral Single Nucleotide Polymorphisms (SNPs) from 25 sites (n = 426 individuals). Resistance modelling showed cooler mean annual temperatures limited genetic connectivity linearly, with greater resistance at colder range limit sites. Relative site abundances were reduced along the sampled gradient; however, genetic diversity showed no significant change, suggesting recent colonisations. Results were consistent with female-biased dispersal, with females showing reduced temperature resistance to gene flow and a small, almost linear effect of land cover type that was not observed in males. Female, but not male, wing length also increased towards the range limit. Our findings demonstrate sex-specific morphological and landscape genetic responses during a climate-change induced range expansion.

Key words: range expansion, *Ischnura elegans,* landscape genetics, climate change, morphology, sex-biased dispersal

Introduction

Contemporary range expansions due to rapid climate change, have been observed in many plant and animal species from every continent, but are especially common in higher latitudes and elevations (Bradshaw & Holzapfel, 2006; Buckley et al., 2012; Hill et al., 2011; Hoffmann & Sgrò, 2011; Lavergne et al., 2010; Parmesan, 2006; Parmesan & Yohe, 2003; Pateman et al., 2012). Despite warming climates facilitating the colonisation of new environments, there are often environmental pressures at the range edge that are not present in the core, pre-adapted climate (Lancaster et al., 2015). This can lead to a gradient of declining habitat suitability that reduces gene flow making it difficult for species to adapt and persist in these novel climatic conditions (Halbritter et al., 2015). Individuals colonising these new areas are usually genetically, demographically and morphologically non-representative of the broader population (Excoffier et al., 2009; Hill et al., 1999; Miller & Inouye, 2013) potentially due to within-species variation in dispersal capacity, life-history, or selection pressures. Variation in these traits can lead to differential responses to novel environmental conditions during range expansion. (Duckworth & Badyaev, 2007; Hill et al., 1999).

Behavioural and phenotypic variation between males and females can create sex-specific responses, such as sex-biased dispersal, which can affect range expansion patterns. Sex-biased dispersal is a well-documented characteristic particularly in birds (Clarke et al., 1997; Greenwood, 1980), mammals (Dobson, 1982; Greenwood, 1980) and insects (Beirinckx et al., 2006). This research has led to many proposed hypotheses that address the differential costs and benefits of dispersing for both sexes (Beirinckx et al., 2006). For example, resource defence by males often favours female sex-biased dispersal (Nagy et al., 2007), scramble mate competition often favours male sex-biased dispersal (Höner et al., 2007; Spritzer et al., 2005), while inbreeding avoidance can involve biased dispersal in either sex (Pusey, 1987). Sex-biased dispersal can create spatial clines in sex-ratios and may reduce reproductive output at the leading edge where the more dispersive sex is over-represented (Miller & Inouye, 2013; Miller et al., 2011). Reduced reproductive output combined with novel environmental conditions at the range edge can strongly affect the velocity of spread and dynamics of range expansions (Kot et al., 1996; Miller et al., 2011). For example, Miller and Inouye (2013) used experimentation and simulation to demonstrate that increasing the dispersal of females relative to males accelerated the spread of the bean beetle, *Callosobruchus maculatus*. Female-biased dispersal will accelerate the speed of a range

expansion as females, being the regenerative sex, will disperse farther, until the point where the negative effects of male limitation exceed the positive effects of female dispersal (Miller & Inouye, 2013). It is, therefore, crucial to consider sex-specific dispersal behaviours when making predictions about the rate and dynamics of range expansions. Sex-specific landscape genetic responses is a rarely considered way of understanding sex-biased dispersal and by examining landscape genetic effects on males and females separately, processes that would be otherwise hidden can be revealed.

Only a few studies have examined the effect of sex-specific dispersal patterns on landscape genetic structure, despite often disproportionate contributions of the sexes to gene flow in many species (Amos et al., 2014; Coulon et al., 2004; Harrisson et al., 2014; Harrisson et al., 2013; Shafer et al., 2012; Tucker et al., 2017). Quantifying sex-specific environmental effects on genetic connectivity via landscape genetic approaches offer an informative way to examine this (Coulon et al., 2004; Manel & Holderegger, 2013). For instance, sex-biased dispersal may result in differential landscape genetic responses in males versus females, but fail to be detected as one sex masks the influence of landscape features on the other (Tucker et al., 2017). Landscape genetic studies have found strong responses to habitat fragmentation when conducting analyses by sex but had inconclusive results when analysing both sexes together (Amos et al., 2014; Coulon et al., 2004; Harrisson et al., 2014; Harrisson et al., 2013). These studies have also been conducted on species of conservation concern (Tucker et al., 2017) and when looking at sex-specific responses to summer and winter landscapes (Shafer et al., 2012) both finding differential responses between the sexes. This growing body of evidence highlights that failing to account for sex-biased dispersal in landscape genetic analyses may result in misidentifying climatic or landscape influences on genetic connectivity, which may have conservation management implications. Sex-specific landscape effects are also crucial considerations in range expanding systems as declining habitat suitability will likely exaggerate the differential responses between males and females.

Sex-specific morphological variation along range expansion gradients is another factor that been little explored, yet has implications for traits such as dispersal capacity due to variable environmental conditions and their associated selection pressures. Individuals at the leading edge of a range expansion often show morphological traits that are distinct from those closer to the centre of the historic range (Krause et al., 2016). For example, spatial sorting of individuals with dispersal-enhancing traits during range expansion can lead to more dispersive genotypes at the range limit and declining dispersal ability towards the core

(Krause et al., 2016; Phillips et al., 2006; Shine et al., 2011; Travis & Dytham, 2002). On the other hand, suboptimal and novel environmental conditions at the range limit can reduce the body condition of individuals in edge populations (Hardie & Hutchings, 2010; Swaegers et al., 2015), which may affect dispersal capacity. For example, an increase in flight muscle mass, related to flight endurance (Therry, Gyulavári, et al., 2014), was found in range expanding damselflies, along with reduced body condition at sub-optimal thermal regimes, suggesting strong selection pressure on dispersal traits to overcome environmental pressures (Therry, Zawal, et al., 2014). Despite this, many insect studies have found dispersal related morphological traits to be associated with range expansions (Haag et al., 2005; Hill et al., 1999; reviewed in Hill et al., 2011; Niemelä & Spence, 1991; Simmons & Thomas, 2004; Thomas et al., 2001). Sex-biased dispersal over generations can lead to the more dispersive sex contributing more genetic diversity to local gene pools. Combining spatial sorting of dispersal-enhancing traits during range expansion with sex-biased genetic dispersal may lead to phenotypic divergence between the sexes at the range limit. Sex-specific increases in dispersal capacity at the range edge offers intriguing insights into the evolutionary consequences of the range expansion process.

Insects are a model taxonomic group for studying range expansions, landscape effects and sex-specific dispersal patterns. Sexual size dimorphisms and sex-specific behaviours and life-histories are frequent throughout insect species and have the potential to create sex-biased differences in dispersal which may become inflated during a range expansion (Hill et al., 1999; Teder & Tammaru, 2005). Furthermore, as ectotherms, insects are particularly responsive to environmental change as locomotion, growth and reproduction are directly influenced by ambient temperatures (Deutsch et al., 2008; Paaijmans et al., 2013; Sánchez-Guillén et al., 2016). Their short generation times, high reproductive rates and the high mobility of many taxa allow them to undergo rapid range expansions (Bale et al., 2002; Gullan & Cranston, 2010; Ott, 2010; Settele et al., 2008). For example, range expansions have been documented in 22 of 35 European butterfly species (Parmesan et al., 1999), 38 of 41 UK odonate species (Hickling et al., 2005), as well as in heteropterans, neuropterans and orthopterans (Hickling et al., 2006). These characteristics of insects also enable them to be responsive at shorter time scales (i.e. to recent landscape change) and to be limited by finer scale landscape features which assist detection of dispersal barriers in landscape genetic analyses. In non-range expanding species, a variety of landscape features have been found to influence gene flow between insect populations, including topography (Phillipsen et al.,

2015), agriculture (Wallace, 2013), residential areas (Watts et al., 2004) and forests (Keller et al., 2012) as well as biotic variable such as the presence of heterospecifics (Medley et al., 2015). These interactions with landscape and climatic variables may be inflated towards the range edge as habitat suitability declines and gene flow is reduced (Dudaniec et al., 2018; Lancaster et al., 2015). Insects make ideal taxa for identifying sex-specific landscape effects during range expansions, however, only a few studies have explored sex-biases in dispersal in insects (Beirinckx et al., 2006; Caudill, 2003; Heidinger et al., 2018; Lagisz et al., 2010; Markow & Castrezana, 2000; Sundström et al., 2003). These studies find evidence of sex-biased dispersal but there seems to be no consistent trend among insects, with biases in dispersal being dependant on the species and system in question.

Studies that have explored general patterns of sex-biased dispersal in insects have revealed complex interactions between life-history, morphology and density (Beirinckx et al., 2006; Chaput-Bardy et al., 2010). Damselflies have been used as model invertebrates for dispersal studies as they are relatively easy to handle and mark individually (Bohonak & Jenkins, 2003; Wellenreuther et al., 2011). Meta-analyses determining the probability of each sex dispersing to a new pond in multiple damselfly species found variation in the magnitude of sex-biased dispersal between species and families (Conrad et al., 1999) but overall, found that males are more likely to be recaptured from the same pond (Beirinckx et al., 2006). This would suggest female-biased dispersal, however, factors such as male harassment, mating systems and differential survival rates need to be considered (Anholt et al., 2001). For example, Chaput-Bardy et al. (2010) found female-biased dispersal in the banded damselfly and attributed it to the territorial behaviour of males in a lek mating system, as well as site density and habitat suitability. These factors encourage female dispersal through the surrounding landscape and may increase the chance of detecting sex-specific landscape effects on movement and genetic connectivity. The influence of sex-biased dispersal on landscape genetic connectivity has rarely been explored in insects and never, to our knowledge, in a range-expanding insect.

The blue-tailed damselfly, *Ischnura elegans,* is a range expanding species in northern Europe and has expanded its range northwards by 143 km in the UK over the last 50 years (Hickling et al., 2005). This species has been well investigated including studies conducted along the range expansion axis of *I. elegans* from the south of Sweden to its northern range edge (Dudaniec et al., 2018; Lancaster et al., 2016; Lancaster et al., 2015, 2017). These studies identified that the recent colonisation of higher latitudes in Sweden by *I. elegans* occurred

under strong selection on cold tolerances, imposed by greater climatic variability at recently colonised, higher latitude sites (Lancaster et al., 2015). They further confirmed that heat tolerance mechanisms are largely conserved with latitude in this species, whereas gene expression associated with cold stress is more latitudinally variable (Lancaster et al., 2016). Colour morph frequencies along the latitudinal gradient were found to be best explained by positive social interactions facilitating range expansion and counteracting expected patterns associated with thermal tolerant phenotypes (Lancaster et al., 2017). Most recently, this study system has revealed rapid adaptations associated with the range expansion process including genes under selection associated with thermal response, mate discrimination and salinity tolerance (Dudaniec et al., 2018). Together these studies describe a complex system where environmental, social, demographic and genetic processes interact and present novel findings that contrast with expected patterns from stable, non-expanding populations. One remaining knowledge gap is understanding the influence of landscape and environmental variables on neutral genetic connectivity, shifts in relative site abundance and the influence of sex-biased dispersal and morphological variation along the range expansion axis.

Here we integrate both ecological and neutral genetic information with a landscape genetic resistance approach to address questions regarding gene flow patterns along the expansion gradient. Using neutral Single Nucleotide Polymorphisms (SNPs) from Restriction-site Associated DNA sequencing (RAD), morphological data and measurements of relative site abundance, we elucidate demographic and sex-biased mechanisms underlying landscape genetic relationships in *I. elegans.* Specifically, we test: i) whether genetic diversity and relative site abundance declines along the range expansion axis, in accordance with expectations under range expansion into less suitable habitat; ii) whether landscape and climatic variables differentially affect males and female patterns of neutral genetic connectivity along the range expansion axis; and iii) if there are sex-specific shifts in body size or wing length towards the range limit, indicative of spatial sorting or selection for dispersal-enhancing traits along the range expansion gradient. Our study elucidates interactions between landscape genetic connectivity, morphological variation and sex-biased dispersal in contributing to a successful range expansion under climate change.

Materials and Methods

Sampling and study design

Ischnura elegans (Coenagrionidae: Odonata*)* has a widespread distribution throughout Europe and Asia. Its northern range extends to the southern coastal areas of Scandinavia and the north of the United Kingdom (Dijkstra & Lewington, 2006). Adult *I. elegans* thrive in disturbed areas such as agricultural ponds subject to frequent vegetation removal (Hofmann & Mason, 2005; Okuyama et al., 2013) and prefer open and light waterbodies with abundant reed growth to shady areas with overhanging canopy (Dijkstra & Lewington, 2006). Our study area spans a latitudinal gradient of approximately five degrees of latitude in Sweden (latitudinal range: 55.53˚ to 60.58˚), extending 583km. In the summer months of July-August 2013, 25 sites were sampled for *I. elegans* following a paired gradient sampling design that spanned the southern core region to the northern range edge (Figure 1). Adult *I. elegans* were caught near reed beds and vegetation using sweep nets within 10m of water bodies including ponds, lakes and coastal inlets as described in Dudaniec et al. (2018). Searches were performed by 2-3 people simultaneously and catching time was calculated across all searchers as the total minutes spent searching. Data for relative abundance and morphology were conducted on a larger sample size of sites (n = 61 for relative abundance and n = 41 for morphology) which included sites for which genetic data were not obtained. Relative site abundance was inferred from capture rates as the total number of *I. elegans* caught at a site divided by the number of minutes spent searching (i.e. 'capture rate per minute'). The date, time of day and time spent searching were also recorded for each site. Sex and life stage (adult or teneral) information were recorded for each damselfly collected. Teneral (i.e. immature, newly emerged) individuals were identified as having brownish soft wings. Morphological data were acquired by scanning images of live individuals placed dorso-ventrally in a petri-dish, between the two plates of the dish, with one side inverted so that the damselfly was in a straight and stationary position. The dishes were then placed on a Canon portable scanner with images saved as jpeg files. Only adults were used for morphology scans. Details on morphological analyses are below. Finally, damselflies were preserved in 90% ethanol for DNA preservation. All procedures were conducted according to the ethical guidelines of Lund University in Sweden, and sampling permissions were obtained from local authorities and landholders.

