

**Geographic Variation in Male Agonistic Display
among Three Populations of the Lizard
Amphibolurus muricatus: the Role of Habitat
Structure, Predation Risk and Temperature**



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This thesis is presented for the degree of Doctor of Philosophy

February 2014

“In the realm of ideas everything depends on enthusiasm...
in the real world all rests on perseverance”

Johann Wolfgang von Goethe

Dedico este trabajo a mis dos familias, vieja y nueva. A mi mamá, papá, hermana, hermano y sobrinas, quienes son parte de mi corazón. A Viviana y mi hijo Julián, quienes son parte de mi alma. Mi vida no tendría sentido si ellos no estuviesen conmigo.

I dedicate this work to my old and new families. To my mum, dad, sister, brother and nieces, whom are part of my heart. To Viviana and my son Julián, whom are part of my soul. My life is meaningless without all of you.

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SUMMARY

Signal diversification is often the product of sexual or natural selection and may be accompanied by genetic differentiation or reflect a plastic response to environmental variables. Visual displays of lizards performed during communication with conspecifics can be affected by a multitude of factors including habitat light and habitat structure, predators, and the thermal environment. Widely-ranging species occupying different habitat types and environmental conditions including predator type and density are predicted to exhibit greater variation in signal form and function. This geographic variation can manifest itself in differences in communication and even population-level aggression and dominance. I use an agamid lizard from Australia, the Jacky dragon (*Amphibolurus muricatus*) to: 1) examine geographic variation of intrinsic features such as genetic structure, morphology, performance capacities, body temperature and signaling behaviour; 2) determine the variation of extrinsic factors such as the characteristics of the habitats, predation risk and ambient temperature experienced by these populations; and 3) establish the relationships between intrinsic and extrinsic factors and the repercussions for the communicative system of the species. I sampled three populations (Yarratt State Forest, Royal National Park and Cann River State Forest) across the range of the species (at least 280 km apart) and collected data in the field on morphological traits and body temperatures of lizards, as well as the structure and complexity of the habitat. I also performed lab experiments to record the variation in signaling behaviour, performance capacities and preferred body temperature of animals.

I found that individuals from two more closely-related populations were also more similar in morphology and field body temperatures than lizards from a third, more distant population. All three populations differed in performance capacities and

characteristics of the signaling behaviour. Variation in visual displays and habitat use was unrelated to habitat structure and complexity. Predation risk and thermal environment were highly variable along the distribution of the Jacky dragon and both factors have an effect on the properties of the signaling behaviour. No significant differences in aggression were found during intra- and inter-population contests, although one population seemed to be dominant over the others and different rules among populations seemed to govern the outcome of contests. I argue that display variation might be a consequence of behavioural plasticity and that, despite difference in genetic structure, morphology and behaviour, the species retains a communicative cohesion.

DECLARATION

I certify that the work in this thesis entitled “Evolution and function of visual signals in a widely-ranging lizard species complex” has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree to any other university institution other than Macquarie University.

I certify that this thesis is an original piece of research and that it has been primarily written by me. Where co-authored chapters exist my contributions and those of all other authors have been clearly indicated. Any help and assistance that I have received has been appropriately acknowledged.

I certify that all information sources and literature used are indicated in the thesis.

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ACKNOWLEDGEMENTS

I am very grateful to my supervisors Martin Whiting (Macquarie University) and Richard Peters (La Trobe University), who guided me all the way through during these years here in Australia. The long hours they spent discussing, reviewing and improving my project were an invaluable help for me to get to the end of the road.

Many people also helped me in different ways during my PhD. My lab mates, Dani Chandrasoma, Dan Noble, Alana Mailey and Siobhan Dennison, became part of my life as colleagues, advisors and mates. A big thanks to Fernando Soley, Seppi, Yian Yian Dam, Rowan McGinley and Pau Carazo, your friendship helped me to make this a great experience. I would also like to extend my gratitude to the awesome research assistants: Danny Boerritger, Moniek Poppe, and Aljoscha Kubassa, no words are good enough to thank you for the incredible enthusiasm and hard work you guys spent with me both in the field and the lab, even under difficult conditions and my grumpy mood. I also want to thank to all the great volunteers, interns and students that have been involved with the Lizard Lab, all did an amazing job and shared many experiences with me.

Several institutions supported me in different ways. For their financial and logistical support I am thankful to Macquarie University (with special thanks to the admin of the departments of Brain, Behaviour and Evolution and Biological Sciences), University of Costa Rica, and Ministerio de Ciencia y Tecnología de Costa Rica. For their collaboration in my process of data collection I thank to the Australian Museum (Ross Sadler and Cecilie Beatson), Victoria Museum (Wayne Longmore, Karen Roberts and Katie Smith), and the departments of Sustainability and Environment of Victoria and Environment and Heritage of NSW. For their

participation in some of the experiments I am grateful to the Keogh's Lab (Scott Keogh and Mitzy Pepper) and Peters Lab.

Finally, I would like to thank my family and friends from Costa Rica for all their support. Special thanks to Logan Arroyo, Julio Brenes and Randall Arguedas for their help in securing financial support from the University of Costa Rica. The unconditional love of my mother, the always appropriate advice of my father (he is still teaching me from a remote place) and the joyful way and affection of my siblings, were so essential and inspiring that without them I wouldn't have been able to finish this stage. Viviana Arguedas decided to leave everything she had in Costa Rica and follow me in this adventure, and just for that I am especially grateful. She constantly helped me with work-related matters and provided me with extremely useful advice, but more importantly is that she became the best mother my son could have. I also thank my son Julián, who became my biggest motivation to finish my PhD.

INTRODUCTION

Population is the key evolutionary unit for most organisms. Each population of the same species follows a particular evolutionary pathway, experiencing changes in morphological, ecological, physiological and behavioural features that make one population different from another (Reznick et al. 1997). Eventually, if the frequency of particular characteristics in one or more populations is accentuated through time, this could lead to the rise of new species in a process called speciation. Therefore, as Darwin and others have noted, it is variation within and among populations the key factor to produce new species (Darwin 1859; Mayr 1999). Some of this variation is genetically based and some is produced by the specific environmental conditions where each population inhabits (Dillon 1984; Martin & McKay 2004; Oufiero et al. 2011). However, identifying the role that both genetics and environment play to account for the variation observed has proven difficult, especially for very labile traits as behaviour.

Animal signaling behaviour is particularly fascinating because of the vast range of signals used to communicate with members of the same species or of different species (Bradbury & Vehrencamp 1998). Individuals may use visual displays, sounds, vibrations, chemicals or a combination of these signals to convey information to heterospecifics and conspecifics. For example, many species of snakes can produce odoriferous secretions and adopt aggressive postures to deter predators (interspecific communication) (Greene 2000), and most birds use specific songs and ritualized displays to attract mates (intraspecific communication) (Searcy & Andersson 1986). It is well known that animal communication systems (i.e. the mechanisms and structures involved to produce and receive different types of signals [Endler 1993]) are species-specific (How et al. 2009); however, the extent to which a

communication system varies across populations of the same species remains poorly known for most organisms.

The signals used in a communication system are affected by the characteristics of: a) the emitter, b) the medium of transmission, and c) the receiver. All these components are constrained by the specific environmental conditions where the system is used, such as temperature, spectral properties, background noise, and obstacles (i.e. structure of the habitat) (Endler 1992, 1993; Boughman 2002). For instance, turbid conditions reduce visibility in some fishes and thus relax the effort of high signaling (Wong et al. 2007). In addition, emitters and receivers are affected by intrinsic body conditions (e.g. physical limits, physiological state, and genotype) and the presence of unwanted receivers (e.g. predators and parasites) (Endler 1991). Therefore, factors such as the current state of nourishment, number of parasites, and predation risk, can have a strong effect on the properties of the signals transmitted.

In order to communicate effectively, emitters and receivers have to overcome environmental constraints by using several strategies. Some of these strategies involve: a) using signals in locations where the habitat allows a clear transmission (e.g. less obstacles, appropriate light conditions, etc.), b) using signals during times when the conditions are suitable (e.g. less wind, less turbidity, etc.), and c) changing the properties of the signals (e.g. frequency of transmission, duration, intensity, etc.) (Endler 1992, 1993) to suit prevailing environmental conditions. The use of one or more of these strategies can bring both positive and negative effects. For example, animals could be able to communicate in different habitat types, allowing individuals to disperse and thus avoid the problems of overcrowding. However, animals might also become more exposed to predators and parasites depending on the time and place chosen to signal. Hence, animals face a trade-off: making signals conspicuous enough to be detected by conspecifics, but not as conspicuous that will attract the

attention of unwanted heterospecifics (Stuart-Fox et al. 2004; Fowler-Finn & Hebets 2011).

Three interrelated processes affect signal conspicuousness: a) degradation through habitat, b) perceptual tuning, and c) signal matching. Degradation of a signal occurs with increasing distance and the number of obstacles between the emitter and the receiver (Endler 1992). The higher the degradation of a signal, the less effective the message it conveys. Thus, a signal can be conspicuous in an open-vegetation habitat, for example, but be cryptic in a closed-vegetation one. Also, perception plays an important role to detect and process a signal. Because signals are subjected to local environmental conditions, local adaptation in perception is necessary to identify relevant signals from the background noise (Fleishman & Persons 2001; Woo & Rieucou 2013). For instance, the call produced by a frog in a pond can easily reach conspecifics when no other species are around, but can break down if the site is shared with other frogs calling at the same time (Wollerman & Wiley 2002). Finally, variation in a signal due to local adaptation can pose a problem for individuals from different locations to interpret the message being transmitted and respond accordingly. A matching between the intended message and the information perceived is required to establish an effective communication (Colbeck et al. 2010).

Lizards have been frequently used as model systems to explore the mechanisms of speciation, since they use a wide diversity of signals, including sounds (Marcellini 1977), chemical secretions (Cooper & Vitt 1987), and visual displays (Ord et al. 2002a). Many species produce motion-based signals, which are composed of discrete and sequential movements that are performed in a ritualistic fashion (Carpenter & Ferguson 1977). Phylogenetic analyses have determined that evolutionary changes in the structure of these displays can occur rapidly (Martins et al. 2004). Furthermore, selection pressures may vary between populations of the

same species (Martins et al. 1998). The aim of my thesis is to quantify the geographic variation of visual displays of the Jacky dragon (*Amphibolurus muricatus*) and the factors involved causing this variation. I am interested in answering the following general questions: 1) what is the degree of lability of the signal structure across populations of the same species?; 2) what are the effects of different selective pressures on the divergence of signal structure?; 3) are closely-related populations also more similar in their signal design?; and 4) does divergence in signaling behaviour promote the ability to discriminate individuals from the same and from different populations?

The signaling behaviour of the Jacky dragon has received significant attention (Carpenter et al. 1970; Peters & Ord 2003; Watt & Joss 2003). Several authors have explored the behavioural response of captive individuals to looming predators (Carlile et al. 2006) and computer animated, displaying conspecifics (Peters & Evans 2007; Van Dyk & Evans 2007; Woo & Rieucan 2008), as well as the effect of environmental cues to the properties of signals (Peters & Davis 2006; Peters et al. 2008). Despite the different habitat types that the species occupies through its wide distribution (Cogger 2000), no study has been conducted to account for population differences in visual displays. My thesis is intended to fill this gap and is structured into five chapters described below.

Chapter 1. Geographic variation in the signaling behaviour of the Jacky dragon

In this chapter I examine the relationships between genetic structure, morphology and signaling behaviour. My aim is to determine whether a concordant pattern in these three biological aspects occurs across populations of the Jacky dragon and whether individuals are able to recognize conspecifics from different

populations based on morphological and behavioural traits. This is a draft of a manuscript with Martin Whiting and Richard Peters as co-authors. My contribution is as follows: 80% experimental design; 70% data collection; 90% data analysis and 90% writing.

Chapter 2. Signal repertoire and contest outcome in the Jacky dragon

Dominance is key to defending territories and keeping rivals at bay. Morphological, physiological and behavioural traits used during these interactions may vary among populations and individuals from a particular population may on average dominate individuals from other populations. Therefore, I compare populations of the Jacky dragon to determine the relationship between morphology, performance, signaling behaviour and contest outcome, as well as the rules followed to establish dominance. Martin Whiting is a co-author of this draft manuscript. My contribution is as follows: 80% experimental design; 100% data collection; 100% data analysis and 85% writing.

Chapter 3. Influence of habitat on signal structure of the Jacky dragon

The characteristics of a habitat have been shown to significantly influence the expression of phenotypes, physiological performance capacities and the properties of signals used during communication. However, the extent to which habitat variation affect these biological aspects in an integrative framework is still poorly understood. I ask whether variation across populations in morphology, performance and signaling behaviour is a function of variation in the structure and complexity of habitat. Martin

Whiting is a co-author of this draft manuscript. My contribution is as follows: 70% experimental design; 100% data collection; 100% data analysis and 85% writing.

Chapter 4. Effect of temperature on the visual displays of the Jacky dragon

Temperature is fundamental to physiological processes and behavior in ectotherms such as lizards. However, the effect of temperature on motion-based signals has received little attention. In this chapter I examine geographic variation in the thermal environment and body temperatures of the Jacky dragon, and determine the relationship between ambient temperature and signaling behaviour. Martin Whiting and Richard Peters are co-authors on this draft manuscript. I contributed as follows: 60% experimental design; 100% data collection; 100% data analysis and 90% writing.

Chapter 5. Do Jacky dragons trade-off signal efficacy against survival?

Predation risk can change across the range of a species. Hence, populations of the same species might use different predator avoidance strategies. My aim in this chapter is to quantify predation risk across three populations of the Jacky dragon and determine whether anti-predator tactics change along the range of the species. I also examine the influence of predation risk on signaling behaviour. Martin Whiting is a co-author of this draft manuscript. I contributed as follows: 70% experimental design; 100% data collection; 100% data analysis and 85% writing.

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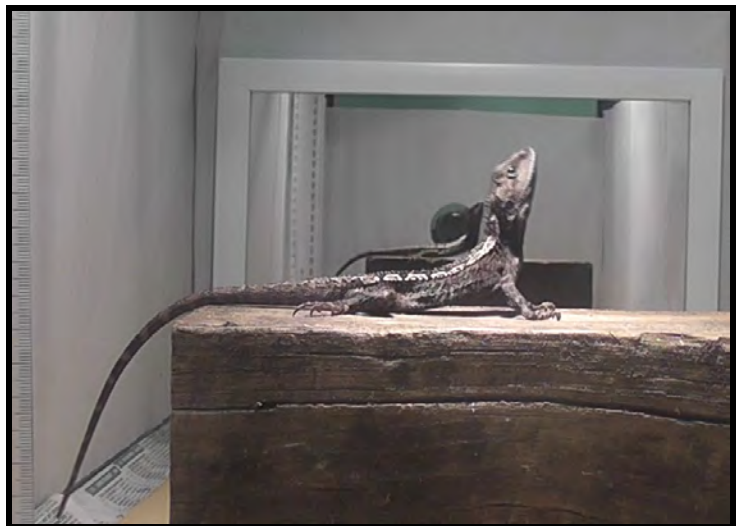
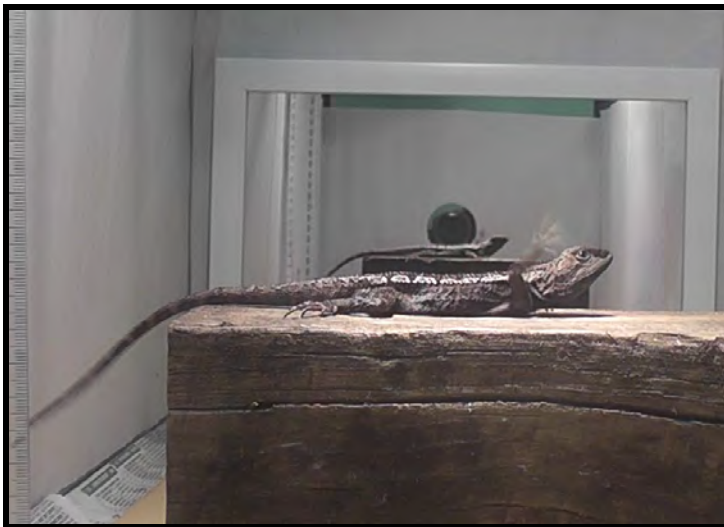
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CHAPTER 1

Geographic variation in the signaling behaviour of the Jacky dragon

Marco D. Barquero, Richard Peters and Martin J. Whiting



Abstract. Animal communication systems can be extremely complex and diverse. Signal diversification is often the product of sexual or natural selection and may be accompanied by genetic differentiation or simply reflect a plastic response to environmental variables. We use an agamid lizard endemic to Australia, the Jacky dragon (*Amphibolurus muricatus*), to examine the relationships between genetic structure, morphology and signaling behaviour. We also investigate the consequences of this variation on the ability of males to discriminate conspecific rivals. We studied three populations, two of which belong to the same genetic clade. We found that individuals from the more closely-related populations were also more similar in morphology than lizards from the third, more distant population. However, all three populations differed in the characteristics of the signaling behaviour. In addition, animals from all populations showed no differences in the levels of aggression when matched with individuals from the same or different populations in staged trials. We argue that display variation might be a consequence of behavioural plasticity and that, despite difference in genetic structure, morphology and behaviour, the species retains a cohesive communication system.

Animal communication is a rich and challenging field in large part because of the immense diversity of signals in a wide range of taxa. Animals make use of odours, sounds, vibrations, colours, postures and movements when transmitting a message (Bradbury & Vehrencamp 2011). This vast range of signals is believed to have evolved through several mechanisms, including natural selection, sexual selection, and genetic drift (Endler 1992, 1993; Endler et al. 2005). However, the roles that each of these mechanisms plays in the evolution of signal structure, as well as interactions between them, are still poorly understood. The evolution of signal diversity is, therefore, a complex matter that requires integrative studies to disentangle the effects of each mechanism.

Although many studies have focused on the vocal and visual communication of several taxa (e.g. birds: Todt & Naguib 2000, fishes: Rowland 1999, frogs: Narins et al. 2007, mammals: Osorio & Vorobyev 2008), the visual displays of lizards have also captivated the attention as a highly variable communicative system (Persons et al. 1999). Lizards can signal using colour patches, body posturing, and dynamic visual signals such as tail-flicks, arm waving and gular extensions (Carpenter & Ferguson 1977; Jenssen 1977). Across-species variation is well known and both natural and sexual selection have been invoked to explain signal diversity in a phylogenetic context (Ord et al. 2002; Stuart-Fox et al. 2007). However, intra-specific variation has received much less attention and relatively few studies have accounted for variation in visual signal structure across populations of the same species (e.g. Ferguson 1971; Jenssen 1971; Martins et al. 1998; Leal & Fleishman 2004; Bloch & Irschick 2006). While we predict that widely-ranging and ecologically variable species will show high variation in signal repertoire (Ord et al. 2002), this remains to be tested.

Unlike other communication systems, such as the calls of birds, frogs and many insects, visual signals are difficult to measure in the wild. Vegetation structure, the

mobility of animals, and weather conditions interfere in the quantification of visual displays, especially motion-based signals (Ord et al. 2007; Peters 2008). In addition, many species can adjust the intensity of these signals according to ambient conditions by reducing or increasing the properties of a signal when conditions are adverse (Peters et al. 2007). For example, Endler (1987) found that male guppies (*Poecilia reticulata*) displayed more often when light intensity was reduced, so that they could avoid predators. Measuring visual signals in the wild can be challenging because social and environmental conditions are highly variable. To circumvent this constraint, it is common practice to study signaling behavior under controlled conditions (e.g. temperature, lighting, social context) in captivity.

The signaling environment can vary dramatically for widely ranging species (e.g. for background noise, vegetation structure, and predation risk), so that differences in visual signals among populations could be a result of local adaptation (i.e. via natural selection) (Podos 2001). Furthermore, differences in signaling behaviour across the range of a species could also emerge as a result of differences in female preferences during sexual selection (Uy & Borgia 2000) or through a non-adaptive process such as genetic drift (Hill 1994). All these processes may or may not generate genetic differentiation and therefore, it is not clear whether signal diversification may be an expression of genetic differences or the direct result of selection.

Whether a trait is the result of genetic divergence or direct selection is of key importance to disentangle the role of the mechanisms involved in signal evolution and to understand the consequences for the species as a whole. For example, if the expression of signals is not subjected to selective pressures (e.g. the structure of the habitat) and they are only the result of genetic differences among populations of the same species, then dispersing animals would not be as constrained by the conditions

present at different habitats to perform their visual displays. At the same time, if the variation on genetic structure and signal repertoire is high across the range of a species, then dispersing animals could face problems to convey a message to local individuals since signals might not be recognized. Therefore, despite the opportunity to display freely under different environmental conditions, signal diversification can cause reproductive isolation and, eventually, speciation (Bensch et al. 1998; Leal & Fleishman 2004).

Visual signals may also be constrained by morphological characteristics of the individuals in a population (Podos 2001). If body parts used to produce visual signals differ among populations of the same species, then a concordant pattern of variation in the signaling behaviour is predicted. For instance, if the tail of an animal is used to communicate with conspecifics and tail length varies among populations, then some properties of the signal, such as duration and frequency, could also be divergent (Barnard 1991; Young et al. 1994). However, variation in signaling behaviour is not always explained by variation in morphology (Ferguson 1971; Irwin et al. 2008). Therefore, when comparing populations of the same species, it is important to consider how variation in genetics, morphology and signaling behaviour are related. Table 1 shows the outcome and associated explanation of the geographic variation in signaling behaviour when morphology and genetics are considered but not environmental factors.

Here, we test variation in morphology and signaling behaviour among populations of the Jacky dragon (*Amphibolurus muricatus*) reflects genetic differentiation. We predict that greater divergence in genetic structure between populations should produce the same effect in morphology and signaling behaviour. We also asked whether variation in signaling behaviour has an impact on the ability of individuals to recognize conspecifics from different populations. We expect that

higher divergence in the signaling behaviour should reduce the levels of aggression between animals from different populations. This means that we predict higher levels of aggression during intra-population contests compared to inter-population contests. Our rationale is that animals would not respond to unfamiliar signals as aggressively as to familiar ones (although see Dunbrack & Clarke 2003).

Table 1. Molecular, morphological and signaling behaviour variation when populations of the same species are compared. Variation, when present, can be seen as Genotype (or Phenotype or Behaviour type) 1 in population A, Genotype 2 in population B and so on. A potential explanation of the variation in signaling behaviour as a function of molecular and morphological variation is included.

Variation in			Signaling behaviour results of:
Genetics	Morphology	Signaling behaviour	
No	No	No	Lack of genetic and morphological variation
		Yes	Phenotypic (behavioural) plasticity
	Yes	No	Lack of genetic variation Behavioural stability
		Yes	Phenotypic plasticity (morphological and/or behavioural)
	No	No	Lack of morphological variation Behavioural stability
		Yes	Genetic differentiation Phenotypic (behavioural) plasticity
Yes	Yes	No	Behavioural stability
		Yes	Genetic and morphological differentiation Phenotypic (behavioural) plasticity

Jacky dragons are a good model system because they are widely-ranging and relatively abundant species (Cogger 2000), and the signaling behaviour has been described in detail (Carpenter et al. 1970; Peters & Ord 2003; Watt & Joss 2003). Animals reach sexual maturity at a snout-vent length of 72 mm and can grow up to 120 mm (Harlow & Taylor 2000), with a tail that is 1.5-2 times the body size. The dorsal colouration ranges from pale grey to dark brown with two light stripes at each side (Cogger 2000). Jacky dragons use motion-based signals to communicate between conspecifics and males have readily identifiable aggressive and submissive signals. Aggressive displays consist of five components performed in a ritualistic fashion: an introductory tail-flick, a backward foreleg-wave, a forward foreleg-wave, a push-up, and a body-rock (Peters & Ord 2003). The submissive display involves a slow circumduction of one of the forelimbs.

The Jacky dragon is an endemic Australian lizard extending over 2,000 km along the eastern and southeastern coast of the country. Despite its large distribution and well known signaling behaviour, geographic variation of the aggressive and submissive displays has never been measured in this species. Moreover, the Jacky dragon was one of the first Australian reptiles to be described (White 1790) and it has been extensively studied under different biological contexts, yet genetic variation along its range has only recently been reported (Pepper et al. 2014). Molecular analyses revealed the existence of five clades along the distribution of the species, although these were not supported by morphological data (Pepper et al. 2014).

Methods

Study populations and captive animals

We visited three sites along the range of the species: Yarratt state forest (31°48'17.4" S, 152°25'57.5" E), Royal National Park (34°04'49.7" S, 151°05'39.6" E), and Cann River state forest (37°36'25.9" S, 149°09'05.8" E), henceforth referred to as Yarratt, Royal and Cann River respectively. Animals from two populations, Yarratt and Royal, are members of the same genetic clade, whereas lizards from Cann River are genetically more distant (see below) (Pepper et al. 2014). Ten to fifteen males were collected from each population and transported to Macquarie University, where they were housed individually in indoor bins for 3-4 weeks as a quarantine period before being moved to outdoor pens. The outdoor enclosures (180 L x 180 W x 88 H cm) were made from metal sheets and a divider (45 cm high) was used to split them in half. Each half consisted of a sand substrate, branches suitable for basking, screen for sun cover, and plastic containers as refuges and water receptacles. Lizards were fed twice weekly with crickets dusted with calcium and vitamin supplements (Repti-Vite® and Repti-Cal®) and mealworms, and water was provided *ad libitum*. The Macquarie University's Animal Ethics Committee and the National Parks and Wildlife Service of New South Wales and Victoria approved all housing and experimental procedures.

Genetic relatedness and morphological variation

Using the uncorrected genetic distances generated by Pepper et al. (2014) for two mtDNA genes (ND2 and ND4) and three nDNA genes (RAG1, PRLR and BDNF), we established the genetic relatedness among our study populations by calculating pairwise average distances. We used these averages to generate separate distance matrices for mtDNA genes combined and nDNA genes combined.

We examined morphological variation by collecting the following morphometric data from males caught in the field: snout-vent length (SVL), tail length, interlimb length, head length, width and height, forelimb (length of humerus, length of radius and total length), hindlimb (length of femur, length of fibula and total length), and mass. All morphometrics, except head width, were taken on the right side of the animal. SVL, tail length and total lengths of both limbs were measured with a ruler (to the nearest 0.5 mm), whereas mass was measured with a digital balance (to the nearest 0.1 g) and the remaining measurements with a digital caliper (to the nearest 0.1 mm). We also recorded the number of femoral and anal pores from both sides (right and left) of an animal's body.