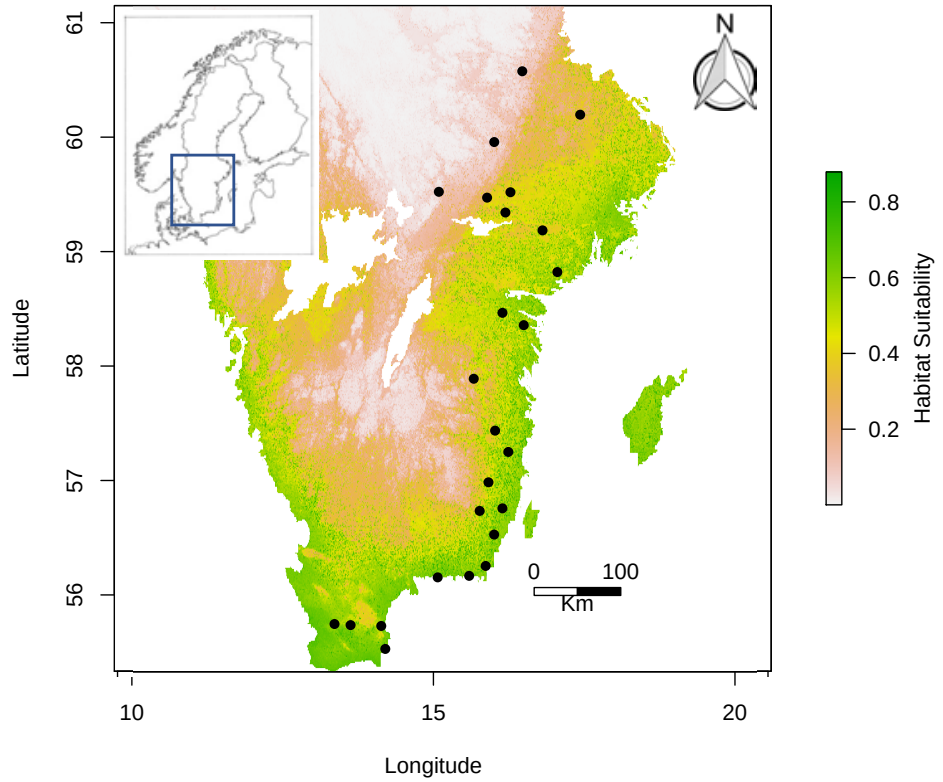

Figure 1: Sites sampled for *I. elegans* (n=25) along a latitudinal gradient between 55.53˚ and 60.57˚ in southern Sweden, overlayed on a habitat suitability model published by Lancaster et al. (2015). The inset shows the study area within Scandinavia.

DNA sequencing, bioinformatics and SNP characterisation

DNA was extracted from the head, thorax and legs of 432 *I. elegans* across 25 sites (10–20 individuals per site, mean 17.04 ± 0.72) using a DNeasy Blood and Tissue extraction kit (Qiagen). Extracted genomic DNA was quantified using a QUBIT 2.0 Fluorometer (Life Technologies) and processed into paired-end RAD libraries that were sequenced on an Illumina Hiseq 2500 as outlined in Dudaniec et al. (2018). Bioinformatics analyses performed on the data are described in detail in Dudaniec et al. (2018). Briefly here, raw reads were demultiplexed using the *process_radtags* program in STACKS v.1.40 (Catchen et al., 2013; Catchen et al., 2011). The final sample size of individuals retained for analyses was 426 across the 25 populations, as six samples were excluded due to low coverage. This included 209 males and 217 females (Table S1). A draft *I. elegans* genome (available on DRYAD: https://doi.org/10.5061/dryad.8s449qb) was used to align duplicated reads using BOWTIE2 v.2.2.5 (Langmead & Salzberg, 2012). Aligned reads from BOWTIE2 were analysed

in the *ref_map* program in STACKS to build the initial consensus catalogue of SNPs, resulting in 3,452,911 loci. SNPs were further filtered using the *rxstacks* corrections model, which removes excess haplotypes and confounded loci (Catchen et al., 2013). An initial minimum depth of coverage of 5x was specified for each SNP-containing RAD locus with a minor allele frequency (MAF) of 0.05. Additionally, a locus was only included if it occurred in 22/25 populations and in at least 80% of individuals within each population to ensure wide representation of data for each SNP across all samples and sampling locations (recommended in Paris et al., 2017). After filtering loci using the STACKS *populations* program, 13,612 SNPs were retained. To minimise the inclusion of closely linked SNPs we filtered one SNP per RAD tag using the 'write_single_snp' option, which resulted in 3809 SNPs. Furthermore, to minimize the inclusion of putative loci under selection from our analyses, F_{ST} outlier loci, identified using both BAYESCAN (Foll & Gaggiotti, 2008) and OUTFLANK (Foll & Gaggiotti, 2008; Whitlock & Lotterhos, 2015), were removed from the dataset (n = 255 SNPs), resulting in a final dataset of 3554 'neutral' SNPs for analysis (see Dudaniec et al., 2018). Genetic structure analysis was then undertaken with this final SNP dataset using the R package ADMIXTURE (Alexander et al., 2009) as in Dudaniec et al. (2018) (Figure S1 and S2).

Genetic distance, diversity and sex-biased dispersal

Genetic distance was calculated between the 25 populations using Nei's pairwise F_{ST} (Nei, 1977) from the R package ADEGENET (Jombart, 2008) and Hedrick's $G`_{ST}$ (Hedrick, 2005) using the R package MMOD (Winter, 2012). Hedrick's $G`_{ST}$, a standardised version of G_{ST}, has been recommended as an appropriate measure of genetic differentiation as it takes into account different levels of within-population genetic diversity and can so is effective when comparing across multiple populations or sampling sites (Heller & Siegismund, 2009). Geographic distance (km) between sites was calculated using the R package GEOSPHERE (Hijmans et al., 2015). Mantel tests were conducted in the R package ECODIST (Goslee & Urban, 2007) to test for patterns of isolation by distance (IBD) for the total dataset and a spatial autocorrelation analysis was conducted in GENALEX v 6.5 (Peakall & Smouse, 2006) using a distance class of 50km. To examine for patterns of sex-biased dispersal, IBD and spatial autocorrelation analyses were repeated for males and females separately (e.g. as in Banks & Peakall, 2012).

Latitudinal effects on genetic diversity and relative abundance

Observed heterozygosity estimates were calculated using the R package ADEGENET (Jombart, 2008) and allelic richness was calculated using the R package POPGENREPORT (Adamack & Gruber, 2014). We ran linear regression models in the R package STATS to test for changes in allelic richness and observed heterozygosity with latitude. To test for decreasing abundance towards the range edge we ran a linear mixed-effects model (LMM) on relative abundance between sites. Data for relative abundance was available at sampling sites that were not included in the genetic analysis and so the LMM was expanded to include 61 sites to increase the sample size for this analysis. The statistical significance was examined using a likelihood ratio test within ANOVA by comparing fitted models with the null model (excluding fixed effects). Insignificant fixed effects were removed from the model by applying the "analysis of deviance test" using the drop1() command with a Chi-square test and once removed, the model was refitted. We included latitude, day of the year and mean annual temperature as fixed effects and sampling site as a random effect and used the lmer() function within the lme4 R package (Bates et al., 2014). Day of the year was included due to the potential influence of climate variability between sampling days on the relative abundance recorded at each site.

Temperature and land cover data

The effect of landscape and climatic variables on neutral genetic dispersal was tested using two raster datasets for, 1) mean annual temperature and 2) land cover. Mean annual temperature (BIO1) was obtained from the WorldClim Version 1.4 database (Hijmans et al., 2005) at a cell resolution of 1km using a WGS84 projection system and showed a three degree change along the sampling gradient (Figure 2). Mean annual temperature was chosen due to its well-known effects on physiology, growth and reproduction in ectothermic insects (Sánchez-Guillén et al., 2016). Mean annual temperature also encompasses temperatures exposed to both larva and adults and is commonly associated with species distributions (e.g. Evangelista et al., 2011; Lancaster et al., 2015; Zhu et al., 2012). Furthermore, previous research on the same study system created niche models using both climatic and non-climatic variables and found mean annual temperature to be the best predictor of habitat suitability explaining most (62.1%) of the variation in habitat suitability for *I. elegans* in the study area (Lancaster et al., 2015). Temperature (maximum mean summer temperature; BIO5) was also

found to be strongly associated with signatures of selection in *I. elegans* using the same dataset as the current study (Dudaniec et al. 2018). Finally, temperature is particularly important given that climate calculations predict an increase in mean annual temperature throughout Sweden during the current century, with the largest increase expected to occur in the north of Sweden (Eklund et al., 2015). Such shifts in temperature are likely to facilitate the continued expansion of *I. elegans* into previously uninhabitable areas.

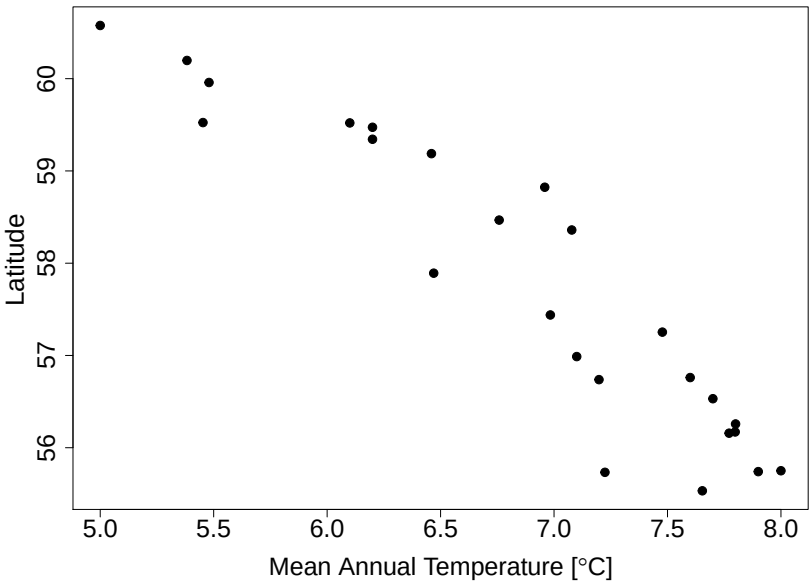

Figure 2: Mean annual temperature data for each sampling site (n = 25) plotted against latitude shows a decrease of 3°C along the sampling gradient.

Land cover data were obtained from the Corine Land Cover database (Büttner & Eiselt, 2013) and the dataset contained 44 land cover variables in our study extent, which we collapsed into seven categories considered relevant to the dispersal of *I. elegans* (Table 1). Generally, we predicted that lakes, rivers and agricultural land use would offer lower resistance than developed (i.e. urban or industrial) and forested areas (temperate coniferous) (Figure 3) for *I. elegans* genetic dispersal. Categorical variables were represented in one resistance surface and were ranked from least to most resistant to dispersal based on expert opinion and findings from the published literature (Dijkstra & Lewington, 2006; Dudaniec et al., 2018; Dunson, 1980; Okuyama et al., 2013; Sato et al., 2008; Swaegers, Janssens, et al., 2014; Wellenreuther et al., 2011). For example, *I. elegans* prefer open and light areas such as agricultural ponds to areas with overhanging and dense canopy (Dijkstra & Lewington, 2006; Okuyama et al., 2013). They have also been demonstrated to be much more salinity tolerant

than other damselfly species (Dunson, 1980), occurring in both freshwater and brackish water (Barnes & Barnes, 1994) and studies on other *Ischnura* species find much greater population genetic differentiation in urban areas (Sato et al., 2008). The seven categories, in order of low to high resistance, were: 1) inland wetlands and waterbodies, 2) marine wetlands and waterbodies, 3) agriculture, 4) scrubland (e.g. grasslands and heathland, 5) forests (three types: broad-leafed, coniferous and mixed), 6) open areas (e.g. beaches and glaciers) and 7) developed areas (including urban, industrial and mining areas) (Figure 3, described in Table 1). A 500m buffer was placed around the coastline for category 2 because *I. elegans* can inhabit both freshwater and coastal, brackish environments, but only close to the shore, with larger expanses of water representing a barrier (Dijkstra & Lewington, 2006). Land cover data were processed using the R package RASTER (Hijmans et al., 2016), at a 100m cell resolution using the ETRS89 projection system. Both mean annual temperature and land cover datasets were cropped to the same spatial extent.

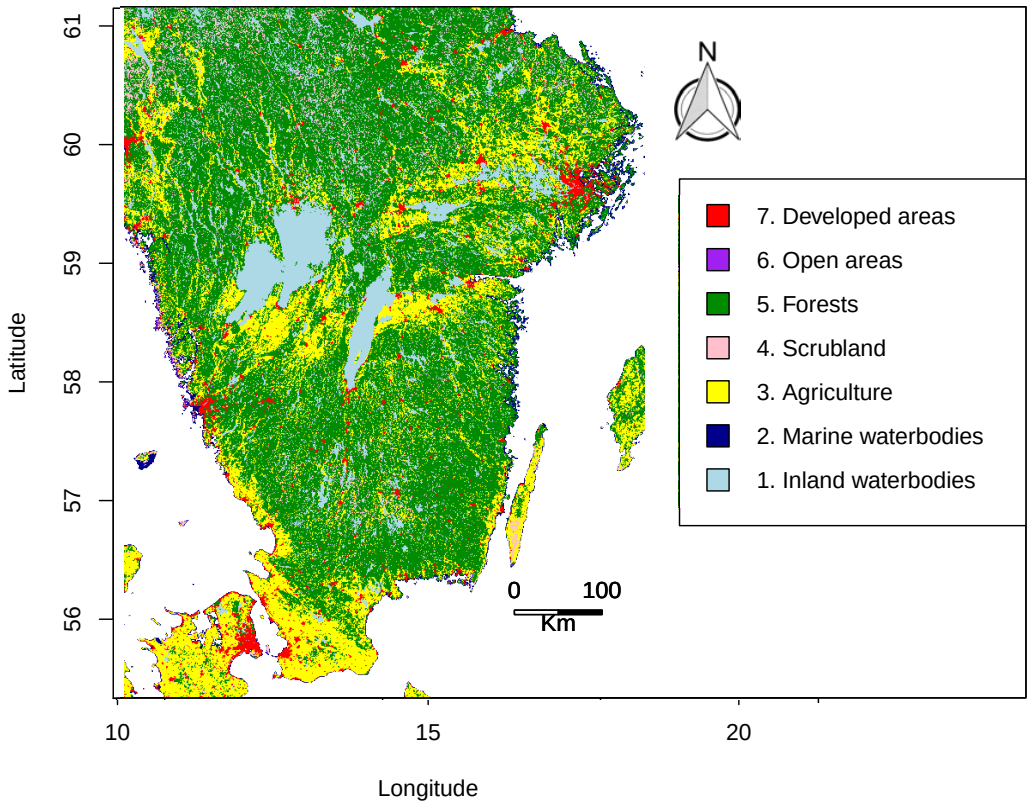

Figure 3: Seven categories of land cover relevant to the dispersal of *I. elegans* ranked from 1 (least resistance) to 7 (most resistance). Data sourced from the Corine Land Cover database (Büttner & Eiselt, 2013).

Table 1: The 44 Corine land cover variables collapsed into seven categories relevant to the dispersal of *I. elegans* and ranked from least resistant (1) to most resistant (7) to dispersal. Data source: Corine land cover database (Büttner & Eiselt, 2013).

44 Land Cover Variables	7 variables relevant to the dispersal of *I. elegans*
Inland marshes Peat bogs Water courses Water bodies	1. Inland Waterbodies
Salt marshes Salines Intertidal flats Coastal lagoons Estuaries Sea and ocean	2. Marine Waterbodies
Non-irrigated arable land Permanently irrigated land Rice fields Vineyards Fruit trees and berry plantations Olive groves Pastures Annual crops associated with permanent crops Complex cultivation patterns Land principally occupied by agriculture, with significant areas of natural vegetation Agro-forestry areas	3. Agricultural Areas
Natural grasslands Moors and heathland Sclerophyllous vegetation Transitional woodland-shrub	4. Scrubland
Broad-leaved forest Coniferous forest Mixed forest	5. Forests
Beaches, dunes, sands Bare rocks Sparsely vegetated areas Burnt areas Glaciers and perpetual snow	6. Open Areas
Continuous urban fabric Discontinuous urban fabric Industrial or commercial units Road and rail networks and associated land Port areas Airports Mineral extraction sites Dump sites Construction sites Green urban areas Sport and leisure facilities	7. Developed Areas

Landscape resistance surfaces

To evaluate climatic and landscape effects on genetic distance in *I. elegans* we use a resistance approach based on circuit theory (McRae & Beier, 2007) combined with a multimodel inference approach similar to that used in previous studies, which is a rigorous and effective way of testing for non-linear isolation by resistance relationships (Dudaniec et al., 2013; Dudaniec et al., 2016; Shirk et al., 2010). Shirk et al. (2010) used an approach based on Mantel tests and correlation coefficients which was built upon by Dudaniec et al. (2013) and Dudaniec et al. (2016) who used linear regression and log-likelihood to evaluate multiple competing resistance models.

We created different resistance surfaces by varying the resistance relationships determined by the slope (γ) and intercept (α) parameters following a similar method to Dudaniec et al. (2013); Dudaniec et al. (2016). Here, we calculate resistance surfaces for temperature and land cover separately, as follows:

$$ri = 1 + \alpha\,(Ti - 1 / max - 1)^{\gamma} \qquad [1]$$

$$ri = 1 + \alpha\,(Li - 1 / max - 1)^{\gamma} \qquad [2]$$

where r_i is the resistance of cell i, T_i is the mean annual temperature 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. The parameter α determines the maximum possible resistance and γ is the parameter that determines the shape of the relationship. This equation explicitly assumes that the effect of temperature on resistance is negative (as temperature decreases, resistance increases) and that the effect of land cover is positive (as land cover rank increases, resistance increases) in accordance with our resistance predictions based on expert opinion and previous findings (Dudaniec et al., 2018; Keller et al., 2012; Sato et al., 2008; Wellenreuther et al., 2011).

We tested for an effect of landscape resistance on genetic distance and chose a range of values for α and γ that test non-linear relationships. Values were 0, 5, 10, 100, 1000 for α and 0.1, 0.2, 0.5, 1, 5, 2 and 10 for γ. For each combination of these values (i.e. 29 resistance surfaces), we calculated a resistance surface, each representing a unique test of how the environment influences gene flow. This resulted in a total of 29 resistance surfaces for mean annual temperature data (Figure 4a) and 29 resistance surfaces for land cover data (Figure 4b). Note that where α = 0, all cells were assigned a value equal to one and is, therefore, equivalent to a model of isolation by geographic distance.

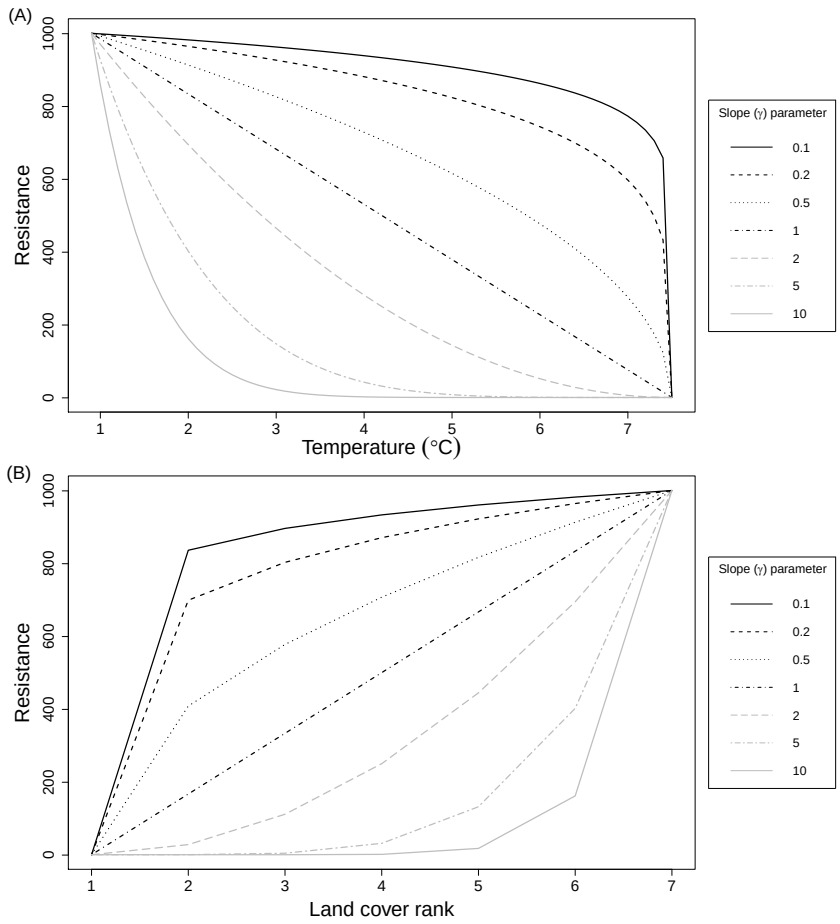

Figure 4: Shapes of the isolation-by-resistance relationships tested for the effect of temperature (A) and land cover (B) on genetic distance. All γ values are displayed, representing the seven slopes, however, for simplicity, only α = 1000 is displayed, representing the maximum resistance. Maximum mean annual temperature was 7.6°C. Ranked land cover variables are described in Table 1.

Resistance distance matrices were calculated for the 29 transformed raster layers for both mean annual temperature and land cover using the program CIRCUITSCAPE (McRae & Beier, 2007). CIRCUITSCAPE uses electrical circuit theory to model pairwise resistance among sampling locations on a given landscape and calculates the average cumulative resistance of all possible paths between each pair of sampling sites (Shah & McRae, 2008). We used the four-cell connection scheme in CIRCUITSCAPE such that each raster cell was connected to its four surrounding neighbour cells (Shah & McRae, 2008). Each resistance matrix generated for the 29 surfaces was then each treated as a predictor of genetic distance between sites.

Landscape genetic modelling

We used the R packages RESISTANCEGA (Peterman, 2014) and M_UMI_N (Bartn, 2013) to conduct AIC model selection and multimodal inference to determine which resistance surface(s) best explained genetic distance, for both temperature and land cover. All resistance surfaces were evaluated against the null, isolation by geographic distance, model. To estimate the relative support of each parameter combination of α and γ we used Akaike Information Criterion (AIC) scores, with the lowest score representing the best-selected model and the difference between the scores (Δi) indicating the strength of the model with the lowest score in comparison to other models (Akaike, 1998; Bozdogan, 1987). The larger Δ_i is, the less plausible it is that the fitted model is the best model (Burnham & Anderson, 2003). Burnham and Anderson (2003) recommend that when $\Delta_i < 2$ the model has substantial support, when Δ_i is between 4-7 the model has much less support and when $\Delta_i > 10$ the model has essentially no support. Therefore, we only discuss results with $\Delta_i < 10$.

Morphological analysis

Morphological analyses were conducted on a sample size of 41 sites, which included additional sites for which genetic data were not obtained. In total, we analysed 374 females and 533 males from these 41 sites (Table S2) and ran a linear regression for sex-ratio with latitude. From each of the scanned images of live individuals (Figure S3), the program ImageJ (Rasband, 2018) was used to measure six body size variables (mm) including: total length, wing length, abdomen length, thorax length, thorax width and S4 width (width of abdomen segment 4). If the dorsal side of an individual was not entirely visible, or the individual was laying on its side, the image was excluded from analysis. We tested for morphological changes along the range expansion axis using linear mixed-effects models (LMM) and generalized linear mixed-effects models (GLMM) using the lme4 (Bates, et al. 2014) and M_UMI_N (Barton & Barton, 2018) R packages. To test for body size change along the range expansion axis, we used wing length, total length and a principle component variable incorporating all six body size features. The principle component analysis (PCA) was conducted using the prcomp() function within the STATS R package for the total group and for males and females separately. The first axis of each of the PCAs captured the majority of the variance when analysing males (64%), females (63%) and the total group (66%), and the variable 'PC1 body size' was subsequently used in analyses (Table S3). Latitude and mean annual temperature were assigned as fixed effects and site ID and sex as random effects in the mixed-effects model.

Results

Genetic distance and sex-biased dispersal

Pairwise F_{ST} ranged from 0.01 - 0.04 and pairwise $G`_{ST}$ ranged from -0.002 - 0.098. There was a significant isolation by distance (IBD) relationship for both genetic distance measures when analysing the total dataset ($F_{ST}$: $R^2 = 0.47$, $P = 0.001$; $G`_{ST}$: $R^2 = 0.5$, $P = 0.001$; Figure S4 & S5). An isolation by distance relationship was also found for males ($R^2 = 0.26$, $P = 0.003$; Figure S6) and for females separately ($R^2 = 0.20$, $P = 0.01$; Figure S7), however, females showed a weaker relationship. Spatial autocorrelation analysis of all individuals using distance classes of 50km found significant genetic relatedness of individuals up until 200km ($\omega = 163.014$, $P = 0.001$; Figure S8). This pattern did not differ when analysing males and females separately despite some slight deviations between the sexes at some distance classes (Figure S9).

Genetic diversity and relative abundance

No change in allelic richness (Adj $R^2 = -0.02$, $P = 0.45$, Figure 5a) or observed heterozygosity (Adj $R^2 = 0.04$, $P = 0.172$, Figure 5b) was found across the latitudinal gradient when analysing all 25 sites. This contrasts with a significant decline in relative site abundance with increasing latitude (accounting for day of year) as determined using LMM ($\chi_{(1)} = 16.8$, $P = <0.001$, n = 61, Figure 6).

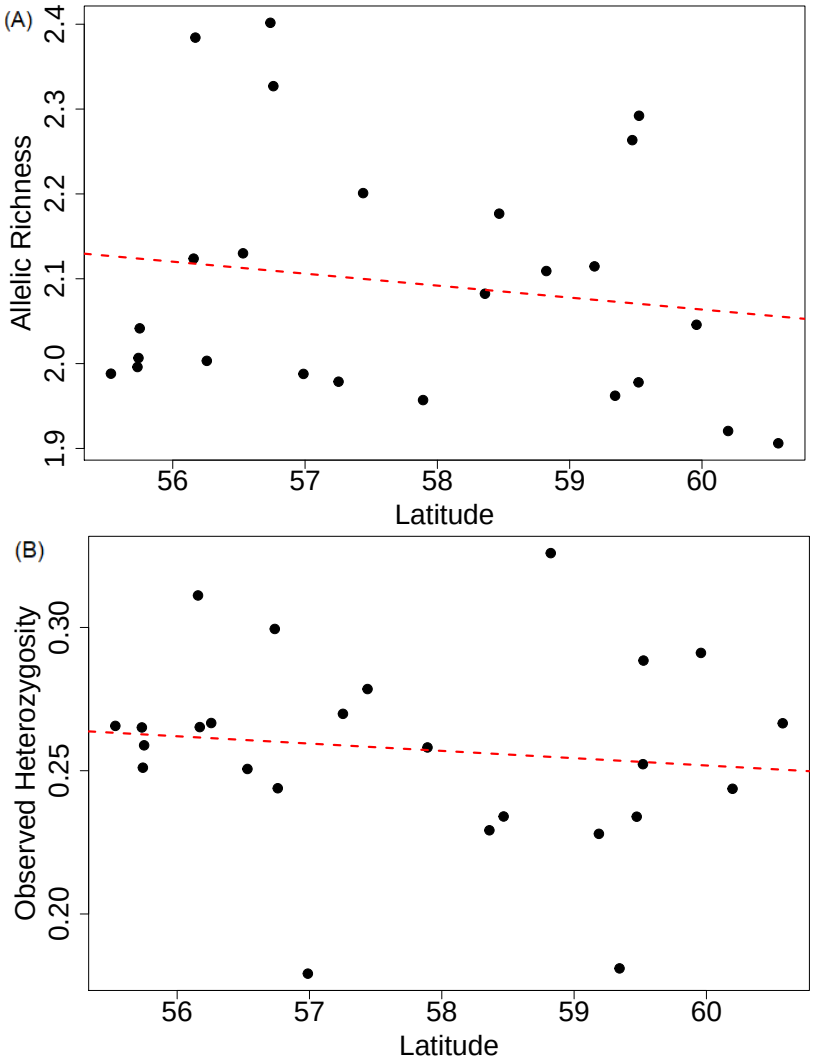

Figure 5: Allelic richness (A, Adj R^2 = -0.02, P = 0.45) and observed heterozygosity (B, Adj R^2 = 0.04, P = 0.172) showed no significant change between each of the 25 sampling sites along the latitudinal gradient.