Male-male contests and video analysis

Trials were carried out during summer (January and February) of 2012 and 2013, at least four months after collection of individuals. Two captive males from the same (intra-population contests) or different (inter-population contests) populations were transferred to a neutral arena similar to the outdoor enclosures described above, but containing only one T-shaped wooden block (50 cm high) as a perch site. One or both individuals were temporarily marked with a Xylene-free paint pen and then placed at the same time within the arena. Interactions were filmed for up to 90 min using a Panasonic HDC-HS60-K digital video camcorder mounted on a Manfrotto tripod. The equipment was placed behind a thick black mat to avoid disturbing the subjects. Although not in consecutive sessions or days, each captive male had 1-4 intra-population and 1-2 inter-population encounters with different opponents and a total of 88 sessions were recorded. The neutral arena was sprayed with water after each session in order to prevent any chemical signal interfering with the behaviour of a new pair of subjects.

Videos were analyzed using JWatcher Video version 1.0 as an event recorder program (Blumstein & Daniel 2007), scoring the following behaviours: tail-flick (TF), aggressive display (AD, lizard arm-waves and does push-ups together), submissive display (SD), bite-attack (BA, lizard lunges towards an opponent with an open mouth), chase (C), dorsal grasp (DG, defined as an individual grasping another from the back), tail lift (TL), substrate lick (SL) and locomotion bout (LB). All videos were viewed twice, scoring the behaviours for one subject at a time. The following information was then obtained for all the contests in which each animal participated: average latency to the first TF, AD and SD, average number of TFs, ADs and SDs, and average duration of inter-TF, inter-AD and inter-SD intervals. Inter-display intervals were calculated as the time elapsed between the start of two behaviours (e.g. time between AD1 and AD2). Because it has been demonstrated that TFs and ADs in the Jacky dragon usually occur in bouts and pauses between bouts can last for 6-12 s (Ord & Evans 2003), we also calculated the average number of bouts and the average inter-bout interval.

Discrimination of individuals

To determine whether animals were able to discriminate conspecifics from their own and different populations, we created an index of aggression weighing each behaviour described above as follows: TF 1, AD 2, DG 3, C 4 and BA 5. We excluded TL as the meaning of this signal was not clear, and SL and LB as they were neutral signals. Because individuals participated in up to three intra-population contests, we used an average score of these contests for each animal. We also used separate scores for each animal during inter-population contests (i.e. one animal from Cann River had a score for a contest against an animal from Royal and another score for a contest against an animal from Yarratt).

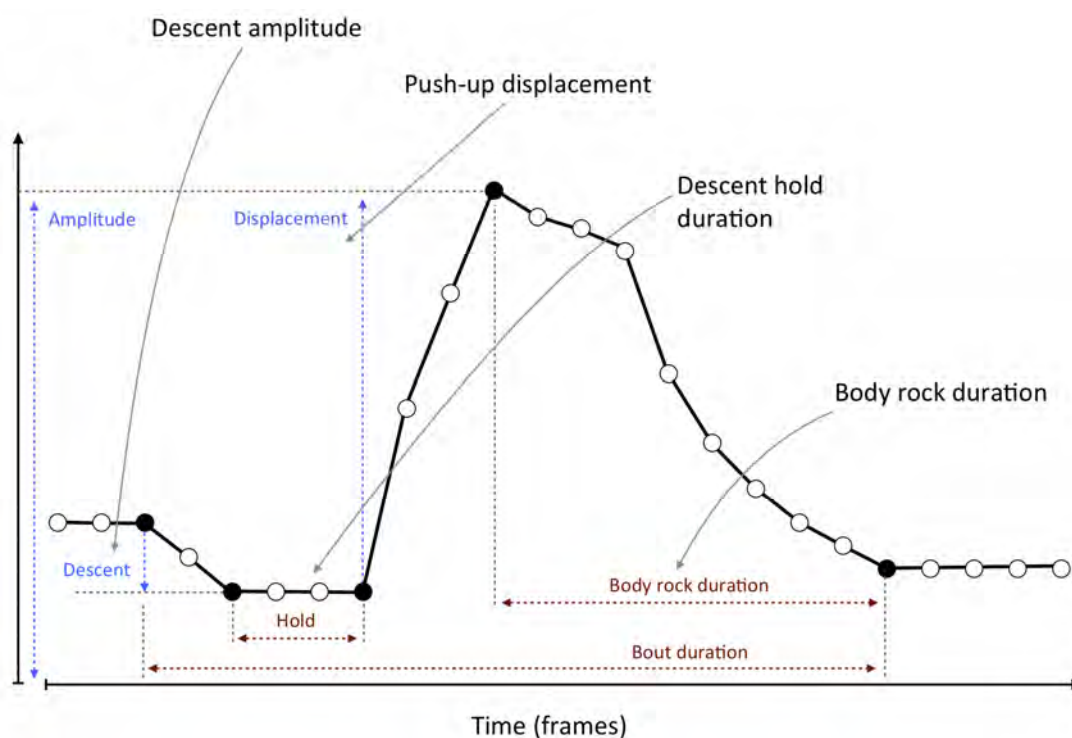


Figure 1. Sequence of the movements involved in the push-up display of the Jacky dragon.

Display-action-patterns (DAP)

We designed an experiment to quantify the variation in the components of the aggressive display of the Jacky dragon and create DAP profiles for each population. Each captive male was transferred to an indoor aquarium (91 L x 35 W x 38 H cm) containing a wooden block as perch site, a mirror at one of the sides and a ruler placed vertically in one of the sides of the aquarium. A heat lamp was suspended above the aquarium and the walls of the aquarium were covered with paper to avoid disturbing the subject, with one side having a viewing window for filming. The mirror was covered when a subject was placed inside the aquarium and was uncovered after 10 min, leaving enough time for the animal to first warm-up. Each subject was filmed 1-3 times for up to 60 min each time using a Panasonic HDC-HS60-K digital

video camcorder mounted on a Manfrotto tripod. Thirty-five individuals (12 from Cann River, 12 Royal, and 11 Yarratt) were tested during 94 sessions. A total of 165 displays were analyzed using custom written code in Matlab (R. Peters, LaTrobe University), tracking the eye of an individual in each frame of a video to define a DAP and using the ruler to scale measurements (pixels to mm). The following measurements were recorded: descent amplitude, push-up displacement, descent hold time and body-rock duration (Fig. 1).

Statistical analysis

In order to quantify whether variation in morphology across populations of the Jacky dragon, we used 13 morphometric and two meristic traits collected on adult males in the field. Both sets of variables were analyzed separately. First, we regressed all morphometric variables against SVL to remove the effect of size and then used the residuals to perform a multivariate analysis of variance (MANOVA) with population as a fixed factor. Bonferroni post-hoc tests were used to determine which populations differed. We compared the average number of femoral and anal pores across populations using separate Kruskal-Wallis tests.

To analyze the signaling behaviour of the Jacky dragon, we first tested the willingness to display (i.e. number of displaying and non displaying animals) either aggressively or submissively within each population using separate Binomial tests (one for AD and one for SD for each population). We also determined whether variation in characteristics of the signaling behaviour occurs among populations. From the data generated during the male-male contests, we first log-transformed only those behavioural variables (average number of TFs, ADs, SDs and Bouts) that did not fit a normal distribution. Four individuals (1 from Cann River, 1 from Royal and 2 from Yarratt) produced no displays of any kind and were excluded from further

analyses. We then performed a MANOVA on the 11 behavioural variables using population as a fixed factor. Bonferroni post-hoc tests were used to determine which populations differed from each other. From the data used to create the DAP graphs, we performed linear mixed effects models (LME) in R 2.13.0 (R Development Core Team 2011), with population as a fixed effect and lizard identity as a random effect to control for multiple observations from the same lizard. We determined the significance of the full model for each variable as well as investigating pairwise comparisons.

We were interested in determining the relative contribution of morphological and behavioural traits and whether they could be used to classify animals according to population. In order to do this, we only used the behavioural data from male-male contests. We first removed the effect of size by regressing all morphometric variables against SVL and then using the residuals. We then reduced the number of variables (13 morphometric and 11 behavioural extracted from the contests) by performing a factor analysis with principal components and varimax as the extraction and rotation methods respectively. We used the scores of the factors (principal components [PC]) extracted as predictor variables in a discriminant function analysis (DFA), with population as the grouping variable.

We used an analysis of covariance to determine whether the levels of aggression among populations differed. We averaged the scores of the aggression index and used the log-transformed variable as the dependent variable. We also averaged the scores and the number of submissive displays of the rivals that each animal confronted and used the log-transformed variables as covariates. The population of the individual and population of the rival were used as fixed factors. Unless otherwise specified, all analyses were carried out using SPSS Statistics version 20.0 (IBM©).

Results

Genetic and morphological variation

We found higher average genetic distances for both mtDNA genes and nDNA genes, when individuals from Royal and Yarratt were compared to those from Cann River. Comparisons between Royal and Yarratt yielded similar averages (Table 2). We found significant differences for most morphometric traits and for both femoral and anal pores (Tables 3 and S1) across populations. Post-hoc tests revealed that animals from Cann River differed significantly from those of Yarratt and Royal for most of the morphological traits (Table 3). Animals from Royal and Yarratt differed only in the relative length of the hindlimb (Table 3).

Table 2. Uncorrected P distance matrices for three populations of the Jacky dragon showing the averages of two mtDNA genes and three nDNA genes. Range (min-max) shown in parentheses.

mtDNA	Cann River	Royal	Yarratt
Cann River	0.0011 (0 – 0.0029)		
Royal	0.0280 (0.0229 – 0.0304)	0.0035 (0 – 0.0081)	
Yarratt	0.0298 (0.0281 – 0.0315)	0.0067 (0.0026 – 0.0092)	0.0040 (0 – 0.0080)
nDNA	Cann River	Royal	Yarratt
Cann River	0.0004 (0 – 0.0008)		
Royal	0.0014 (0.0004 – 0.0033)	0.0015 (0 – 0.0034)	
Yarratt	0.0017 (0.0004 – 0.0029)	0.0015 (0.0004 – 0.0032)	0.0009 (0.0004 – 0.0020)

Variation in signaling behaviour

Animals from the three populations differed in their willingness to display, either aggressively or submissively, towards conspecifics. Similar proportions of contests with and without displays were observed for lizards from Cann River (ADs: $P = 0.092$, SDs: $P = 0.366$) and Royal (ADs: $P = 0.358$, SDs: $P = 1.000$). Animals from Yarratt produced no displays in most of the contests (ADs: $P = 0.005$, SDs: $P = 0.025$).

Differences between populations were also detected in all behavioural traits extracted from male-male contests (Table 4). Overall, animals from Cann River started to display sooner and produced more displays and bouts with shorter inter-display intervals compared to lizards from Yarratt (Fig. 2). Cann River and Royal were not significantly different for any display variable. Individuals from the latter population produced ADs and SDs sooner and with shorter inter-display intervals than those from Yarratt (Fig. 2).

Lizards from all populations produced aggressive displays using the same components in a stereotyped fashion (Fig. 1). However, we found differences among populations in the descent amplitude ($F_{2,10} = 5.87$, $P = 0.021$) and body-rock duration ($F_{2,10} = 5.38$, $P = 0.026$). Pairwise comparisons showed that individuals from Yarratt performed displays with smaller amplitudes and shorter body-rocks (Fig. 3).

Table 3. Results of the statistical tests applied to morphometric (Univariate Fs extracted from a MANOVA) and meristic (Chi-square extracted from Kruskal-Wallis tests) traits to compare between three populations of the Jacky dragon (CR = Cann River [n = 35], R = Royal [n = 29], Y = Yarratt [n = 20]). *P*-values of pairwise post-hoc tests using Bonferroni are also shown for morphometric traits. Ranks for each population are shown for each meristic trait. Statistically significant results are shown in bold.