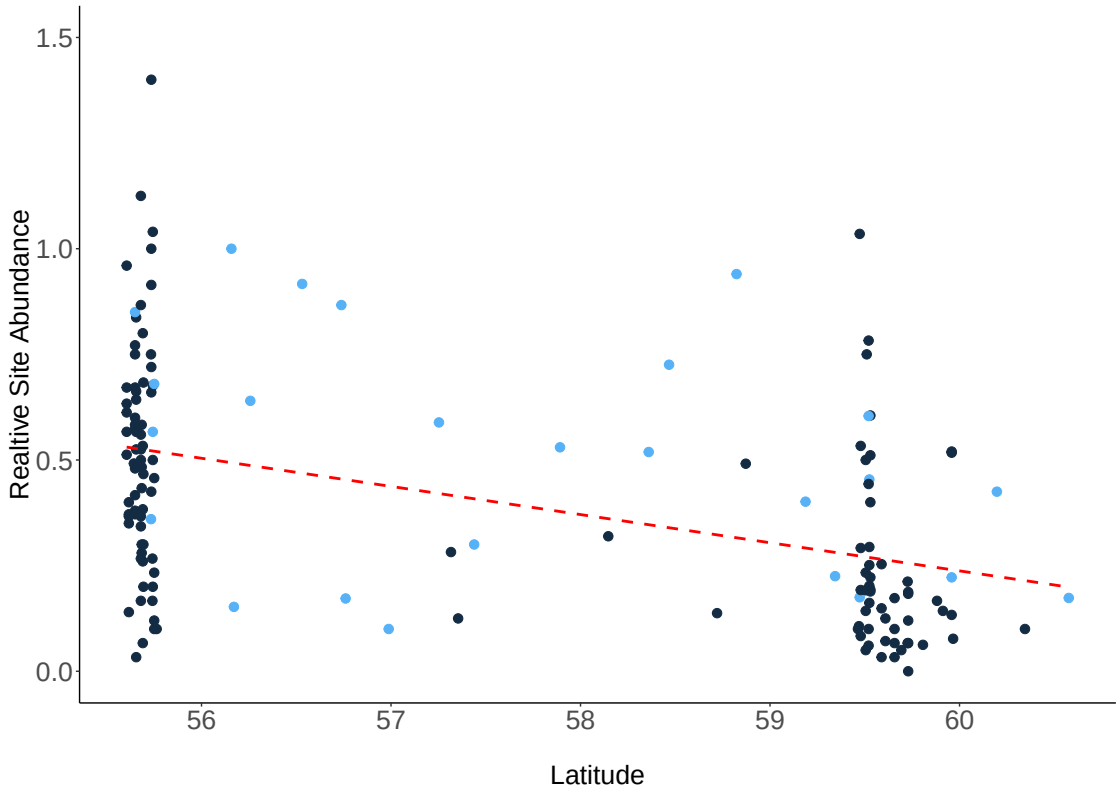

Figure 6: Relative site abundance was found to decrease with latitude ($F_{(38)}$=7.5, *P* = <0.001). This data included extra sites that did not have genetic data. Light blue points are the 25 sites used in the genetic analyses.

Effect of temperature on genetic connectivity

Mean annual temperature significantly influenced genetic connectivity in *I. elegans* using both F_{ST} and G'_{ST} as response variables, when analysing the total dataset. For $F_{ST,}$ the parameter values that gave the lowest AIC scores were those with $\gamma = 1$, $\alpha = 1000$, which indicates that resistance decreases linearly with temperature, with a maximum resistance 1000 times greater than zero (Figure 7). However, using multi-model inference, the α values 1000 and 100 were indistinguishable (as $\Delta AIC < 2$) and altogether the two resistance surfaces explained 95% of the variation in genetic distances (Table S4). These two models differed from the null model (isolation by distance, where alpha = 0) by $\Delta AIC = 56.3$ (Table S4). The analysis using $G`_{ST}$ selected the same two models with slightly stronger weighting (98%) and differed from the null model by $\Delta AIC = 57.2$ (Table S5). This suggests that genetic connectivity decreases towards the range limit due to increasing resistance as a result of cooler temperatures in the north (Figure 8).

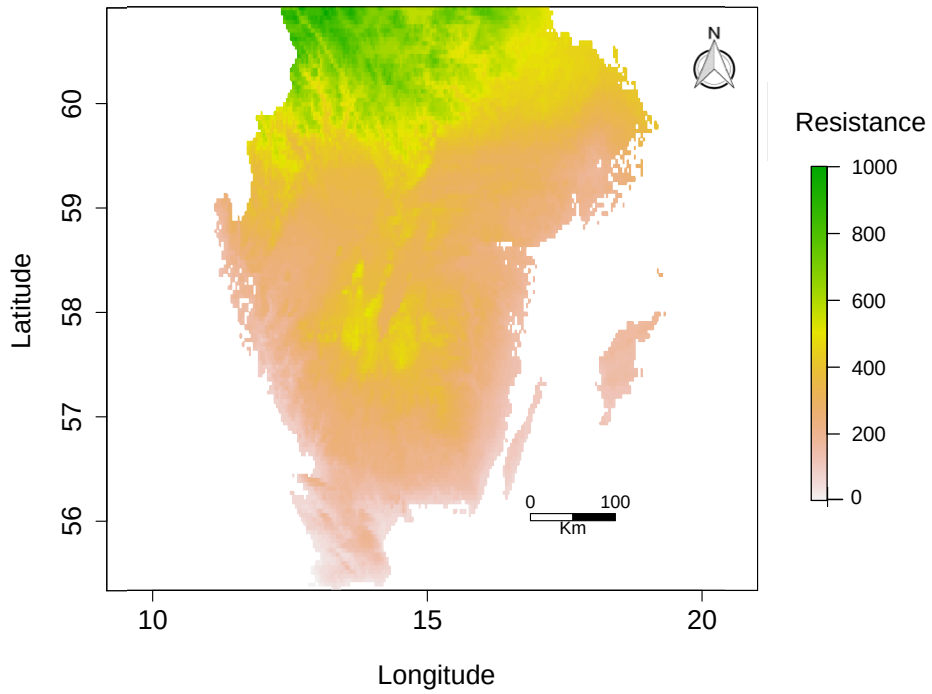

Figure 7: The best-selected resistance model for mean annual temperature that explains genetic connectivity between the 25 sampling locations (analysis of the total dataset). The resistance surface represents parameter values of $\gamma = 1$, $\alpha = 1000$, and shows that landscape resistance increases linearly with decreasing temperatures towards the northern range limit.

Sex-specific effects of temperature on genetic connectivity

Males (n = 209) showed a similar pattern of high landscape resistance at colder northern sites with a linear decrease in resistance as temperature increased in the south. As for the total dataset, the best-selected model using F_{ST} was also $\gamma = 1$ and $\alpha = 1000$, but this was indistinguishable from both $\gamma = 1$, $\alpha = 100$ and $\gamma = 2$, $\alpha = 10$ (as $\Delta AIC < 2$). Together these three resistance surfaces explained 73% of the variance and differed from the null IBD model by $\Delta AIC = 24.4$ (Table S6). The analysis of G'_{ST} selected the same first two models as F_{ST} with the most supported models being: $\gamma = 1$, $\alpha = 1000$ and $\gamma = 1$, $\alpha = 100$, which explained 87% of the variance and differed from the null model by $\Delta AIC = 33.8$ (Table S7).

The best-selected model for females (n = 217) indicated lower resistance to mean annual temperature than in males and for the total dataset. For F_{ST}, the best selected model was $\gamma = 2$, $\alpha = 100$, though $\gamma = 1$, $\alpha = 1000$ was also supported with $\Delta AIC = 1.6$ between the two resistance surfaces, which together explained 83% of the variation in genetic distance. The

null model differed from the best-supported model by ΔAIC = 55.5 (Table S8). The analysis of $G`_{ST}$ selected the model γ = 2, α = 100 on its own as all other models were > ΔAIC = 2. This model explained 62% of the variance and differed from the null model by ΔAIC = 44.2 (Table S9). Females, therefore, had a less sensitive, and non-linear response to mean annual temperature (Figure 4) when compared with males and the total dataset.

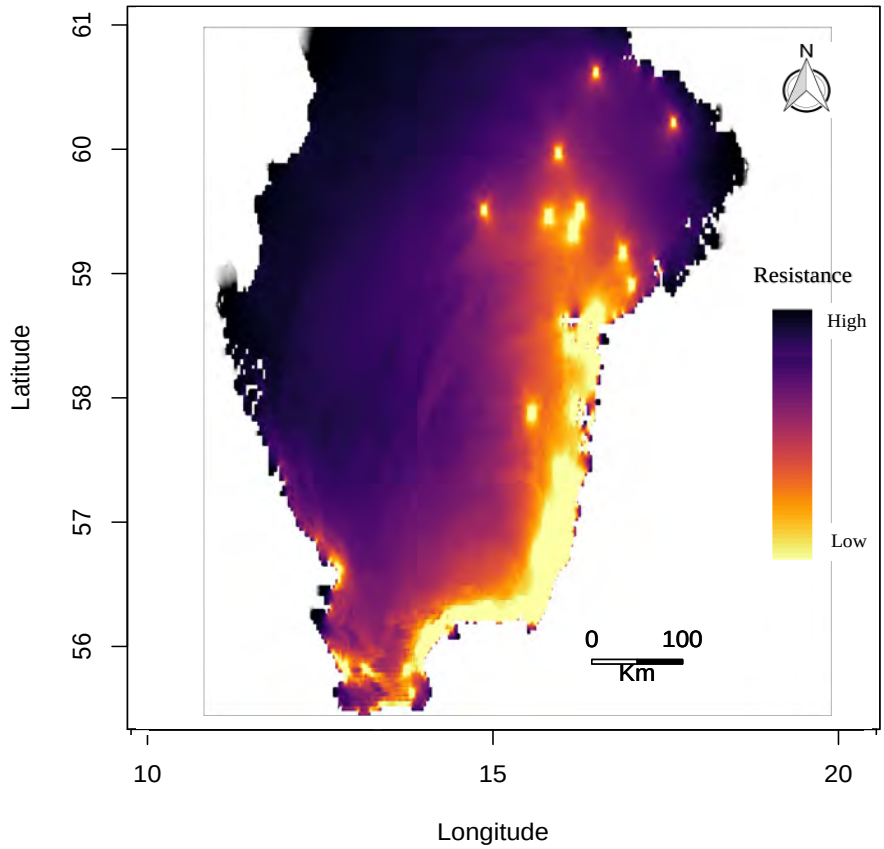

Figure 8: Based on the best selected model of γ = 1, α = 1000 using the total dataset, this resistance map (based on the cumulative current map generated by CIRCUITSCAPE) highlights the reduced genetic connectivity between northern range edge sites, and increased connectivity of coastal sites in *I. elegans* (n = 25 sites) due to resistance from mean annual temperature.

The influence of land cover on genetic connectivity

When analysing the total dataset, there was no effect of land cover on genetic connectivity. Using F_{ST} as the response variable, the best-selected model was the null isolation by distance model, which explained 71% of the variation in genetic distance. The next best-selected models were all > ΔAIC = 2 reflecting little support for other resistance surfaces. The models

between ΔAIC = 2 and ΔAIC = 10 included surfaces for the lowest maximum resistance value tested (i.e. α = 5 with γ = 1, 2,5 and 10), with one model for α = 10 with γ = 2 (Table S10). Analysis of $G`_{ST}$ also show no effect of land cover on genetic connectivity with the null model explaining 61% of the variance. The next best-selected models were between ΔAIC = 2 and ΔAIC = 10 and had maximum resistances of α = 5, with γ = 1, 2, 5 and 10, and two showing α = 10, with γ = 2 and 5 (Table S11).

Sex-specific effects of land cover rank on genetic connectivity

When analysing males alone, there was no effect of land cover on genetic connectivity. For the analysis of F_{ST}, the null isolation by distance model explained 89% of the variance. The 11 next best-selected models were between ΔAIC = 2 and ΔAIC = 10 and showed maximum resistances of α = 5 with γ = 0.1, 0.2, 0.5, 1, 2, 5 and 10 and α = 10 with γ = 0.5, 1, 2 and 5 (Table S12). A similar result was found with $G`_{ST}$ with the null model explaining 92% of the variance. The 8 next best-selected models between ΔAIC = 2 and ΔAIC = 10 showed maximum resistances of α = 5 with γ = 0.5, 1, 2, 5 and 10 and α = 10 with γ = 1, 2 and 5 (Table S13).

In contrast, resistance to gene flow due to land cover was detected in females, but only when analysing G'_{ST} (Figure 9). For F_{ST} the best-selected model was γ = 2, α = 5 and explained 30% of the variance, but this was not distinguishable from the null model, which had a ΔAIC = 0.02 (Table S14). For $G`_{ST}$ the best selected model was also γ = 2, α = 5, and was indistinguishable from three other models: γ = 2, α = 10, γ = 5, α = 5 and γ = 5, α = 10 which also had ΔAIC < 2. Together these models explained 83% of the variance and differed from the null model by ΔAIC = 2.78. Both : γ = 2 and γ = 5 indicate reduced sensitivity to land cover than a linear resistance relationship (Figure 4), and α = 5 indicates that land cover contributes approximately 20 times less resistance to gene flow than mean annual temperature, for which most models supported α =100. There were five other models between ΔAIC = 2 and ΔAIC = 10 which showed maximum resistances of α = 5 with γ = 0.5, 1 and 10 and α = 10 with γ = 1 and 10 (Table S15).

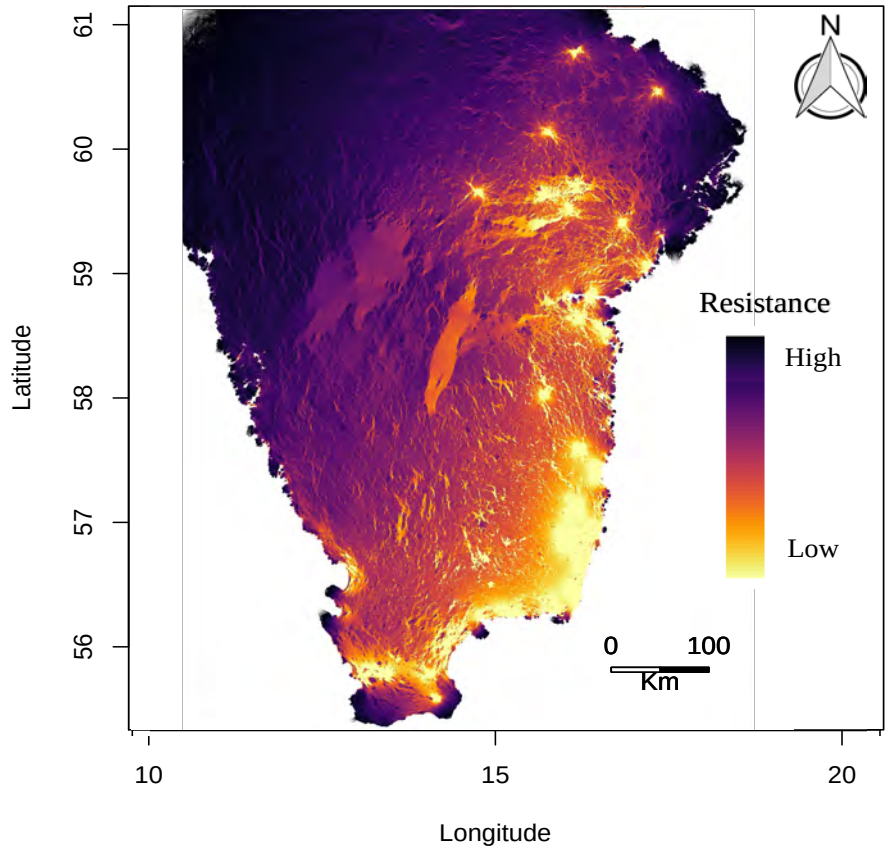

Figure 9: The best selected model explaining the influence of land cover on female genetic connectivity was $\gamma = 2$, $\alpha = 5$ which reflects low resistance that increases as land cover rank increases at an almost linear rate. This map shows fine-scale genetic connectivity of females due to land cover, with increased effects of open and developed areas in both southern and range edge regions.

Morphological analyses

There was a higher proportion of males than females at most sites (Table S2) and no association between sex ratio and latitude (Adj R2 = 0.02, $P = 0.05$). Wing length was positively associated with latitude (LMM: $\chi_{(1)} = 5.27$, $P = 0.02$, n = 907), however a large amount of the variance was attributed to sex (Figure 10). When analysed independently, female wing length was positively associated with latitude (LMM: $\chi_{(1)} = 7.54$, $P = 0.006$, n = 374), whereas male wing length was not (LMM: $\chi_{(1)} = 2.2$, $P = 0.14$, n = 533). However, the interaction between sex and latitude was not significant (LMM: $\chi_{(1)} = 2.5$, $P = 0.11$, n = 907).

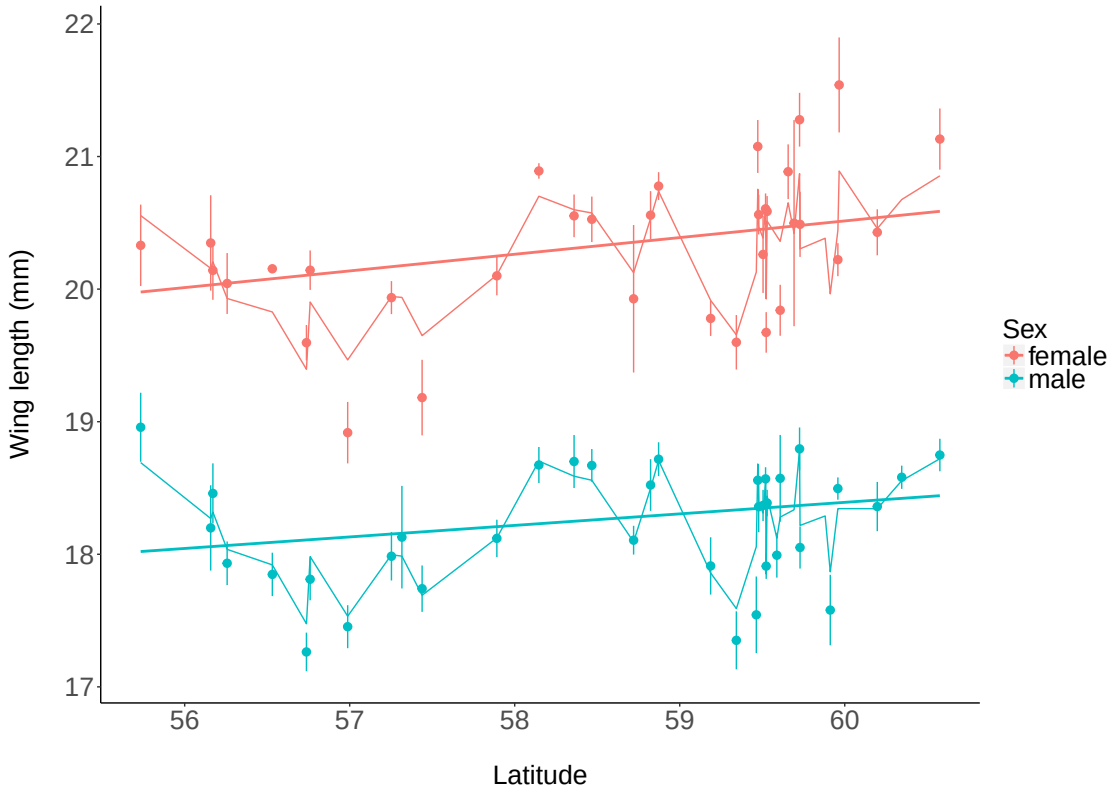

Figure 10: The relationship between wing length and latitude for males (n = 533) and females (n = 374) across 25 sampling sites along the *I. elegans* range expansion gradient.

No significant association was found between total body length and latitude (LMM: $\chi_{(1)}$ =1.11, P = 0.29, n = 907), or mean annual temperature (LMM: $\chi_{(1)}$ =0.13, P = 0.72, n = 907). There was no sex-specific total body length change with latitude when analysing males (LMM: $\chi_{(1)}$ =2.03, P = 0.15, n = 533) and females (LMM: $\chi_{(1)}$ =0.17, P = 0.19, n = 374) separately. PC1 body size (derived from the first axis of PCA incorporating 6 body size variables) showed no significant association with latitude (LMM: $\chi_{(1)}$ =1.92, P = 0.26, n = 907) or with mean annual temperature (LMM: $\chi_{(1)}$ =0.22, P = 0.64, n = 907). There was no change in sex-specific PC1 body size with latitude when analysing males (LMM: $\chi_{(1)}$ =0.6, P = 0.44, n = 533) and females (LMM: $\chi_{(1)}$ =2.3, P = 0.12, n = 374) separately.

Discussion

Our study suggests that colder mean annual temperatures are reducing the genetic connectivity of *I. elegans* at the range expansion front with evidence for sex-specific morphological and landscape genetic responses. Furthermore, in accordance with expectations of recent colonisation at the range edge, we found that genetic diversity did not significantly change towards the range edge, but relative abundance declined. Our results provide evidence for a subtle pattern of sex-biased dispersal in *I. elegans* along its range expansion gradient via patterns of isolation by distance, but more notably, via sex-specific landscape genetic relationships and morphological variation in wing length. Female genetic connectivity was limited by resistance due to mean annual temperature 10 times less than in males, and a small effect of land cover was evident that was not observed in males. Morphological analysis showed that wing length increased significantly with latitude in females, but not in males. In addition, total body length and PC1 body size did not increase with latitude, indicating possible selection on this dispersal trait towards the range limit within the more dispersive sex. These findings demonstrate how differential morphological and landscape genetic responses among males and females may be integral to successful range expansion under climate change.

Genetic diversity and abundance along the range expansion axis

It is often expected that genetic diversity declines along a range expansion axis, eroding the ability of populations to adapt to new environments, increasing the risk of local extinction (Reed & Frankham, 2003; Song et al., 2013). Due to founder effects and the loss of genetic diversity through population bottlenecks, the successful spread and adaptive capacity of expanding species may be surprising (Kolbe et al., 2004). Some processes, however, can lead to an opposing pattern, whereby genetic diversity is maintained at the range limit (Song et al., 2013). These include recent colonisations or long-distance dispersal events that lead to the admixture of genes from multiple source locations, resulting in high genetic diversity at the expanding edge (Leydet et al., 2018). Increased dispersal capacity at range edges through the spatial sorting of dispersal capacity can also help to maintain high levels of genetic diversity at the expansion front by enabling high gene flow (Shine et al., 2011). High gene flow is often thought to have a constraining effect on local adaptation, however, many recent studies have found substantial evidence for local adaptation despite high gene flow (Jacob et al., 2017; Moody et al., 2015), including at species' range edges (Dudaniec et al., 2018;

Halbritter et al., 2015). *I. elegans* has high dispersal rates making it possible for genetic diversity to be maintained across the range expansion axis through recent and ongoing colonisation from southern sites. Concordantly, we find that allelic richness and observed heterozygosity showed no significant change with latitude from the south of Sweden to those at the range limit (Figure 6a,b). This consistent allelic richness is likely to serve as a source of genetic variation on which selection can act at the range limit. For example, Dudaniec et al. (2018) found evidence for local adaptation in *I. elegans*, with many genes showing strong environmental selection signatures and thresholds of allelic turnover using 13,612 SNPs from the same individuals analysed in the current study. SNPs under putative selection along the gradient were annotated to genes with functional roles in thermal stress (i.e. heat shock proteins), visual processing and salinity tolerance (i.e. ion transport). Whilst genetic diversity was maintained across the range expansion axis, relative site abundance declined (Figure 7). Reduced abundances are often observed at species range edges where habitat suitability is reduced (Eckert et al., 2008; Guo et al., 2005; Murphy et al., 2006). This may suggest a constraining effect of novel climatic conditions or overall reduced habitat suitability, which may influence the dynamics and success of future range expansion (Bennie et al., 2013). For *I. elegans*, such affects do not appear to be limiting the capacity of this species to adapt and expand during climate change (Dudaniec et al., 2018; Hickling et al., 2005). Our findings, together with the findings from Dudaniec et al. (2018), support the notion that local adaptation during range expansion in *I. elegans* is facilitated by the maintenance of genetic diversity across the range expansion gradient. Our results also support the possibility that individuals sampled at the range limit may be comprised of colonising individuals or their recent descendants, suggesting ongoing colonisation events as *I. elegans* continues its poleward expansion under climate change-induced temperature increases.

Temperature as a driving factor

Temperature is a major determinant of species distributions, particularly those of ectotherms subject to shifting thermal regimes as a result of climate change (Lancaster, 2016). Our landscape genetic resistance modelling for the total dataset indicated that low mean annual temperatures at the range edge are reducing genetic connectivity of *I. elegans*. This result supports previous findings about the effects of temperature on *I. elegans* during its current range expansion in Sweden. For example, Lancaster et al. (2015) created a niche model for *I. elegans* and found that mean annual temperature was the best predictor of habitat suitability, explaining 68% of the model. Furthermore, Lancaster et al. (2015) experimentally

determined the upper and lower thermal tolerances of *I. elegans* along the expansion axis and found that upper thermal tolerances exhibited local adaptation in the core region but was released from thermal selection toward the cooler, northern range edge. They also found that adaptive plasticity at lower thermal tolerances (acclimation ability) increased towards the northern, expanding range edge. These results were strengthened by Lancaster et al. (2016) who found that the level of gene expression and number of genes involved in heat stress has been reduced during the range expansion whereas genes associated with cold stress increased at the range edge. This suggests that some cellular responses originally adapted to heat stress may switch to cold-stress functionality when experiencing cooler climatic conditions and this process may be facilitating rapid poleward expansions in insects. Finally, Dudaniec et al. (2018) found SNPs potentially under selection in *I. elegans* that were associated with maximum summer temperatures with some genes annotated to heat shock proteins. These studies highlight the influence of temperature as a key selection pressure along the range expansion axis of *I. elegans*, which increases in novel climatic conditions at the northern range edge. The increased resistance to gene flow due to lower temperatures we observe in this study further validates the dominating effect of temperature during this range expansion. Other studies on odonates, and insects in general, find temperature to be a driving factor of range expansions, adaptations and genetic connectivity (Buckley et al., 2012; Pateman et al., 2012; Swaegers et al., 2013; Watts et al., 2010). Our findings are informative about the role of temperature in driving the genetic dispersal of *I. elegans*, adding to previous findings about the role of temperature in the species' rapid adaptation during its continuing range expansion.

Sex-specific genetic responses in I. elegans

Mantel tests indicated a pattern of isolation by distance when analysing the total dataset and for males and females independently, however, the relationship was weaker in females, suggesting that male dispersal may be slightly more limited by distance than in females. However, evidence for this was not reflected in the spatial autocorrelation analysis, which showed a pattern of genetic correlation up to 200km and no difference in this relationship between males and females. Evidence for female-biased dispersal has been observed in damselflies and odonates in general, with females being recaptured less often than males in mark recapture studies and are observed spending more time away from the natal pond or stream, generally only visiting when they are receptive (Beirinckx et al., 2006; Chaput-Bardy et al., 2010; Conrad et al., 1999; Conrad et al., 2002; Corbet, 1999). However, there is

evidence of male biased dispersal in some Odonates, such as the non-territorial dragonfly *Sympetrum sanguineurn* (Corbet, 1999). A complex interaction between mating systems, survival rates, dispersal capacities and life histories work in combination to shape patterns of sex-biased dispersal (Beirinckx et al., 2006). Mating systems are important determinants of which sex is the most dispersive, whereby lek mating systems, in which males aggregate, drive female biased dispersal (Chaput-Bardy et al., 2010). Differential survival rates between males and females also influence sex-ratios and the probability of recapture (Chaput-Bardy et al., 2010). The dispersive sex has been found to have higher mortality rates due to the survival costs of dispersal, e.g. increased predation risk (Chaput-Bardy et al., 2010). Higher mortality rates have been detected in female *I. elegans* than males, potentially due to increased female dispersal, or from males have higher mortality during the immature stage, biasing initial sex-ratio estimates (Anholt et al., 2001). Other hypotheses to explain female-biased dispersal in damselflies include: harassment avoidance (Beirinckx et al., 2006; Svensson et al., 2005) the need to mature clutches of eggs away from the natal pond (Conrad et al., 2002; Corbet, 1999) and increased time spent in the dispersive teneral (immature adult) stage (Johnson, 1986). Male mating harassment is a well-documented behaviour in *I. elegans* and is potentially a primary factor driving females away from the pond and increasing their chances of moving to a new site (Gosden & Svensson, 2007, 2009; Svensson et al., 2005). Our isolation by distance and spatial autocorrelation findings indicate weak evidence of female-biased dispersal, however, this is in accordance with the non-directional, passive dispersal suggested in the literature (Beirinckx et al., 2006; Conrad et al., 1999; Conrad et al., 2002).