Morphometric	F_{2,81}	<i>P</i>	CR vs R	CR vs Y	R vs Y
Tail	16.08	<0.001	<0.001	<0.001	1.000
Interlimb	0.14	0.870	1.000	1.000	1.000
Head length	12.37	<0.001	<0.001	0.006	0.929
Head width	4.73	0.011	1.000	0.011	0.062
Head height	2.98	0.056	0.167	1.000	0.088
Humerus	6.61	0.002	0.009	0.010	1.000
Radius	11.94	<0.001	<0.001	0.001	1.000
Forelimb	3.20	0.046	1.000	0.047	0.162
Femur	8.97	<0.001	0.003	0.001	1.000
Fibula	28.71	<0.001	<0.001	<0.001	1.000
Hindlimb	38.48	<0.001	<0.001	<0.001	0.013
Mass	4.23	0.018	0.880	0.014	0.188
Meristic	X²(df=2)	<i>P</i>	CR	R	Y
Femoral pores	38.36	<0.001	61.25	28.10	29.43
Anal pores	8.86	0.012	49.59	35.33	38.78

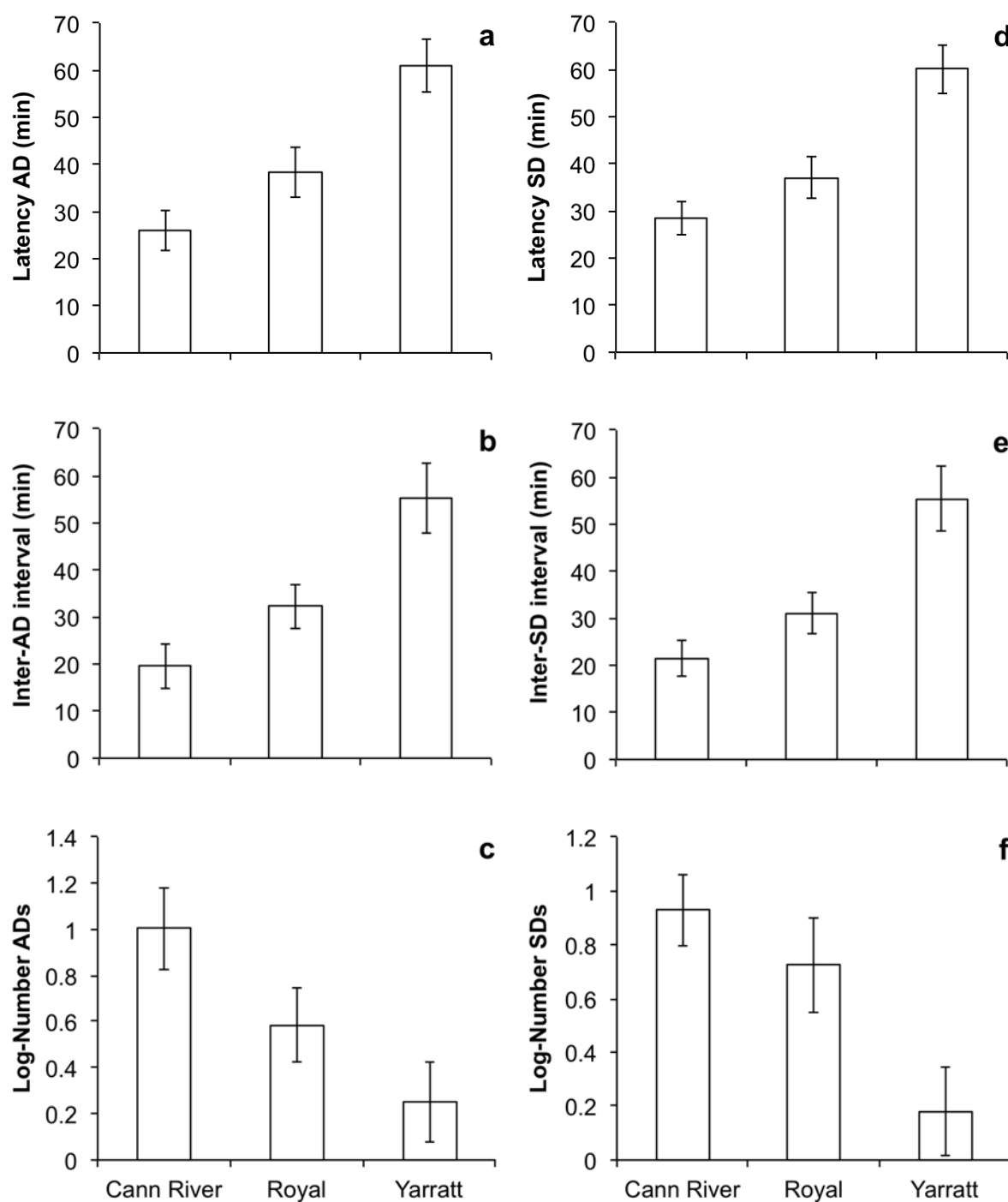


Figure 2. Behavioural characteristics (Mean \pm Standard Error) of three populations of the Jacky dragon. Panels a-c refer to the characteristics of the aggressive display (AD), while panels d-f refer to the submissive display.

Table 4. MANOVA (Univariate Fs) and pairwise post-hoc tests using Bonferroni (*P*-values) performed on behavioural traits comparing between three populations of the Jacky dragon (CR = Cann River [n = 12], R = Royal [n = 12], Y = Yarratt [n = 12]). Statistically significant results are shown in bold.

Trait	F _{2,33}	<i>P</i>	CR vs R	CR vs Y	R vs Y
Latency TF	6.60	0.004	0.240	0.003	0.231
Latency AD	12.13	<0.001	0.279	<0.001	0.011
Latency SD	13.76	<0.001	0.531	<0.001	0.002
Inter-TF interval	7.41	0.002	0.109	0.002	0.321
Inter-AD interval	9.70	<0.001	0.396	<0.001	0.025
Inter-SD interval	11.41	<0.001	0.596	<0.001	0.007
Inter-Bout interval	10.53	<0.001	0.169	<0.001	0.042
Log No. TFs	4.68	0.016	0.122	0.017	1.000
Log No. ADs	4.89	0.014	0.273	0.011	0.530
Log No. SDs	6.02	0.006	1.000	0.006	0.059
Log No. Bouts	4.62	0.017	0.235	0.015	0.714

Morphological and behavioural divergence

The Factor analysis identified five significant PCs (eigenvalues ≥ 1) accounting for 83.23% of the total variance. Morphometrics loaded more strongly on PCs 2, 4 and 5, while behavioural variables loaded strongly on PCs 2 and 3. The DFA produced two significant discriminant functions (Function 1: Wilks' Lambda = 0.26, $X^2 = 41.49$, df = 10, $P < 0.001$; Function 2: Wilks' Lambda = 0.70, $X^2 = 10.92$, df = 4, $P = 0.028$) that classified correctly 69.4% (Fig. 4) of the cases (83% for Cann River, 58% for Royal and 67% for Yarratt).

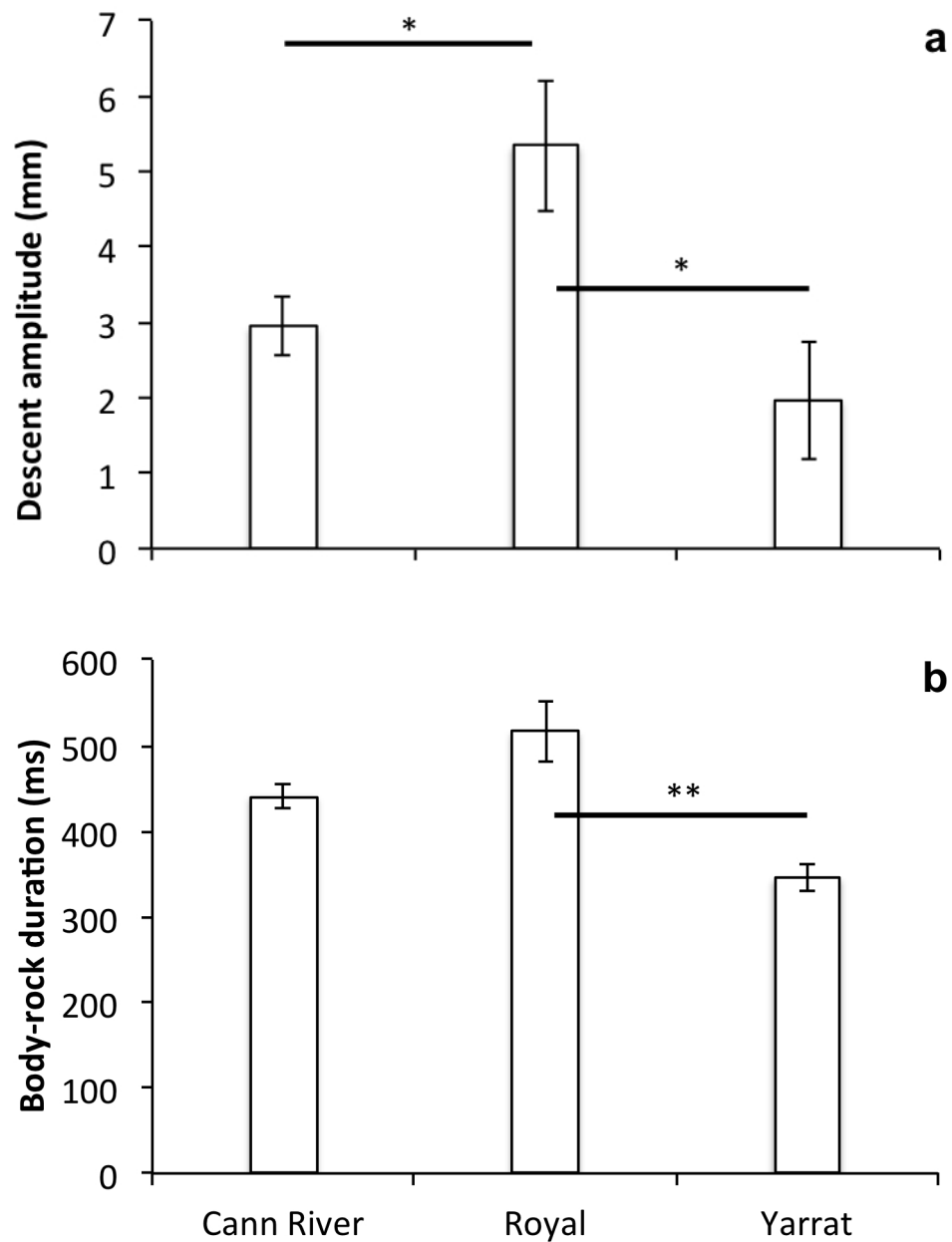


Figure 3. Two characteristics (Mean \pm Standard Error) of the push-up display of the Jacky dragon compared across three populations. Significant results among pairwise comparisons are shown (* $P < 0.05$, ** $P < 0.01$).

Discrimination of individuals

Despite differences in genetics, morphology and signaling behaviour, we found no significant differences in the overall levels of aggression for animals of each population ($F_{4,94} = 0.64$, $P = 0.632$).

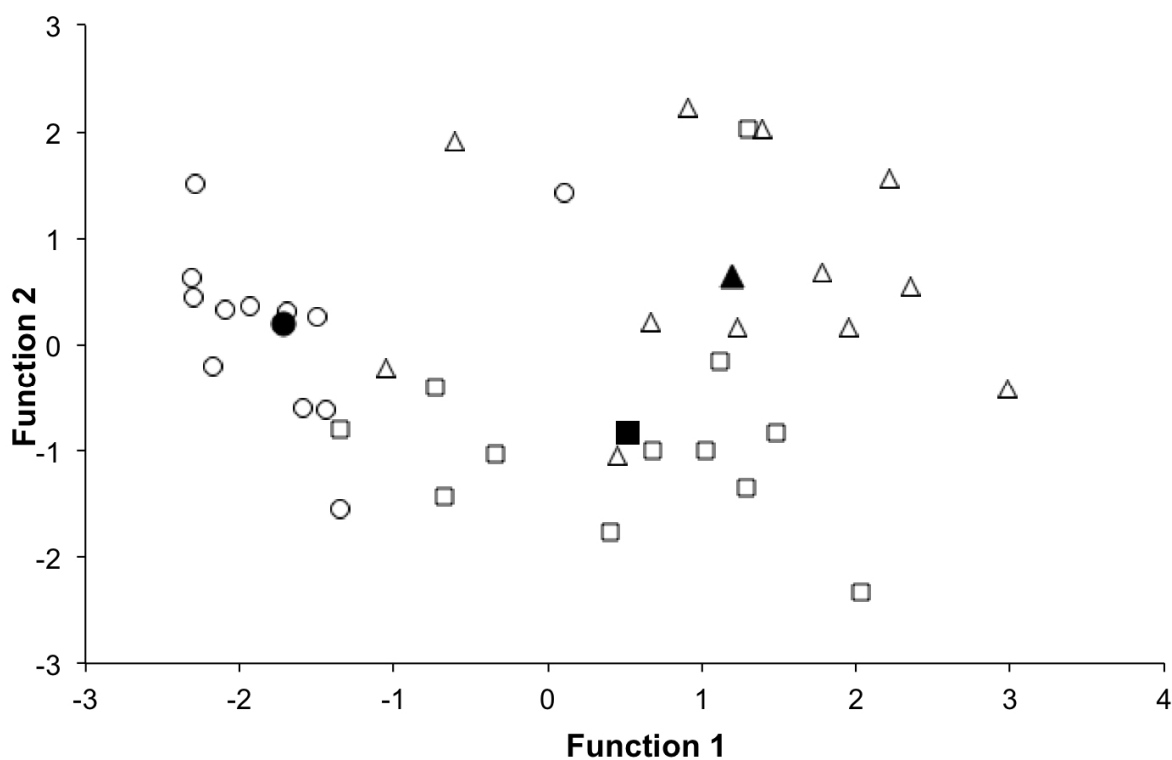


Figure 4. Discriminant scores based on morphological and behavioural variables collected from individuals of three populations of the Jacky dragon (open circles = Cann River, open squares = Royal, open triangles = Yarratt). Solid shapes represent centroids of each population.

Discussion

Our study reveals three main findings. First, genetic, morphological and behavioural variation were evident in the three populations of *A. muricatus*. Second, genetic, morphological and behavioural variation did not covary with populations more closely related also showing greater similarity in morphology but differing in signaling behaviour. Third, males showed similar levels of aggression towards rivals regardless of their population. These results can help to elucidate the complex relationship between genotypic, morphological and behavioural variation.

Interaction of genetics, morphology and behaviour

Patterns of genetic, morphological and signal divergence, although essential for understanding the consequences for processes like speciation, are not commonly addressed together in a single study, especially for motion-based signals. Molecular and morphological divergence is commonly observed in widely-ranging species, with allopatric populations showing higher levels of differentiation (Irwin et al. 2008). Variation in signaling behaviour is more contentious. Both signal lability (Bloch & Irschick 2006) and stability (Lovern et al. 1999) have been reported for species with wide distributions or low gene flow between populations. Genetic, morphological and signal divergence all occur across the sampled range of the Jacky dragon, although in a discordant pattern.

We found that populations that are genetically more closely related (Royal and Yarratt) were also more similar in morphology compared to a genetically more distant population (Cann River). Because the phenotypic characteristics that we measured are used to produce signals (i.e. display morphology), a concordant pattern of variation would reflect more similarities between Royal and Yarratt in the signaling behaviour and different characteristics of the visual displays for animals from Cann

River. However, Cann River and Royal were more similar in both temporal (e.g. latency to display, inter-display interval, body-rock duration) and structural (e.g. descent amplitude) characteristics of the visual displays compared to Yarratt (Figs. 2 and 3). This discordant pattern could be a reflection of behavioural plasticity in this species (Table 1).

Behavioural plasticity can be the result of differences in selective forces across the range of a species (Snell-Rood 2013). Indeed, habitat characteristics have been commonly used to explain geographic differences in the expression of signals between populations of the same species (Leal & Fleishman 2004; Endler et al. 2005; Bloch & Irschick 2006). Previous work on the Jacky dragon (Peters et al. 2007; Peters 2008) and other lizards (Fleishman 1992; Ord et al. 2007) has shown that environmental noise, specifically plant motion, can interfere in the effective transmission of motion-based signals. However, other factors can also be involved in shaping the diversification of signals, such as predation pressure (Stuart-Fox et al. 2003) and differences in density among populations (Bloch & Irschick 2006). For example, by targeting animals performing conspicuous displays, predators can change the display rates observed in a population (Endler 1987). Also, in denser populations the number of displays can increase due to the proximity of individuals and higher encounter rates (Birkhead 1978). Both factors may have an effect on the average phenotype found in a population and therefore, geographical changes of the signaling behaviour might be uncorrelated with changes in display morphology (Wiens 2000).

Interestingly, we found that animals from Yarratt were less willing to display, either aggressively or submissively, took longer to perform the first display and subsequent displays, and overall, their displays were less conspicuous (Table 4). Furthermore, research on variation in habitat characteristics, predation risk and

population density will help to determine the cause of the discordant pattern observed between genetics, morphology and signaling behaviour in the Jacky dragon.

Implications of signal variation

Geographic changes in the signaling behaviour of a species can have significant effects on communication between individuals from different populations. For example, it can be a factor causing reproductive isolation, when signals of dispersing animals are not recognized by local conspecifics, or it can change the preferences of mates, when 'novel' courtship signals are selected. In the case of the Jacky dragon, our study revealed significant geographic variation in signaling behaviour. The variation observed occurred on some temporal and structural characteristics of the core display of the species, which could be affected by changes in environmental conditions. However, the components involved in the displays and the sequence of these components were still highly stereotyped across populations, revealing that these signals must have an underlying genetic basis. Therefore, the signaling behaviour of the Jacky dragon is another example of a species retaining an ancestral motor pattern that has been modified along the range of the species possibly by behavioural plasticity (Foster 1999).

Despite the strong effect of selection as a driver of signal divergence in many species, reproductive isolation and changes in mate preferences can be caused by other factors, such as adaptive differentiation in morphology. For example, Jacky dragons flick their tails as an introductory signal to get the attention of conspecifics. Differences in tail length were found among our study populations, but these differences could simply be the result of selection acting on habitat use, for instance, and not on favour of a more effective communication within each population. Two

questions emerge: 1) to what extent modifications in signaling behaviour will affect communication among conspecifics from different populations? and 2) are differences in morphology stronger than those of signaling behaviour as a cause of population divergence?

Our results show that despite population differences in display behaviour, similar levels of aggression occur between individuals of the same and different populations. This suggests that the species retains cohesion in terms of communication. Also, we found a similar pattern of population divergence when morphological and behavioural traits were considered together compared to the pattern when morphology was considered alone (Fig. 4). This indicates that differences in morphology might have a stronger influence in the direction of selection.

In summary, divergence in signaling behaviour has been commonly invoked as an important factor causing reproductive isolation and speciation. However, it is important to quantify the interaction between genetics, morphology and signaling behaviour to understand the extent to which signal diversification might cause reproductive isolation. We have demonstrated that the display behaviour of the Jacky dragon is quite labile across its range and that behavioural plasticity might be the cause of this variation. Yet factors other than morphology and genetics might have an important role shaping the divergence of signaling behaviour in this species.

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Supplementary material

Table S1. Mean \pm SD of all morphological characters measured for three populations (Cann River $n = 35$, Royal $n = 29$, Yarratt $n = 20$) of the Jacky dragon, *Amphibolurus muricatus*. Minimum and maximum values are included in parentheses.

Character	Cann River	Royal	Yarratt
SVL	80.23 \pm 10.97 (61.5-106)	87.69 \pm 12.41 (66-108.5)	87.23 \pm 8.06 (76-108)
Tail	169.91 \pm 21.08 (135-223.5)	195.91 \pm 28.58 (98-257)	204.35 \pm 20.35 (157.5-257)
Interlimb	40.78 \pm 5.61 (30.9-51.23)	44.38 \pm 6.03 (34.6-55.69)	43.95 \pm 5.21 (36.64-58.89)
Head length	23.43 \pm 2.86 (18.83-31.28)	26.50 \pm 3.50 (20.94-33.2)	26.13 \pm 2.27 (22.64-30.59)
Head width	17.41 \pm 1.86 (14.32-22.84)	18.59 \pm 2.49 (14.45-22.77)	17.98 \pm 1.76 (14.84-21.2)
Head height	11.30 \pm 1.52 (9.26-15.05)	12.11 \pm 1.89 (8.83-15.33)	12.34 \pm 1.25 (10.11-15.01)
Humerus	11.52 \pm 1.31 (9.11-14.42)	12.85 \pm 1.56 (10.34-15.87)	12.83 \pm 1.05 (11.4-14.91)
Radius	10.93 \pm 1.24 (8.56-14.25)	12.36 \pm 1.47 (9.98-15.43)	12.32 \pm 1.14 (10.73-14.66)
Forelimb	22.54 \pm 4.85 (15-32)	25.86 \pm 5.32 (17.5-36.5)	26.80 \pm 3.18 (22.5-35)
Femur	16.88 \pm 1.96 (13.44-20.95)	19.02 \pm 2.36 (15.07-23.32)	19.03 \pm 1.81 (16.56-23.05)
Fibula	18.33 \pm 2.16 (14.78-23.05)	20.96 \pm 2.49 (16.87-25.77)	20.81 \pm 1.77 (17.83-24.73)
Hindlimb	37.46 \pm 4.43 (29.5-46)	42.79 \pm 5.06 (34.5-52)	43.85 \pm 3.50 (38.5-51)
Mass	19.83 \pm 7.77 (10.1-43.4)	24.42 \pm 9.71 (10.1-44.7)	22.89 \pm 7.26 (13.5-42.2)
Femoral pores	8.12 \pm 1.61 (4-11)	5.52 \pm 1.09 (4-8)	5.65 \pm 1.14 (4-8)
Anal pores	4.74 \pm 1.11 (4-9)	4.17 \pm 0.54 (4-6)	4.20 \pm 0.89 (2-6)

CHAPTER 2

Signal repertoire and contest outcome in the Jacky dragon

Marco D. Barquero and Martin J. Whiting



Abstract. Establishing dominance is an important mechanism for determining access to resources based on physical size, physiological performance, and the behaviour of the individuals involved. However, levels of aggression and dominance may vary among populations based on history and variation in individual traits. Here, we use an agamid lizard from Australia, the Jacky dragon (*Amphibolurus muricatus*), to quantify variation in traits influencing the structure of a dominance hierarchy among males of different populations. We measured morphology, physiological performance capacity (sprint speed, endurance, bite force) and visual displays during staged encounters. We found that: 1) morphology, performance and visual displays used during agonistic interactions varied significantly across populations; 2) one population was dominant over the others based on behavioural attributes; and 3) different rules among populations seem to govern the outcome of contests and hence the potential of individuals to become dominant. We discuss these results based on the possible consequences for dispersing animals contacting new populations with differences in signal form and function.

Dominance can be established within a population through direct contact, such as physical fighting, or indirect interactions, such as threatening displays (Kaufmann 1983). Once dominance is established, it is assumed that direct contact is commonly reduced, whereas dominant individuals must keep using signals (e.g. odours, sounds, visual displays) to maintain their status (Goessmann et al. 2000). Although becoming a dominant individual and retaining that status can be costly (Olsson 1992), the benefits (i.e. access to resources) must out-weigh the costs in order to sustain the system (Baird & Sloan 2003). Several studies have explored the effect of the various factors that can affect the establishment and maintenance of dominance in a population, such as differences between individuals in genetic, morphological, physiological and behavioural traits (Kaufmann 1983; Swain & Riddell 1990; Goessmann et al. 2000; Baird & Sloan 2003). For example, Jonart et al. (2007) found that body size, body condition and motivation had a significant impact on the initiation and outcome of contests in the house finch, *Carpodacus mexicanus*.

Wide-ranging species may experience different environmental conditions, habitat and predators, which may shape key features of individuals in different populations (Forsgren et al. 1996; Herrel et al. 2002; Bidau et al. 2011). Since the outcome of a contest may be dependent on physiological (e.g. stamina, strength, speed), the physical attributes (e.g. body size, colour, weaponry) of an individual, and the type and intensity of displays, we might predict population differences in how dominance and social hierarchies are determined (Carpenter 1995; Molina-Borja et al. 1998; Goessmann et al. 2000; Husak et al. 2006). If this is the case, larger body size, a common predictor of contest outcome, could be favoured in some populations. For example, Forsgren et al. (1996) determined that differences in nest-site availability between two populations of sand gobies (*Pomatoschistus minutus*)

led to differences in intrasexual selection, with larger males being favoured only in the population with higher male-male competition.

Thus, a question arises: what happens when individuals from populations with different hierarchical systems come into contact? The answer to this question is crucial for understanding the probability of dispersing animals establishing themselves in a new environment and attaining reproductive success. Three potential results for a dispersing animal emerge: 1) the individual fails to settle in the new population; 2) the individual succeeds in settling in the new population but with low reproductive success; and 3) the individual succeeds in settling in the new population and increases its reproductive success.

The first scenario could be a consequence of a dispersing animal having morphological and/or behavioural traits (e.g. visual displays) that are not recognized by resident individuals (both males and females) of the new population and thus reproductive isolation occurs (Massot et al. 1994). The second result could occur when the dispersing individual integrates into the new population but as a subordinate. Thus, an animal that was dominant in its population of origin can become a subordinate if arrives at a population with different social drivers (Dunbrack & Clarke 2003). The last result happens when the dispersing animal carries features that confers it an advantage in the new populations, such that the individual is more likely to be dominant or females tend to prefer it (Robertson & Rosenblum 2010). Hence, the type and intensity of the signals used during agonistic interactions can be of major importance to determine the outcome of a contest, the social system in a population, and the fate of dispersing animals.

A growing body of literature on lizards has demonstrated the links between social dominance and morphology (Whiting et al. 2006; Sacchi et al. 2009), performance (Robson & Miles 2000; Husak et al. 2006) and display behaviour

(Carpenter et al. 1970; Molina-Borja et al. 1998). For example, Lailvaux et al. (2004) found a positive relationship between body size, head size and bite force in males of *Anolis carolinensis*, so that larger males with high bite forces also won more size-matched contests.

The Jacky dragon (*Amphibolurus muricatus*) is a relatively abundant agamid lizard that occupies a range of habitats over a distribution of > 2,000 km along the southeastern coast of Australia (Cogger 2000). Hatchling Jacky dragons have been shown to disperse relatively long distances (up to 160 m) in a few months (Warner & Shine 2008) and the species is able to quickly recolonize and thrive in altered habitats (e.g. post-fire or logged forests) (Letnic & Fox 1997). Therefore, the chances that animals from different populations converge in neutral habitats could be high. In addition, the signaling behaviour of the species has been extensively studied (Carpenter et al. 1970; Peters & Ord 2003; Watt & Joss 2003) and two behaviour types during agonistic interactions have been described: an aggressive display and a submissive display. The former involves five movements performed in the following order: tail-flick, backward arm-wave, forward arm-wave, push-up and body-rock (Peters & Ord 2003). The submissive displays include a slow circumduction of one of the forelegs (Carpenter et al. 1970) and some authors have also mentioned a head-bow (Ord et al. 2002; Watt & Joss 2003; Van Dyk et al. 2007). However, the signal repertoire could include other displays that have been reported for other agamids (Carpenter et al. 1970) and that could be used to settle the outcome of contests between individuals.

Here, we use the Jacky dragon to determine whether: 1) morphology and physiological performance capacity predicts contest outcome; 2) the rules used to establish dominance and hierarchy formation are the same for all populations; and 3) individuals retain the rank obtained in their population of origin when confronted with

animals from different populations. We built upon recent research that has revealed differences among populations of the Jacky dragon in morphological traits and the properties of signaling behaviour (Chapter 1). We first compared morphology and performance in the lab for males from three populations. Then we staged contests among these individuals in a neutral arena to quantify the signaling behaviour, identify winners and losers and determine the best predictors of contest outcome. We also include a description of signals not previously mentioned in the literature, so that we can use these signals for population comparisons. Finally, we establish the relationship of signal use among winners and losers and the resulting hierarchies of each population.