Sex-specific landscape responses in I. elegans

The sex-specific landscape genetic responses we found further indicate differential genetic dispersal processes occurring in male and female *I. elegans*. Specifically, females showed much lower resistance to mean annual temperature than males and a small effect of land cover type, which was not observed in males. Importantly, the spatial pattern of resistance to mean annual temperature (i.e. the slope of the relationship) was similar between males and females, with females showing slightly less resistance than the linear pattern observed in males, and the maximum resistance value was 10 times less in females. These findings are consistent with a pattern of female-biased dispersal as they show reduced sensitivity to temperature but greater fine scale sensitivity to land cover features. Mean annual temperature encompasses temperatures exposed to both larva and adults, is less spatially variable than

land cover and is commonly associated with species distributions indicating it's influence on long term, broad scale dispersal dynamics (Evangelista et al., 2011; Lancaster et al., 2015; Zhu et al., 2012). This suggests genetic connectivity in males is determined to a larger extent by broader scale patterns of mean annual temperature, perhaps reflecting greater passive dispersal and/or slower dispersal processes than females. Conversely, female dispersal may be less passive, reducing the effect of temperature but increasing the fine-scale land cover effect. Specifically, our land cover results suggest that the chances of a female encountering a new breeding area will be lowered if the surrounding environment is comprised of land covers ranked with higher resistance (i.e. forest, open areas, or developed areas) (Figures 3 and 9). Females are perhaps more sensitive to land cover as they spend more time away from the breeding grounds; foraging and dispersing more actively in the surrounding landscape than males. However, evidence for the impact of land cover type on female genetic connectivity was weak and difficult to distinguish from a pattern of isolation by distance in the case of F_{ST} (Table S14), but less so for $G`_{ST}$ (Table S15). Overall, we find evidence for sex-specific variation in genetic connectivity in relation to landscape variables.

Sex-specific morphological changes in I. elegans

Individuals at the leading edge of a range expansion often show morphological traits that are distinct from those closer to the centre of the historic range (Krause et al., 2016). Sex-specific morphological variation, particularly on dispersal related traits, may arise along range expansion gradients due to variation in life history or dispersal capacity, leading to differential patterns of genetic dispersal between the sexes. We found sex-specific changes in morphology along the *I. elegans* range expansion axis, with evidence for female wing length, but not male wing length, increasing towards the range limit. Importantly, this increase in wing length was independent of total body length and PC1 body size. However, we failed to find an interaction between sex and latitude in the analysis of wing length, suggesting that although patterns with latitude were evident independently, this effect was not evident when analysing males and females together; therefore, additional investigations are required. The disproportionate increase in wing length in relation to body size with latitude provides some evidence that directional selection may be operating on this dispersal-related trait in females during range expansion. Wing length in insects is frequently associated with dispersal distance and capacity (Delettre, 1988; Guerra, 2011; Southwood, 1966; Zickovich & Bohonak, 2007). Increased dispersal capacity has previously been observed in range expanding damselflies with increased relative investment in flight muscle mass in males and

females found at the range edge (Swaegers et al., 2015; Therry, Zawal, et al., 2014). Additionally, flight muscle mass was found to positively covary with flight endurance (Therry, Gyulavári, et al., 2014) and was associated with allelic frequency changes along the expansion axis (Swaegers et al., 2015). To our knowledge, sex-specific morphological changes have not been previously documented in range expanding insects. Evidence for a stronger increase in wing length in females, the more dispersive sex, suggests that sex-specific behavioural and ecological differences may result in differential selection pressures during the range expansion process. For example, increased female wandering behaviour may facilitate dispersal into novel, cooler, less suitable northern habitats (Lancaster et al. 2015), and select for larger wing length in order to increase the chances of colonising more distant and suitable habitats. This finding is firstly consistent with our isolation by distance analysis, with females showing a weaker relationship than males, and secondly, our landscape genetic analysis, with females showing reduced resistance to mean annual temperature and an effect of land cover.

Conclusion

The damselfly *Ischnura elegans* is undergoing a rapid poleward range expansion in response to climate-change-induced temperature shifts with evidence accumulating for rapid adaptation to novel thermal conditions (Dudaniec et al., 2018; Lancaster et al., 2016; Lancaster et al., 2015). Here we combine landscape genetics with demographic and morphological analyses to disentangle factors affecting connectivity and gene flow in *I. elegans* during its ongoing, poleward range expansion in Sweden. Our results indicate sex-specific responses during the *I. elegans* range expansion and are supported by morphological and behavioural variation between the sexes with shifting environmental conditions towards the range edge. Differential effects of range expansions on the genetic connectivity of males and females are little studied, and here, we complement this with a concurrent examination of landscape and climatic drivers and an examination of morphological changes of dispersal related traits. This study demonstrates that these factors can be strongly related and is a call to account for sex-specific processes in an effort to understand range expansion processes.

Acknowledgements

This work was supported by Macquarie University (to AC), an EU FP7, Marie Curie International Incoming Fellowship (to RYD; project code 'MOVE2ADAPT'), a Wenner-Gren Foundation Postdoctoral Stipend (to RYD), the Oscar and Lili Lamm Foundation (to RYD, BH), Biodiversity and Ecosystem Services in a Changing Climate (BECC; a joint Lund-Gothenburg University initiative) (LL), the Swedish Research Council (EIS, BH), and the Crafoord Foundation (EIS, BH). We would like to thank Hanna Bensch and Paul Caplat for assistance with the collection of samples in the field and the Grimso Research Station and Mikael Akesson for logistical support. We would also like to thank Hanna Bensch and John Waller for assistance with morphological measurements. We thank Martin Andersson for assistance with DNA extraction, and Julian Catchen, Martin Stervander, Dag Ahren and Maren Wellenreuther for bioinformatics advice and helpful discussion. We thank Sonu Yadav for assistance with analyses.

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Supplementary material

Genetic structure
Detailed information on DNA sequencing, bioinformatics, SNP characterisation and genetic structure analysis can be found in the related paper, Dudaniec et al. (2018): https://onlinelibrary.wiley.com/doi/10.1111/mec.14709. Genetic structure analysis was undertaken previously and described in Dudaniec et al. (2018), who used the R package ADMIXTURE (Alexander et al., 2009) which uses a cross-validation procedure to determine genetic structure in large autosomal SNP data sets. ADMIXTURE was run for 1-25 potential ancestral populations (K) with a 5-fold cross-validation (CV) error and four genetic clusters were chosen where the cross-validation error was minimised (Figure S1 and S2).

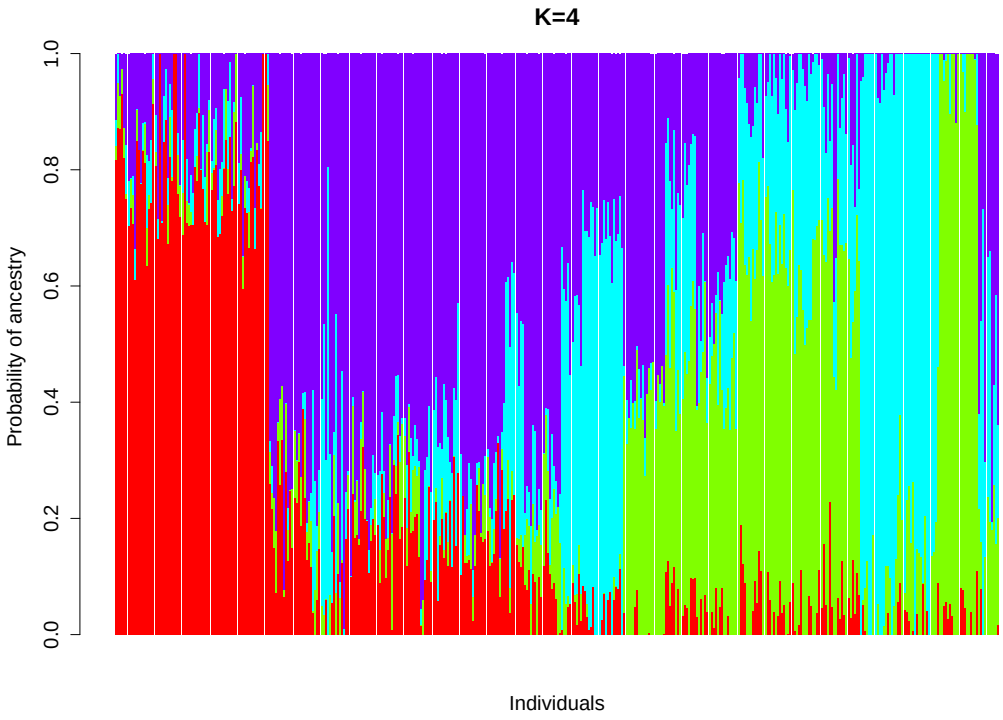

Figure S1: Barplot of assignment probability to each genetic cluster for *I. elegans* in Sweden using the most likely K derived from the lowest cross-validation error in ADMIXTURE (K = 4). Each bar represents the assignment probability of each individual to each genetic cluster. The analysis was performed using 3809 unlinked randomly selected SNPs across all 25 populations and 426 *I. elegans* individuals.

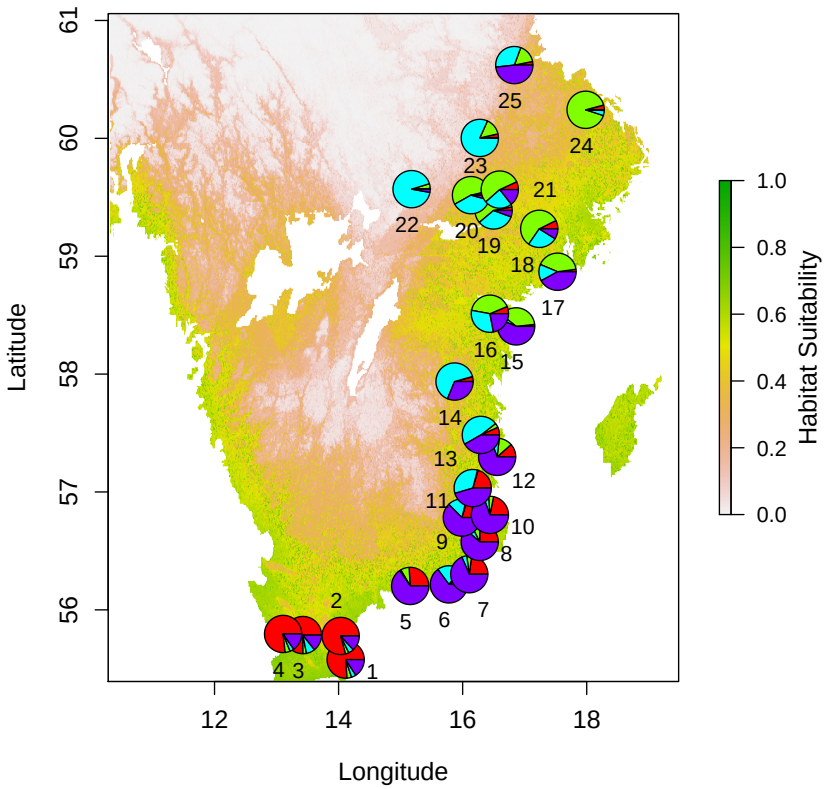

Figure S2: Genetic structure of *I. elegans* across the environmental gradient in southern Sweden. The probability of *I. elegans* genetic cluster assignment (K=4) is shown at the population level on a habitat suitability map in Sweden (published in Lancaster et al. 2015). The proportion of each color within each pie chart indicates the mean assignment probability of individuals to a genetic cluster in that population, displayed for 426 individuals across 25 populations.

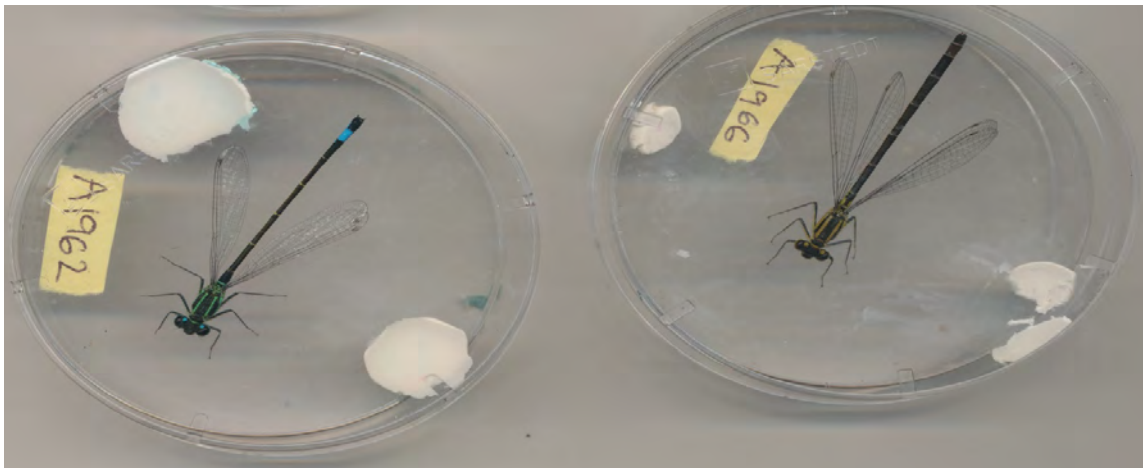

Figure S3: Images taken of male (left) and female (right) *I. elegans* (n = 907) for 41 sites along the range expansion gradient to be used in the morphological analysis.

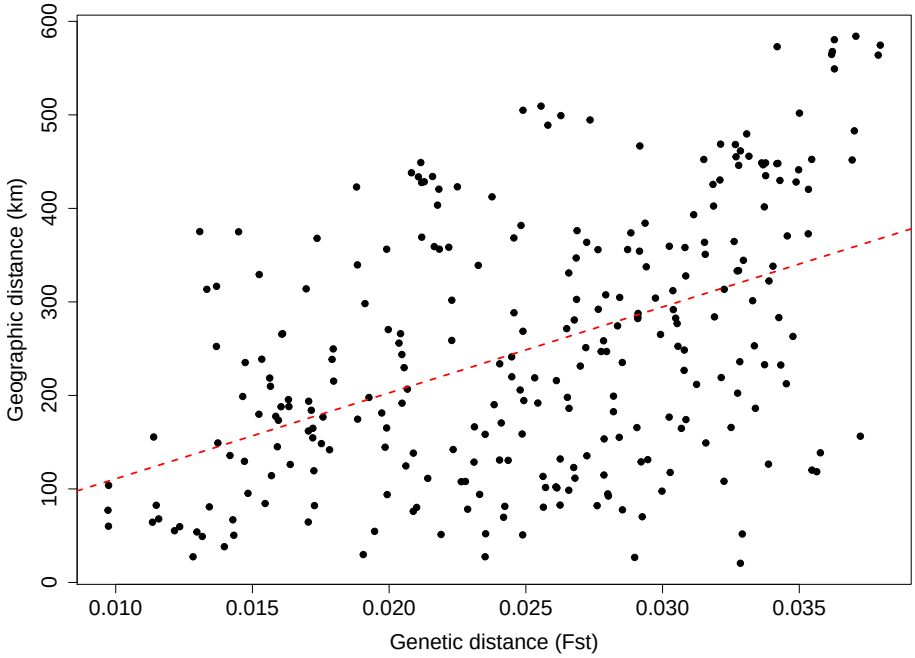

Figure S4: The relationship between pairwise genetic distance (F_{ST}) and geographic distance (isolation by distance) between each of the 25 sampling sites for all 426 individuals of *I. elegans*.

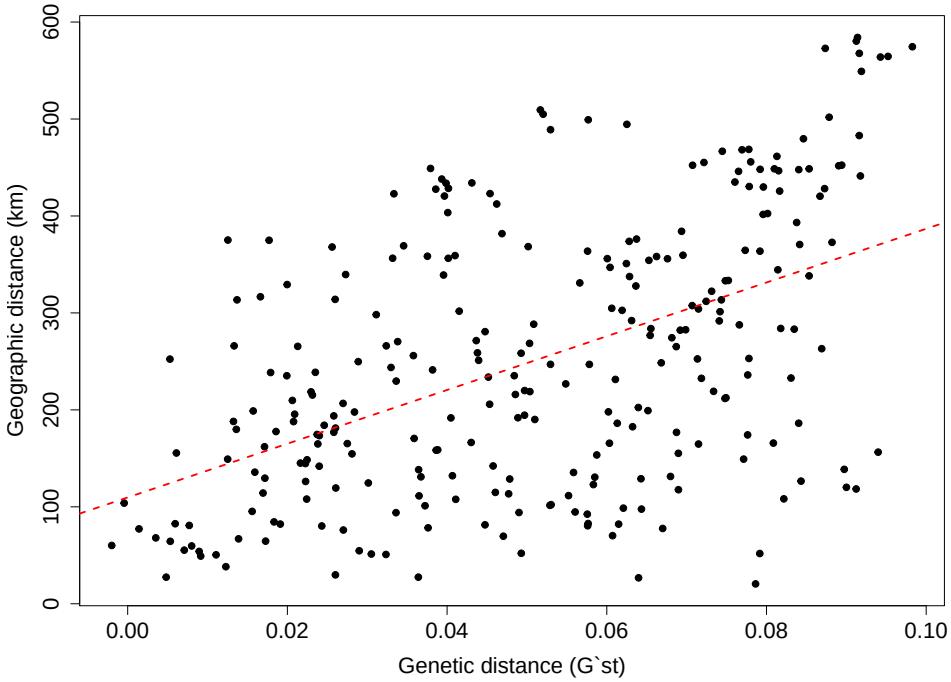

Figure S5: The relationship between pairwise genetic distance ($G`_{ST}$) and geographic distance (isolation by distance) between each of the 25 sampling sites for all 426 individuals of *I. elegans*.

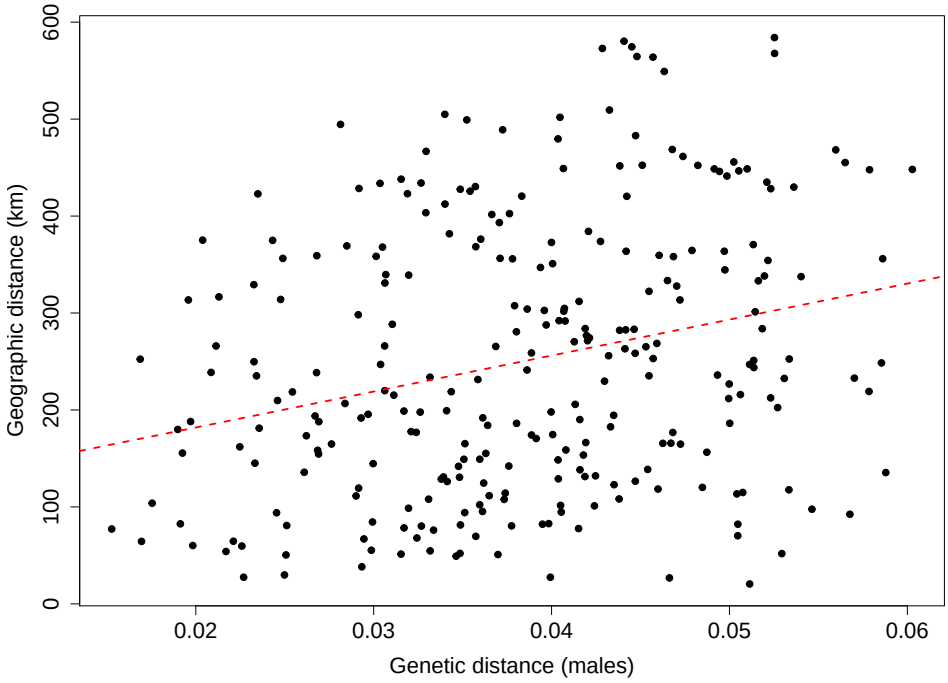

Figure S6: The relationship between pairwise genetic distance (F_{ST}) and geographic distance (isolation by distance) between each of the 25 sampling sites for 209 male *I. elegans*.

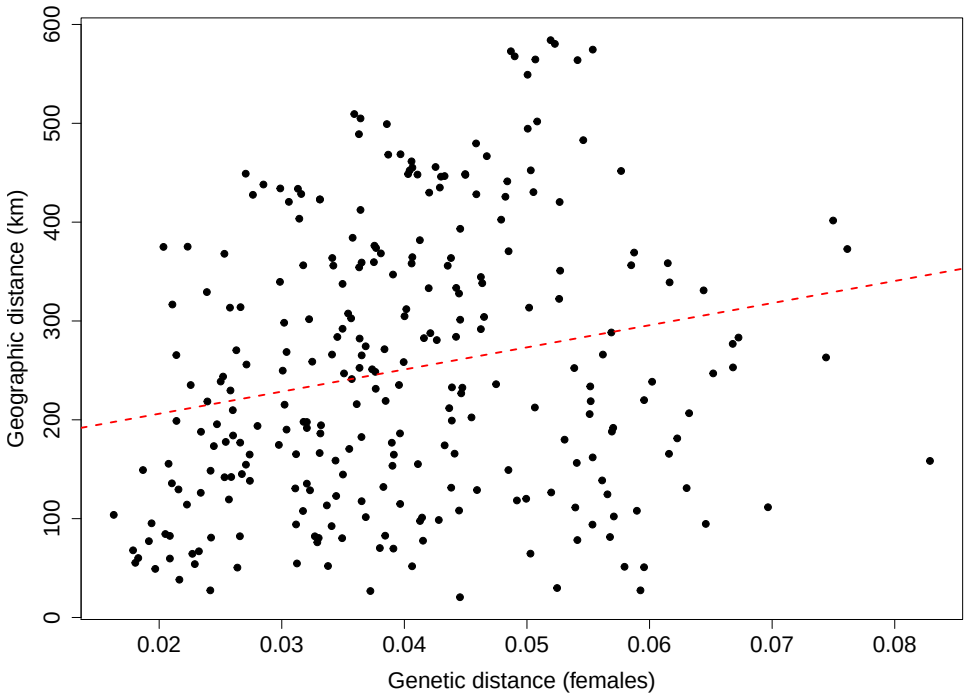

Figure S7: The relationship between pairwise genetic distance (F_{ST}) and geographic distance (isolation by distance) between each of the 25 sampling sites for 217 female *I. elegans*.

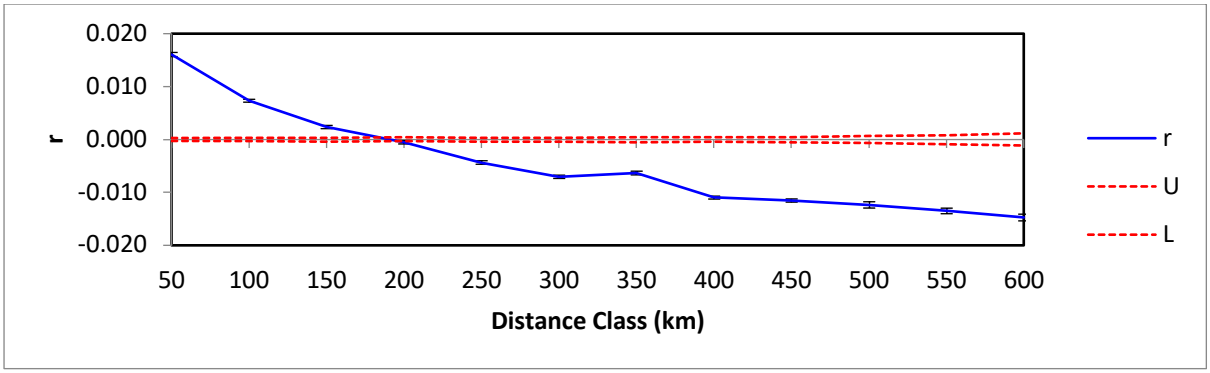

Figure S8: Spatial autocorrelation (*r*) estimates for 50km distance classes from 50 up to 600 km, for all samples of *I. elegans* (n = 426). Error bars show the 95% confidence intervals estimated from 1000 bootstrap resampling. The dotted lines represent the upper and lower 95% confidence intervals from the null model of no spatial structure, determined by 999 permutations.

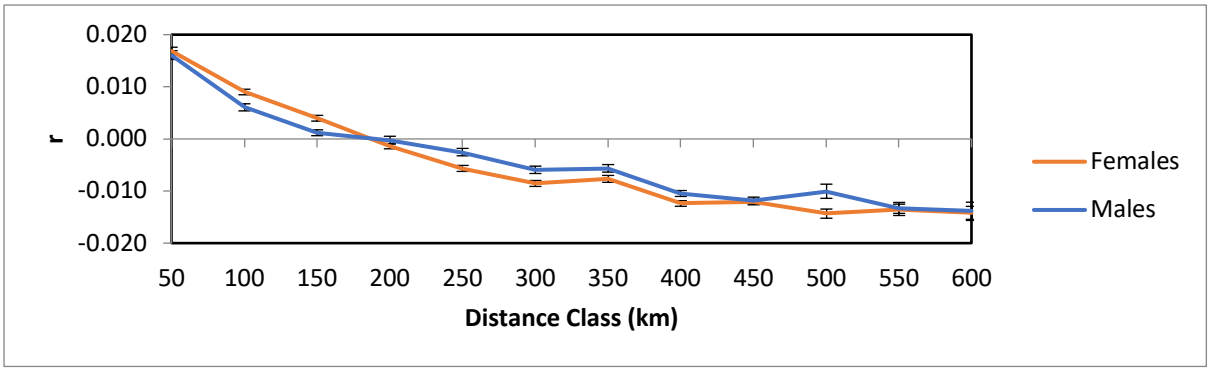

Figure S9: Spatial autocorrelation (*r*) estimates for distance classes 50 km up to 600 km. for male (blue line; n = 209) and female (orange line; n = 217) *I. elegans*. Error bars show the 95% confidence intervals estimated from 1000 bootstrap resampling.

Table S1: Number of males and females of *I. elegans* sampled from in each site that were genotyped and used in landscape genetic analyses.

Site ID	Latitude	Longitude	N females	N males	N
LUN	55.6497	13.3324	12	5	17
1D	55.7335	13.9645	10	9	19
FLY	55.7410	13.3560	11	9	20
BOG	55.7504	13.0365	11	7	18
2B	56.1578	15.0847	11	9	20
3A	56.1710	15.7097	8	12	20
3B	56.2575	16.0390	10	8	18
3E	56.5313	16.2053	7	8	15
4A	56.7379	15.9179	9	11	20
4B	56.7605	16.3696	9	11	20
4D	56.9879	16.0933	2	8	10
5A	57.2530	16.4866	13	5	18
5D	57.4389	16.2244	5	5	10
6C	57.8919	15.8006	12	8	20
K1	58.3598	16.7931	11	9	20
6E	58.4674	16.3704	10	5	15
8A	58.8235	17.4604	10	10	20
8C	59.1874	17.1659	9	6	15
8D	59.3433	16.4295	5	5	10
9A	59.4732	16.0658	8	7	15
G8	59.7937	16.1698	8	11	19
G14	59.5241	15.1078	6	11	17
G17	59.9585	16.2074	13	7	20
G44	60.1973	17.9135	12	8	20
G39	60.5765	16.7638	1	9	10

Table S2: Number of male and female *I. elegans* at each site included in the morphological mixed effects models.