Methods

Study populations and captive animals

We visited three sites along the range of the species: Yarratt state forest (31°48'17.4" S, 152°25'57.5" E), Royal National Park (34°04'49.7" S, 151°05'39.6" E), and Cann River state forest (37°36'25.9" S, 149°09'05.8" E), henceforth referred to as Yarratt, Royal and Cann River respectively. Ten to fifteen males were collected from each population and transported to Macquarie University, where they were housed individually in outdoor enclosures (180 L x 90 W x 88 H cm). Enclosures were made from metal sheeting and consisted of a sand substrate, branches suitable for basking, screen for sun cover, and plastic containers as refuges and water receptacles. Individuals were kept under these conditions for several months prior to the commencement of the trials.

Morphology and performance

The following morphological measurements were collected when animals arrived in the lab and prior to the trials: snout-vent length (SVL), head length, width and height, forelimb length, and hindlimb length. All morphometrics, except head width, were taken on the right side of the animal. SVL and limb lengths were measured with a ruler (to the nearest 0.5 mm) and the remaining measurements with a digital caliper (to the nearest 0.1 mm).

The day before performance trials, we moved lizards to indoor facilities and kept them in a temperature-controlled room at 25 °C. Thirty-six lizards were used during these trials (Cann River: $n = 13$, Royal: $n = 12$, Yarratt: $n = 11$). Thirty minutes before trials, lizards were placed in plastic bags and moved into a large container with water kept at a constant temperature of 36 °C. This temperature allowed the lizards to attain the preferred body temperature of the species (ca. 33 °C; Heatwole et al. 1973). Three measurements were collected for each individual: sprint speed, endurance and bite force. Sprint speed was determined by placing an individual on a 1.75 m racetrack with a rubber substrate. Each animal was gently tapped on the tail base with a brush and the time spent to complete the full length of the racetrack was recorded. Sprint speed for the total length of the racetrack was then calculated. Endurance was measured by placing a lizard on a treadmill at a constant speed of 0.33 m/s. When a lizard was exhausted (i.e. unable to move or respond to taps from the researchers), it was removed from the treadmill and the total time spent moving on the treadmill was recorded. Bite force was measured by placing an individual in front of the plastic plates of a Kistler bite force meter (Kistler Inc., Winterthur Switzerland) and encouraging it to bite them. Three trials were performed for each animal and the hardest bite was selected. Each lizard was measured once per day and three times in total for all three performance measures over consecutive days.

The highest score for each measurement was used for each individual in subsequent analyses (Losos et al. 2002).

Male-male contests

We staged male-male encounters using two contest types: animals from the same population (intra-population) and from different populations (inter-population). We first determined the winners and losers from intra-population contests to establish a hierarchy for each population. Two males were transferred to a neutral, outdoor arena (180 L x 180 W x 88 H cm) similar to the enclosures described above and their interactions were filmed for up to 90 min (see Chapter 1 for details). In order to identify winners and losers, we first selected the winner of a contest, so that the loser was established by default. An individual was declared the winner of a contest if it chased the other animal, performed aggressive displays or showed an aggressive behaviour after a fight. Contests for which no agonistic interaction occurred or no clear winner could be established were declared as 'no outcome' and 'draw' respectively. Each individual participated in 1-4 contests for a total of 60 contests (contests per population: Cann River = 18, Royal = 20, Yarratt = 22), but only those that participated in more than one contest were considered for social dominance analyses. We used the following scoring system to establish a hierarchy for each population: 3 points for a contest won, 1 point for a draw, 0 points for a contest with no outcome and -1 point for a contest lost. Individuals with a final score of 3 or more points were designated as dominants, the rest were considered subordinates.

We also staged inter-population contests and followed the same criteria mentioned above to classify winners and losers and to establish an overall hierarchy including individuals from all three populations. Each individual had two contests, facing an opponent from each of the other two populations each time. Some

individuals used during the intra-population contests were not in sufficient body condition when the inter-population contests were carried out, so that we only used 11 animals per population for the latter. Most encounters were size-matched (60% of intra-population and 56% of inter-population), that is when the difference in SVL between combatants represented $<6\%$ of the body size of each individual.

Signal repertoire

The videos generated from the male-male contests (both intra- and inter-population) were used to review the signal repertoire of the species. The footage was analyzed using JWatcher Video version 1.0 as an event recorder program (Blumstein & Daniel 2007). We scored the visual displays reported previously for the species (i.e. tail-flicks, push-ups and body rocks, and submissive displays). We also accounted for any new display that could be used as a signal directed to conspecifics. Because of the way the camcorder was placed (1 m away to the closest point inside the arena) and that no zoom was possible to film both lizards at the same time, some of the displays were not quantified for comparison purposes and only a description of the behaviour is mentioned. Whenever possible, we counted the number of displays performed by each individual.

Statistical analyses

We determined whether differences in morphology and performance involving the individuals used during male-male contests occurred among populations. We first removed the effect of size by regressing each morphological variable against SVL and then used the residuals. Performance variables were log-transformed to improve normality. We checked for relationships between relative morphological and performance variables using Pearson's correlations. We then tested whether

differences in morphology occur across populations using a MANOVA, with relative morphological variables as the dependent variables and population as a fixed factor. We used a MANCOVA to test for differences among populations in performance, using population as a fixed effect and SVL as a covariate.

We used the Bradley-Terry model to identify the best variables predicting the outcome of a contest based on the fighting abilities of combatants. Specifically, we examined the potential role of morphology (SVL, head length, head width, head height, forelimb length, hindlimb length) and maximal performance capacity (bite force, endurance, sprint speed). The Bradley-Terry model is a form of generalized linear model that has been used previously in other studies involving lizards and incomplete matrices of interactions between individuals (Whiting et al. 2006). We followed a stepwise procedure to select the best model, initially running a 'full model' (i.e. considering all predictor variables using the original raw variables, but excluding population) and then removing the worst predictor until the remaining variables had $P < 0.10$. The remaining predictors were compared among each other based on standardized coefficients. We also ran a separate model including population as the only predictor. The Bradley-Terry models were carried out using the BradleyTerry2 package for R (Turner & Firth 2012). All other analyses were done using SPSS Statistics version 20.0 (IBM©).

Results

Morphology, performance and contest outcome

Morphological variables were highly correlated with each other (all Pearson's $P < 0.003$). Bite force was positively correlated with SVL ($P < 0.001$), relative head length ($P = 0.007$) and relative head height ($P = 0.013$), whereas maximum sprint speed was positively correlated with relative hindlimb length ($P = 0.009$). No other relationship was significant. When morphology and performance were compared across populations, we found significant differences in relative head length, relative hindlimb length, bite force and sprint speed (Table 1). Compared the other two populations, lizards from Cann River had significantly smaller morphological traits and lower performance capacities, even body size was controlled for.

Table 1. MANOVA for morphological variables and a MANCOVA for performance variables comparing three populations of the Jacky dragon, *Amphibolurus muricatus*.

Variable	F	P
<i>Morphology</i>		
SVL	1.53	0.233
Relative Head length	10.90	< 0.001
Relative Head width	0.99	0.384
Relative Head height	0.86	0.432
Relative Forelimb length	0.69	0.508
Relative Hindlimb length	15.67	< 0.001
<i>Performance</i>		
Log (Maximum Bite force)	8.14	0.001
Log (Maximum Endurance)	1.40	0.260
Log (Maximum Sprint speed)	3.69	0.036

A high proportion of intra-population (29/60, 48.3%) and inter-population (10/34, 29.4%) contests produced no outcome and only 2 draws were recorded (one for each contest type) (Table 2). From the contests with a clear winner and loser ($n = 53$), the Bradley-Terry model selected four variables as the best predictors of contest outcome (Table 3). The strongest predictor was hindlimb length, although the relation was negative. Larger males with higher bite force were predicted to win more contests.

Table 2. Number of individuals used during male-male encounters according to the outcome of intra- (marked with *) and inter-population contests. See text for total number of animals used and total number of contests per population.

Population	Outcome	Population of rival		
		Cann River	Royal	Yarratt
Cann River	Winners	13*	7	6
	Losers	13*	4	1
	Draw	2*	0	1
	No outcome	8*	1	4
Royal	Winners	4	12*	3
	Losers	7	12*	2
	Draw	0	0*	0
	No outcome	1	20*	5
Yarratt	Winners	1	2	5*
	Losers	6	3	5*
	Draw	1	0	0*
	No outcome	4	5	30*

Description of signals

Signals that have not been described previously for the Jacky dragon can be classified as motion-based behaviours and postures (also known as modifiers). The former includes:

Dorsal-grasp: An animal grasps another individual by the back using both forelimbs. The grip can be done from the waist or another part of the trunk. The grasping animal can hold the grip for up to 10 s, while the grasped animal can try to escape by running or it can remain still.

Gaping: Mouth is partially or fully opened, exposing the bright yellow colouration of the lining and tongue. The opening and closing of the mouth can be fast (1-3 s) or the mouth can be kept open for several minutes. Not quantified in this study.

In the case of postures, we observed:

Inflation of trunk: Inflation occurs when the air is retained to increase the size of the body trunk. Not quantified in this study.

Tail-lift: Tail can be elevated in two ways: curled-up or straight. It can be maintained in either position for several minutes and even during locomotion bouts.

Signaling behaviour and contest outcome

During both intra- and inter-population contests, winners performed the vast majority of aggressive displays (tail-flicks and push-up/body-rocks) in all populations (Fig. 1), although an increase in the number of displays was recorded only for winners from Cann River during inter-population contests (Figs. 1c and 1d). Conversely, losers from all populations performed most of the submissive displays (Fig. 2). Animals from Royal and Yarratt performed most of the head-bows in both contest types (Figs. 2a and 2c), whereas slow circumductions were more commonly

observed in Cann River and Royal during intra-population contests (Fig. 2b) and more common in Royal and Yarratt during inter-population contests (Fig. 2d).

Table 3. Results of two Bradley-Terry models explaining contest outcome. The first model included the morphological and performance variables and excluded population. The results show only the best predictors obtained from a stepwise procedure. The second model included only population as a predictor. S.E. = standard error.

Predictor	Coefficient	S.E.	Z	P	Standardized coefficient
<i>Population absent model</i>					
SVL	0.29	0.140	2.090	0.037	1.98
Hindlimb length	-0.76	0.297	-2.571	0.010	-2.94
Bite force	0.27	0.132	2.015	0.044	1.72
Endurance	-0.01	0.007	-1.911	0.056	-0.92
<i>Population only model</i>					
Cann River (vs Yarratt)	1.62	0.966	1.676	0.094	
Royal (vs Cann River)	-0.73	0.757	-0.958	0.338	
Yarratt (vs Royal)	-0.89	0.955	-0.935	0.350	

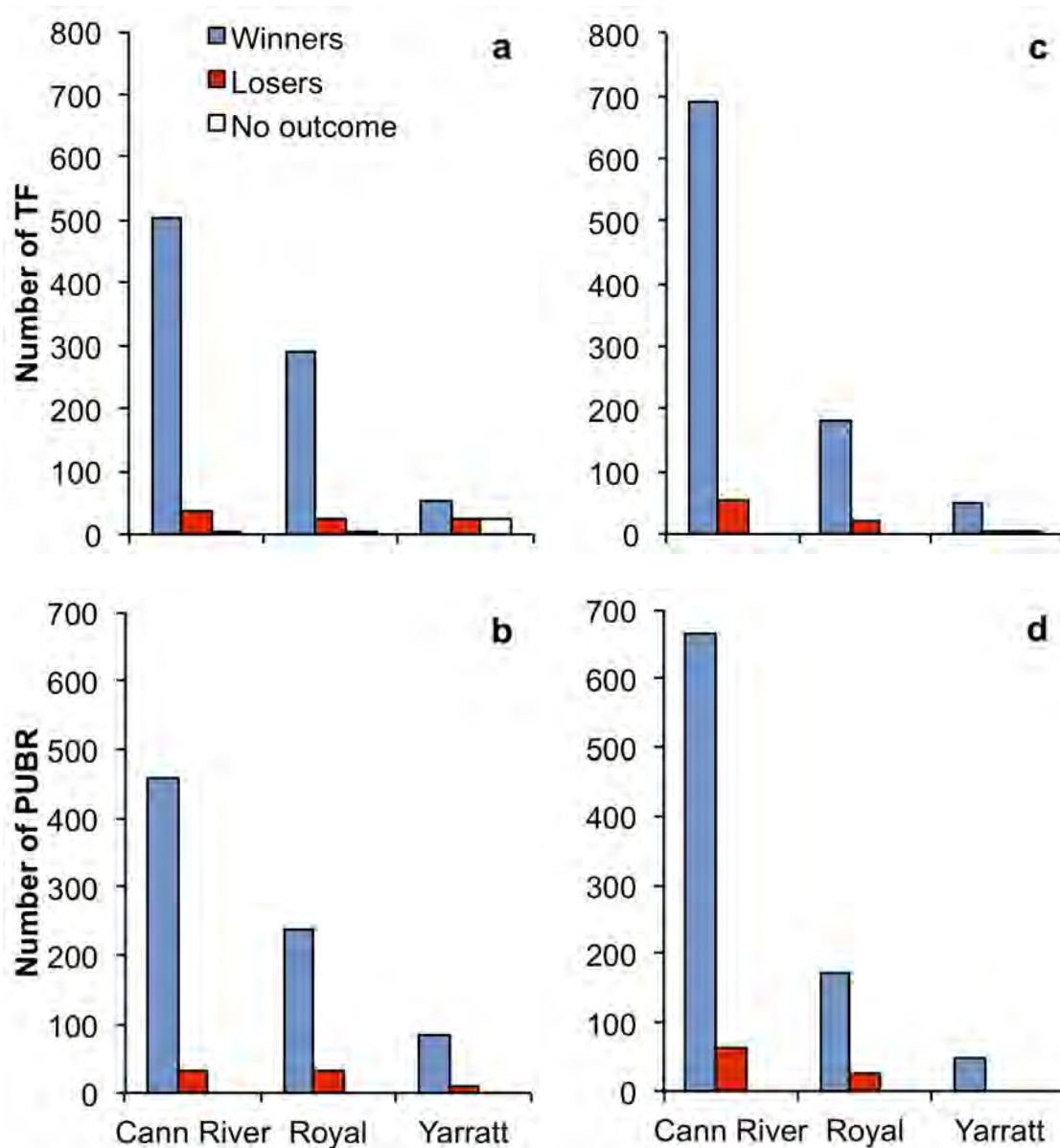


Figure 1. Number of tail-flicks [TF] (a and c) and push-up/body-rock [PUBR] displays (b and d) as aggressive displays recorded during intra-population (a and b) and inter-population contests (c and d).