Site ID	Latitude	Longitude	N Males	N Females	N
1D	55.7335	13.9645	19	13	32
2B	56.1578	15.0847	3	5	8
3A	56.1711	15.7097	10	8	18
3B	56.2575	16.0390	15	14	29
3E	56.5313	16.2053	15	2	17
4A	56.7380	15.9179	19	11	30
4B	56.7605	16.3696	13	8	21
4D	56.9879	16.0933	8	2	10
5A	57.2530	16.4866	20	19	39
5B1	57.3165	16.2740	3	1	4
5C	58.8720	16.1642	1	1	2
5D	57.4389	16.2244	10	4	14
6C	57.8919	15.8006	21	16	37
6D	58.1466	15.6758	13	10	23
6E	58.4674	16.3704	17	19	36
7A	58.8720	17.5764	21	16	37
8A	58.8235	17.4604	13	16	29
8C	59.1874	17.1659	16	16	32
8D	59.3433	16.4295	8	5	13
9A	59.4732	16.0658	20	11	31
9D	59.6089	15.4217	3	2	5
G10	59.5296	14.9987	38	31	69
G12	59.6572	15.2562	0	3	3
G14	59.5241	15.1078	35	14	49
G15	59.4642	15.4597	2	1	3
G17	59.9585	16.2074	20	14	34
G18	59.9124	16.4014	3	1	4
G23	59.6929	15.9181	1	2	3
G24	59.4786	16.0833	24	13	37
G26	59.5050	16.2554	27	13	40
G27	59.7292	15.6917	9	6	15
G3	59.8813	15.6666	1	1	2
G31	59.5886	15.2335	5	0	5
G39	60.5765	16.7638	13	2	15
G4	59.9663	15.7510	0	2	2
G44	60.1973	17.9135	13	12	25
G48	60.3454	18.2580	3	1	4
G8	59.5203	16.5292	33	34	67
G9	59.7263	16.4275	12	3	15
K1	58.3598	16.7931	18	19	37
K3	58.7206	16.1503	8	3	11

Table S3: The proportion of variance explained by each PCA axis for overall body size based on measurements of six morphological features, presented for the total dataset and for males and females separately.

PCA axis	Total	Females	Males
1	0.6552	0.6297	0.6434
2	0.2091	0.1522	0.1561
3	0.0661	0.0908	0.0863
4	0.0415	0.0751	0.0649
5	0.0280	0.0523	0.0493
6	0.0000	0.0000	0.0000

Table S4: The best selected resistance models explaining the influence of mean annual temperature on genetic distance (F_{ST}) for the total *I. elegans* (n = 426), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	logLik	AICc	delta (Δi)	weight
γ = 1, α = 1000	1357.1849	-2706.2343	0.0000	0.5649
γ = 1, α = 100	1356.8080	-2705.4804	0.7539	0.3875
γ = 2, α = 100	1354.4681	-2700.8007	5.4336	0.0373
γ = 2, α = 10	1352.4350	-2696.7343	9.5000	0.0049
γ = 1, α = 10	1352.3451	-2696.5546	9.6797	0.0045
γ = 5, α = 100	1350.3930	-2692.6504	13.5839	0.0006
γ = 2, α = 1000	1348.3071	-2688.4786	17.7557	0.0001
γ = 1, α = 5	1348.2609	-2688.3862	17.8481	0.0001
γ = 0.5, α = 1000	1347.8044	-2687.4733	18.7610	0.0000
NULL	1329.0380	-2649.9404	56.2938	0.0000

Table S5: The best selected resistance models explaining the influence of mean annual temperature on genetic distance ($G`_{ST}$) for the total *I. elegans* (n = 426), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	logLik	AICc	delta (Δi)	weight
γ = 1, α = 1000	975.0888	-1942.0420	0.0000	0.5821
γ = 1, α = 100	974.7067	-1941.2778	0.7641	0.3973
γ = 2, α = 100	971.3048	-1934.4741	7.5679	0.0132
γ = 1, α = 10	970.0957	-1932.0559	9.9861	0.0039
γ = 2, α = 10	969.8616	-1931.5876	10.4543	0.0031
γ = 5, α = 100	966.9871	-1925.8385	16.2034	0.0002
γ = 1, α = 5	965.8895	-1923.6433	18.3986	0.0001
γ = 0.5, α = 1000	965.5305	-1922.9255	19.1165	0.0000
γ = 0.5, α = 100	965.2328	-1922.3299	19.7120	0.0000
NULL	946.4760	-1884.8164	57.2255	0.0000

Table S6: The best selected resistance models explaining the influence of mean annual temperature on genetic distance (F_{ST}) for male *I. elegans* (n = 209), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 1, α = 1000	1312.0713	-2616.0069	0.0000	0.3017
γ = 1, α = 100	1312.0372	-2615.9387	0.0682	0.2916
γ = 2, α = 10	1311.3194	-2614.5031	1.5038	0.1422
γ = 1, α = 10	1310.8104	-2613.4853	2.5216	0.0855
γ = 5, α = 100	1310.6577	-2613.1799	2.8271	0.0734
γ = 2, α = 100	1310.1893	-2612.2429	3.7640	0.0459
γ = 1, α = 5	1309.2781	-2610.4205	5.5864	0.0185
γ = 0.5, α = 1000	1308.8625	-2609.5895	6.4175	0.0122
γ = 0.5, α = 100	1308.7490	-2609.3623	6.6446	0.0109
NULL	1299.8544	-2591.5733	24.4337	0.0000

Table S7: The best selected resistance models explaining the influence of mean annual temperature on genetic distance ($G`_{ST}$) for male *I. elegans* (n = 209), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 1, α = 1000	939.8575	-1871.5794	0.0000	0.4618
γ = 1, α = 100	939.7360	-1871.3365	0.2429	0.4090
γ = 1, α = 10	937.5569	-1866.9783	4.6011	0.0463
γ = 2, α = 10	937.5548	-1866.9740	4.6054	0.0462
γ = 2, α = 100	936.5830	-1865.0305	6.5489	0.0175
γ = 5, α = 100	935.6061	-1863.0766	8.5027	0.0066
γ = 1, α = 5	935.2261	-1862.3165	9.2629	0.0045
γ = 0.5, α = 1000	934.9264	-1861.7173	9.8621	0.0033
γ = 0.5, α = 100	934.7571	-1861.3787	10.2007	0.0028
NULL	922.9587	-1837.7819	33.7975	0.0000

Table S8: The best selected resistance models explaining the influence of mean annual temperature on genetic distance (F_{ST}) for female *I. elegans* (n = 217), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 2, α = 100	1323.0836	-2638.0315	0.0000	0.5771
γ = 1, α = 1000	1322.2652	-2636.3948	1.6368	0.2546
γ = 1, α = 100	1321.7623	-2635.3889	2.6426	0.1540
γ = 2, α = 1000	1318.8300	-2629.5245	8.5071	0.0082
γ = 2, α = 10	1317.5621	-2626.9886	11.0429	0.0023
γ = 5, α = 100	1317.5236	-2626.9117	11.1198	0.0022
γ = 1, α = 10	1317.1571	-2626.1785	11.8530	0.0015
γ = 1, α = 5	1313.3894	-2618.6432	19.3884	0.0000
γ = 0.5, α = 100	1313.0106	-2617.8856	20.1459	0.0000
NULL	1295.3239	-2582.5122	55.5193	0.0000

Table S9: The best selected resistance models explaining the influence of mean annual temperature on genetic distance ($G`_{ST}$) for female *I. elegans* (n = 217), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 2, α = 100	901.6461	-1795.1567	0.0000	0.6260
γ = 1, α = 1000	900.4978	-1792.8601	2.2966	0.1985
γ = 1, α = 100	900.1256	-1792.1156	3.0411	0.1368
γ = 2, α = 1000	898.3367	-1788.5378	6.6189	0.0229
γ = 2, α = 10	897.1473	-1786.1591	8.9976	0.0070
γ = 1, α = 10	896.6446	-1785.1535	10.0031	0.0042
γ = 5, α = 100	896.5861	-1785.0366	10.1201	0.0040
γ = 1, α = 5	893.7537	-1779.3719	15.7848	0.0002
γ = 0.5, α = 1000	893.2737	-1778.4119	16.7448	0.0001
NULL	879.5542	-1750.9728	44.1839	0.0000

Table S10: The best selected resistance models explaining the influence of land cover rank on genetic distance (F_{ST}) for the total *I. elegans* (n = 426), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
NULL	1362.9549	-2717.7743	0.0000	0.7161
γ = 2, α = 5	1361.3517	-2714.5677	3.2066	0.1441
γ = 5, α = 5	1360.6761	-2713.2165	4.5578	0.0733
γ = 1, α = 5	1359.6587	-2711.1819	6.5924	0.0265
γ = 2, α = 10	1359.4270	-2710.7184	7.0558	0.0210
γ = 10, α = 5	1358.6567	-2709.1777	8.5966	0.0097
γ = 5, α = 10	1357.6172	-2707.0989	10.6754	0.0034
γ = 0.5, α = 5	1357.4169	-2706.6983	11.0760	0.0028
γ = 1, α = 100	1357.1647	-2706.1938	11.5805	0.0022
γ = 0.5, α = 5	1355.4767	-2702.8178	14.9565	0.0004

Table S11: The best selected resistance models explaining the influence of land cover rank on genetic distance ($G`_{ST}$) for the total *I. elegans* (n = 426), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
NULL	979.2548	-1950.3740	0.0000	0.6140
γ = 2, α = 5	978.1768	-1948.2179	2.1561	0.2089
γ = 5, α = 5	977.3469	-1946.5582	3.8158	0.0911
γ = 2, α = 10	976.4109	-1944.6861	5.6879	0.0357
γ = 1, α = 5	976.2454	-1944.3552	6.0188	0.0303
γ = 10, α = 5	975.0109	-1941.8862	8.4878	0.0088
γ = 5, α = 10	974.4922	-1940.8488	9.5252	0.0052
γ = 0.5, α = 5	973.7966	-1939.4576	10.9164	0.0026
γ = 1, α = 10	973.7805	-1939.4255	10.9485	0.0026
γ = 0.2, α = 5	971.7097	-1935.2838	15.0902	0.0003

Table S12: The best selected resistance models explaining the influence of land cover rank on genetic distance (F_{ST}) for male *I. elegans* (n = 209), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
NULL	1326.0064	-2643.8772	0.0000	0.5467
γ = 2, α = 5	1324.5591	-2640.9826	2.8946	0.1286
γ = 1, α = 5	1324.1676	-2640.1996	3.6776	0.0869
γ = 5, α = 5	1324.0201	-2639.9047	3.9725	0.0750
γ = 2, α = 10	1323.2638	-2638.3920	5.4852	0.0352
γ = 0.5, α = 5	1323.2589	-2638.3822	5.4950	0.0350
γ = 10, α = 5	1323.0333	-2637.9309	5.9463	0.0280
γ = 1, α = 10	1322.7602	-2637.3849	6.4923	0.0213
γ = 0.2, α = 5	1322.3470	-2636.5584	7.3189	0.0141
γ = 0.1, α = 5	1321.9725	-2635.8093	8.0679	0.0097
γ = 5, α = 10	1321.8451	-2635.5545	8.3227	0.0085
γ = 0.5, α = 10	1321.5814	-2635.0271	8.8501	0.0065

Table S13: The best selected resistance models explaining the influence of mean annual temperature on genetic distance ($G`_{ST}$) for male *I. elegans* (n = 209), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
NULL	948.3129	-1888.4901	0.0000	0.6423
γ = 2, α = 5	946.7186	-1885.3017	3.1885	0.1304
γ = 1, α = 5	946.2378	-1884.3401	4.1500	0.0806
γ = 2, α = 10	945.8476	-1883.5596	4.9305	0.0546
γ = 5, α = 5	945.2492	-1882.3628	6.1273	0.0300
γ = 10, α = 5	944.9689	-1881.8021	6.6880	0.0227
γ = 0.5, α = 5	944.4928	-1880.8500	7.6401	0.0141
γ = 1, α = 10	944.1550	-1880.1745	8.3157	0.0100
γ = 5, α = 10	943.6299	-1879.1242	9.3660	0.0059
γ = 0.2, α = 5	943.2505	-1878.3654	10.1247	0.0041

Table S14: The best selected resistance models explaining the influence of land cover rank on genetic distance (F_{ST}) for female *I. elegans* (n = 217), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 2, α = 5	1327.5046	-2646.8737	0.0000	0.3058
NULL	1327.4929	-2646.8502	0.0234	0.3022
γ = 5, α = 5	1326.9972	-2645.8588	1.0148	0.1841
γ = 2, α = 10	1326.2028	-2644.2699	2.6037	0.0832
γ = 5, α = 10	1325.9247	-2643.7139	3.1598	0.0630
γ = 1, α = 5	1325.3669	-2642.5982	4.2754	0.0361
γ = 10, α = 5	1324.5890	-2641.0424	5.8313	0.0166
γ = 1, α = 10	1323.1312	-2638.1268	8.7469	0.0039
γ = 0.5, α = 5	1323.0519	-2637.9682	8.9054	0.0036
γ = 10, α = 10	1321.4269	-2634.7183	12.1554	0.0007

Table S15: The best selected resistance models explaining the influence of land cover rank on genetic distance ($G`_{ST}$) for female *I. elegans* (n = 217), also including the null (isolation by distance) model. The best selected model has the lowest AIC score and the difference between them, delta (Δi), reflects the strength of the best selected model in comparison to others.

Resistance surface	**logLik**	**AICc**	**delta (Δi)**	**weight**
γ = 2, α = 5	906.7471	-1805.3586	0.0000	0.3306
γ = 2, α = 10	906.2599	-1804.3842	0.9744	0.2031
γ = 5, α = 5	906.0672	-1803.9988	1.3598	0.1675
γ = 5, α = 10	905.8179	-1803.5003	1.8583	0.1305
NULL	905.3573	-1802.5790	2.7796	0.0823
γ = 1, α = 5	904.7343	-1801.3330	4.0256	0.0442
γ = 10, α = 5	904.0647	-1799.9937	5.3649	0.0226
γ = 1, α = 10	903.1781	-1798.2206	7.1380	0.0093
γ = 0.5, α = 5	902.4098	-1796.6840	8.6746	0.0043
γ = 10, α = 10	901.9882	-1795.8408	9.5178	0.0028