Bite-lunges and chases occurred in both intra- and inter-population contests. Both losers (N: Cann River = 14, Royal 13, Yarratt = 10) and winners (N: Cann River = 18, Royal 12, Yarratt = 15) from all populations attempted to bite their opponents during intra-population contests, but the number of bite-lunges of losers from Royal (N = 5) and Yarratt (N = 0) decreased during inter-population ones. Only winners of all populations and in both contest types performed chases. The number of chases performed by individuals from Cann River increased in inter-population encounters (N = 0) compared to intra-population ones (N = 34), whereas the opposite occurred for animals from Royal (N for intra-population = 40, N for inter-population = 9).

New signals described here varied among populations and among contest type. In intra-population contests, lizards from Royal and Yarratt lifted their tails more often than those from Cann River (Fig. 3a) and only winners from Cann River and Royal performed dorsal-grasps (Fig. 3b). In inter-population contests, tail-lifts were still performed more frequently in Royal and Yarratt, but the number of these displays increased for winners from Cann River and Royal compared to intra-population contests (Fig. 3c). Winners from all three populations performed dorsal-grasps, although losers from Royal also performed this behaviour and the number of displays decreased in Cann River compared to intra-population contests (Fig. 3d).

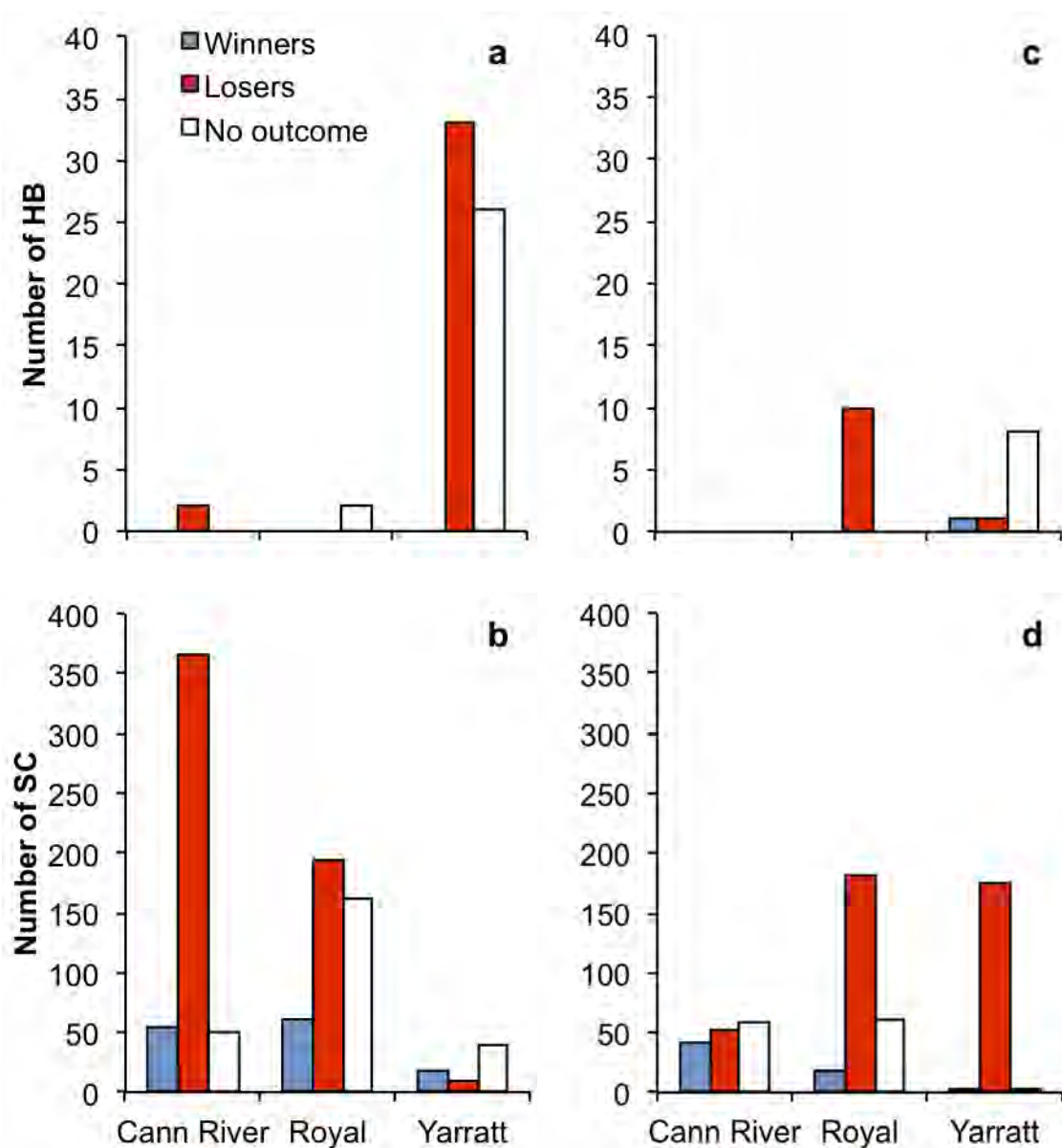


Figure 2. Number of head bows [HB] (a and c) and slow circumductions [SC] (b and d) as bluffing and submissive displays respectively, observed during intra-population (a and b) and inter-population contests (c and d).

Hierarchies

A total of 14 dominant individuals (Cann River = 4, Royal = 6, Yarratt = 4) and 18 subordinates (Cann River = 6, Royal = 5, Yarratt = 7) were identified during intra-population contests (Table 4). After inter-population contests, all subordinates from Yarratt retained this status and 2 dominants were downgraded (Table 4). In Royal, 2 subordinates became dominants and 4 dominants became subordinates (Table 4). In Cann River, 3 subordinates became dominants and only 1 dominant was downgraded (Table 4).

Table 4. Number of individuals distributed according to the social rank obtained during intra- and inter-population contests. The number of individuals classified after intra-population contests are shown in the first column (N), whereas the following columns show the classification after inter-population contests. For example, six lizards from Cann River were classified as subordinates after intra-population contests, of which three became dominant and three remained as subordinates after inter-population contests.

Population	Intra-population dominance	N	Inter-population dominance	
			Dominant	Subordinate
Cann River	Dominant	4	3	1
	Subordinate	6	3	3
Royal	Dominant	6	2	4
	Subordinate	5	2	3
Yarratt	Dominant	4	2	2
	Subordinate	7	0	7
Total		32	12	20

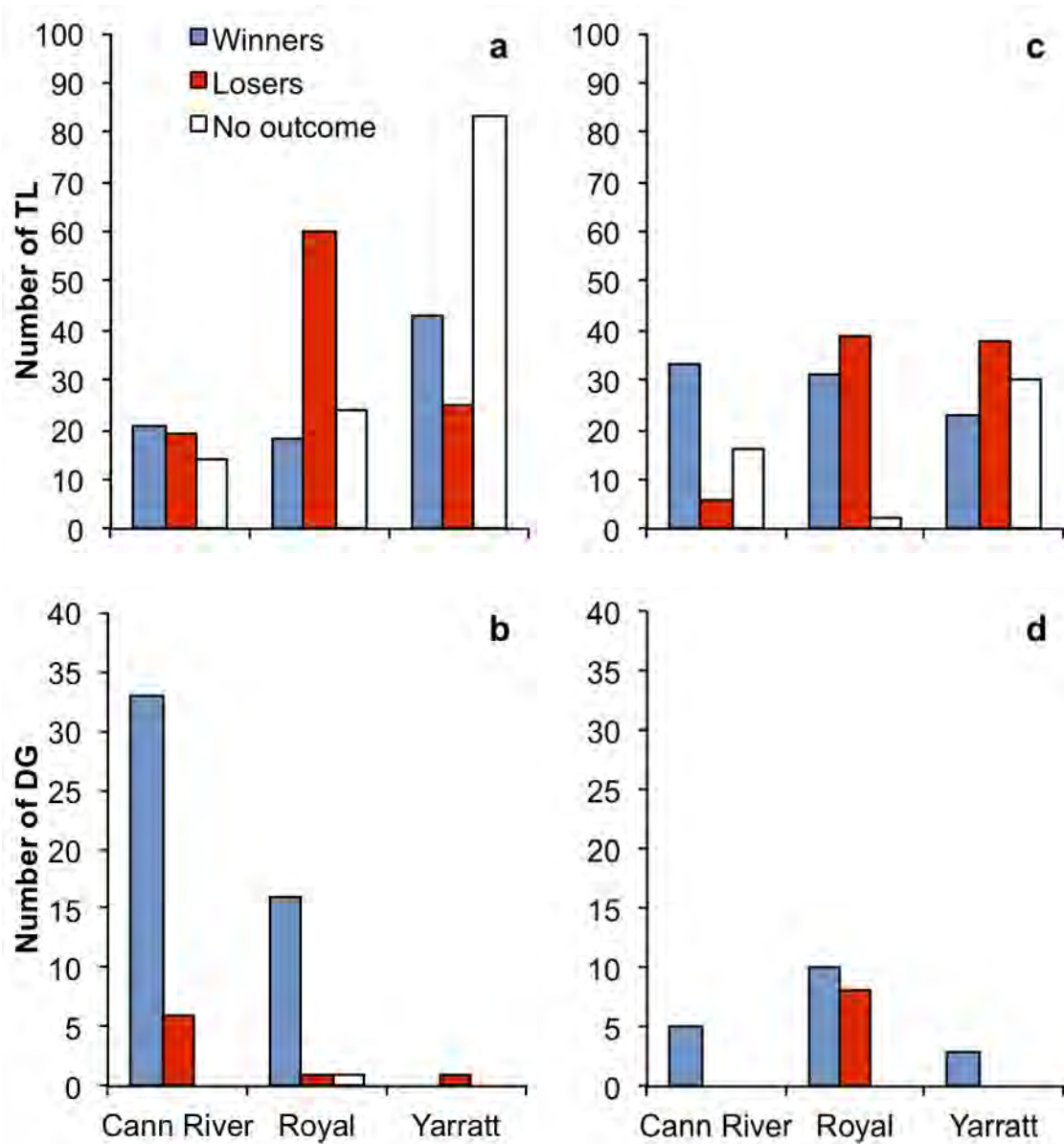


Figure 3. Number of tail-lifts [TL] (a and c) and dorsal-grasps [DG] (b and d) as new behaviours performed during intra-population (a and b) and inter-population contests (c and d).

Discussion

Predictors of contest outcome

Several studies have disentangled the relationships between morphology, maximal performance capacities of animals and the social structure in a population (Robson & Miles 2000; Husak et al. 2006; Whiting et al. 2006; Sacchi et al. 2009). In general, the attributes of an individual serve as honest signals of fighting ability (Tokarz 1985; Hews 1990). We found that two morphological and two performance traits of male Jacky dragons, *Amphibolurus muricatus* that predicted the outcome of contests (Table 3). Our first Bradley-Terry model showed that animals with larger body size and greater bite force won more contests, whereas those with longer hindlimbs and greater endurance lost more. Morphological features and performance capacities of contestants differed across populations (Table 1). Lizards from Cann River having smaller hindlimbs, smaller head lengths, weaker bite force and running more slowly than individuals from Royal and Yarratt. These results suggest that having a longer hindlimb and hence being faster are not good attributes to win a contest for Jacky dragons, but having a longer head and thus biting harder might be.

A second Bradley-Terry model including population as the only predictor, although not fully significant, suggests that the probability of winning a contest increases for animals from Cann River (Table 3). Therefore, it is intriguing that lizards from this population, despite not having the best morphological and performance attributes, could win a contest when confronted with conspecifics from other populations. This apparent discrepancy can have an explanation when the signaling behaviour is examined.

Revised signal repertoire

Two motion-based signals of the signal repertoire of the Jacky dragon have received most of the attention: the typical aggressive display and the submissive circumduction (Ord & Evans 2003; Peters & Evans 2003; Woo & Rieucan 2012). However, the repertoire of the species include some less commonly investigated motion-based behaviours and postures, some of which have been quantified previously (Ord et al. 2002; Watt & Joss 2003; Van Dyk & Evans 2007, 2008; Van Dyk et al. 2007; Warner et al 2010). These include head-bows, lateral body compression, throat extension and crest erection, although we only quantified head-bows. This behaviour has been assumed to be submissive (see Tokarz 1985 for *Anolis sagrei*); however, some of the displays we observed seemed to have an element of aggressiveness, as they were executed with the mouth opened and the body fully raised by all four limbs. Brattstrom (1971) described an aggressive bow down display for *Pogona barbata* (formerly *Amphibolurus barbatus*) similar to the head-bow observed for the Jacky dragon. We suspect that this display might have a dual function depending on the modifiers included, so that it could be not only a submissive display but also serve as a way of bluffing to avoid the attack of an opponent.

In this study we also report two new behaviours (dorsal-grasp and gaping) and two new postures (tail-lift and inflation of trunk) not previously described for the species and that were used during agonistic interactions. Because we were not able to quantify gaping and inflation of trunk we make no further comments on these displays. Tail-lift is a common display in many species of lizards and it is thought to function during predator-prey interactions as a distraction (Downes & Shine 1998) or pursuit-deterrent signal to predators (Cooper 2001) and during courtship as a signal of receptivity to males by females (Radder et al. 2006). We speculate that Jacky

dragons use this display during agonistic interactions among males potentially as a rejection signal to get involved in aggressive behaviour. The dorsal-grasp is a more atypical behaviour. Although a neck-grip is commonly mentioned as a behaviour used by males during courtship and copulation to hold on females (Greenberg 2003), a dorsal-grasp used during male-male encounters has rarely been mentioned. Carpenter (1960, 1963) described a similar behaviour for males of the six-lined racerunner (*Cnemidophorus sexlineatus*) and males of fringe-toed lizards (genus *Uma*). We agree with Carpenter that this is an aggressive behaviour used to establish social dominance.

Signals, contest outcome and population variation

Our results show that variation occurs in the frequency of each signal across populations and according to the outcome of contests. Losers from Cann River performed fewer submissive displays (slow circumduction) during inter-population contests compared to intra-population ones (Fig. 2), whereas winners from this population increased the number of typical aggressive displays (tail-flicks and push-up/body-rocks) (Fig. 1). The opposite occurred for individuals from the other two populations, so that losers performed more submissive displays and winners fewer aggressive ones. This suggests that individuals from Cann River are more aggressive than those from the other populations. This idea is reinforced by the frequency of bite-lunges, an aggressive behaviour that occurred only during escalated fighting. During intra-population encounters, similar numbers of bite-lunges occurred for both winners and losers of all populations, but a decrease was recorded for losers from Royal and Yarratt during inter-population encounters. Robertson & Rosenblum (2010) also found differences in the levels of aggressiveness of *Sceloporus undulatus* towards conspecifics from the same (ecologically similar) and

different (ecologically dissimilar) populations, with one population being more aggressive than the other in both intra- and inter-population encounters.

When other aggressive behaviours are considered, some changes to the above-mentioned pattern emerge. For example, the number of chases increased in Cann River and decreased in Royal during inter-population encounters compared to intra-population ones. This suggests that individuals could be discriminating conspecifics from their own and different populations and using the most appropriate behaviour accordingly. The results for the dorsal-grasp described here seem to support this idea. For instance, individuals from Cann River used this behaviour frequently during intra-population contests but not so much during inter-population ones (Fig. 3). Previous research on the Jacky dragon has shown that individuals are able to discriminate between familiar and unfamiliar intruders using morphological cues (Van Dyk & Evans 2007). However, we go further and show that the behavioural responses during male-male encounters also change depending on the population of origin of each animal.

Finally, it seems that individuals from Yarratt use less aggressive behaviours to settle encounters with conspecifics. For example, animals from this population performed almost all the head bows and a great number of tail-lifts observed during intra-population encounters, including a high proportion of displays in contests where no clear winner or loser could be determined (Fig. 3). Although present, these two signals were less common for lizards from Cann River and Royal. Therefore, the fact that differences among populations exist in the levels of aggressiveness and in the frequency and context in which certain signals are used indicates that animals from each population follow different rules to establish dominance.

Intra- and inter-population dominance

Our presumption that one population, Cann River, is more aggressive than the others is supported when a hierarchy is considered. We observed similar numbers of dominants and subordinates within each population after intra-population contests. However, more dominant animals from Royal and Yarratt became subordinates after inter-population encounters than those from Cann River. Conversely, more individuals from this last population that were classified as subordinates after intra-population contests became dominant after inter-population ones (Table 4). This result is intriguing since animals from Cann River were in comparison smaller and poorer performers than those from Royal and Yarratt, suggesting that the signalling behaviour has a stronger impact on determining the outcome of an encounter between individuals from separate populations than other cues (e.g. morphology).

In addition, based on the use of signals and behaviours across contest types, we consider that each population has a different system to establish dominance. Males from Cann River seem to decide the fate of contests according to the intensity and frequency of motion-based, aggressive signals and direct contact, whereas individuals from Yarratt seem to use less conspicuous (e.g. postures) and less aggressive (e.g. bluffing) displays. A mix of these systems seem to occur in Royal, where individuals used both aggressive behaviours and postures. Therefore, it could be problematic for dispersing animals from any of these populations to get settled somewhere else.

In conclusion, our study demonstrates that variation exists in the physical features, capacities and signals of animals across populations of the Jacky dragon and this variation influences contest outcome. Reproductive isolation could be a consequence for dispersing animals arriving at populations where these rules change drastically. Future research should focus on dispersing animals interacting with novel

populations differing in their communication system and not only on morphological and ecological characteristics.

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CHAPTER 3

Influence of habitat on signal structure of the Jacky dragon

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Abstract. Selection of appropriate habitat can have a significant bearing on fitness because of its link to morphology, performance and communication. Visual displays of lizards performed during communication with conspecifics are particularly affected by factors such as habitat light and habitat structure. We tested whether the structure and complexity of the habitat varied within the range of the Jacky dragon, *Amphibolurus muricatus*, and whether this variation has an influence on behavioural (introductory signals and aggressive visual displays), ecological (perch height) and morphological (limb and tail lengths) traits. We sampled three sites to collect data on the characteristics of the habitat and measure habitat use and morphology of lizards. We also staged male-male contests with individuals from the populations sampled to record their visual displays and measured their sprint speed in the lab. We found significant differences in habitat complexity between sites. Lizards from populations sampled differed significantly in morphology, sprint speed and display behaviour, but in habitat use. Overall, the geographic pattern of habitat variation was discordant with that of both behavioural and ecological variation, suggesting that other selective pressures might have an effect on the variation of the signaling behaviour of this species.

The habitat an organism selects permeates to almost every facet of its biology including finding food, locating a mate, locating shelter, maintaining homeostasis, and communicating with conspecifics. There is also a relatively tight link between an animal's morphology, its mode of locomotion, and the degree to which it is active and moves through a particular habitat. An animal's movements are constrained by habitat characteristics (Bragg et al. 2005; Lindenmayer et al. 2008), which might result in adaptive responses to local conditions. Accordingly, locomotion mode changes when animals move through different habitat types (e.g. open vs. closed vegetation) (Pounds 1988), the time to detect prey increases or decreases depending on the number of irrelevant stimuli (e.g. falling leaves) (Endler 1992), and detectability of conspecific signals decreases as a function of the spectral conditions (e.g. high vs low habitat light) (Leal & Fleishman 2004). Species that occupy a number of different habitats are expected to exhibit more variation in their responses to changes in habitat than those restricted to one habitat type.

The specific characteristics of the habitat selected by each population of one species, hence, can profoundly shape the morphological, physiological, ecological and behavioural features of the individuals (Ord et al. 2013). In particular, signals used to communicate with conspecifics, might vary among populations of the same species as a function of the structural characteristics and the conditions of the habitat (Bloch & Irschick 2006; Derryberry 2009; Eaton & Sloman. 2011). If accentuated over time, changes in signal design across populations can eventually lead to reproductive isolation and speciation (Boughman 2002).

Animals that use visual displays for communication are especially affected by the characteristics of the habitat. In order to be effective, a visual signal must be conspicuous and/or performed frequently enough to be detected by a receiver (Boughman 2002). However, environmental characteristics and conditions can

interfere with the transmission of visual signals to conspecifics by degrading the message being conveyed or decreasing signal detectability. The number and type of obstructions present in a habitat (e.g. bushes, logs, tree trunks) and the arrangement of the vegetation (whether continuous or clumped) have been shown to both reduce the detectability of a signal and degrade visual signals over distance (Endler 1992; Peters & Evans 2007; Derryberry 2009). For example, Tobias et al. (2010) found that the degree of attenuation of acoustic signals of Amazonian birds differed between two habitat types with different structural properties, with higher attenuation in the denser habitat. To overcome these problems, animals can modify the structure of signals in conditions that are not conducive to reliable transmission (Ord et al. 2007; Peters et al. 2007; Eaton & Sloman 2011). Other strategies involve signaling only during periods when conditions are appropriate and selecting microhabitats from where transmission of signals is maximized (Endler & Théry 1996; Heindl & Winkler 2003; Amézquita & Hödl 2004; Ord et al. 2011).

Habitat selection, hence, has a major impact on the communication system of populations of the same species, but signal diversification can also be accompanied by changes in other traits also influenced by habitat. Thus, habitat use, morphology and performance (e.g. locomotor abilities and stamina) have been shown to be interacting traits under which selection operates (Irschick et al. 2005; Calsbeek and Irschick 2007; Goodman 2009). Individuals from different populations of the same species using different strategies to communicate with conspecifics, as those mentioned above, are expected to also vary in these traits. For example, Derryberry (2009) found that variation in song structure among populations of *Zonotrichia leucophrys* was correlated with morphological and environmental variation, such that lower-performance trills and slower trill rates were associated with deeper bills and denser vegetation respectively. It is poorly understood, though, whether changes in

display behaviour are due to the direct effect of local environmental characteristics and conditions or they are a by-product of changes in habitat use, morphology and performance.

In lizards in particular, factors such as habitat light, background noise (i.e. environmental cues that interfere with the transmission of signals) and habitat structure (i.e. density and heterogeneity or diversity of structures [Warfe et al. 2008]), have been shown to modify behaviour, morphology and habitat use of several species. For example, differences in habitat light have been used to explain the evolution of colourful body parts for communication (Macedonia et al. 2003; Leal & Fleishman 2004) and crypsis (Macedonia et al. 2004; Stuart-Fox et al. 2004). Background noise from the movement of windblown vegetation is commonly invoked as the cause of variation in motion-based signals (Ord et al. 2007; Peters et al. 2007; Ord & Stamps 2008). Also, habitat structure has been shown to significantly affect detection and the properties of signals (Bloch & Irschick 2006; Peters 2008; Johnson et al. 2010) as well as habitat use (Pounds 1988; Martín & López 1998).

A good organism to test the influence of these selective forces is the Jacky dragon, *Amphibolurus muricatus*, a widely ranging agamid lizard endemic to Australia. The signaling behaviour of this species has been extensively studied (Carpenter et al. 1970; Peters & Ord 2003; Watt & Joss 2003). Males of the species perform an aggressive display that is composed of five movements performed in a ritualistic fashion: tail-flick, backward arm-wave, forward arm-wave, push-up and body-rock. Most research has focused on variation in signaling behaviour as a function of background noise (Peters & Evans 2003, 2007; Peters et al. 2007; Peters 2008; Woo et al. 2009). However, no research has been done with the Jacky dragon to examine the relationships between display behaviour and habitat light and vegetation complexity. A few studies have explored habitat preferences (Letnic & Fox

1997; Bragg et al. 2005; Lindenmayer et al. 2008) and the link between morphology and performance (Warner & Shine 2006), but none have integrated all these factors.

Here we test two hypotheses using three populations: 1) habitat variation (i.e. the structure and complexity of the vegetation and the ambient light) is concordant only with signaling behaviour variation; and 2) habitat variation is concordant only with ecological variation (i.e. habitat use and associated effects on the phenotype and performance). If the first hypothesis is supported, we predict a negative relationship between the degree of complexity and behavioural traits that ensure communication among individuals, whereas a positive relationship between habitat light and behavioural traits is expected. Previous research on other taxa, such as fishes of the genus *Salmo* (Valdimarsson & Metcalfe 2001; Höjesjö et al. 2004), has shown that decreasing light levels and increasing habitat complexity reduce the levels of aggressiveness among males. Therefore, we expect that an increase in complexity and a decrease in ambient light between sites will cause a reduction in the number of displays or in the time displaying. As the tail has been shown to be an important appendage during communication used to perform the introductory component of the display (Peters & Ord 2003; Peters et al. 2007), we expected that tail length would increase as vegetation complexity increases. If the second hypothesis is supported, we predict that a differential habitat use should occur among sites, with an increase in perch height as complexity increases and habitat light decreases. Animals in more complex and less illuminated habitats are less likely to be detected by predators and therefore they should be able to use higher perches. In addition, the more complex the habitat, the more available perches exist. This change in habitat use should be congruent with changes in limb morphology and maximum sprint speed, so that we expect to find faster animals with longer limbs in less complex and more illuminated habitats.

Methods

Study species and sites

The Jacky dragon is a medium-sized (maximum snout-vent length [SVL] = 120 mm) lizard found along the southeastern coast of Australia. The range extends from southeastern Queensland in the north southwards to eastern New South Wales, and westwards through southern Victoria to eastern South Australia (Cogger 2000). Jacky dragons have a yellow-orange mouth and dark (grey to brown) dorsum with two pale (white to yellow) stripes or blotches along the back. They also have a long tail (approximately twice the SVL), five crests, and numerous spiny projections (Cogger 2000). There is some colour variation among populations. In general, males are darker (Barquero pers. obs.) and have larger heads (Harlow & Taylor 2000) than females. Consequently, individuals can be sexed with relative accuracy at a distance.

We sampled three sites within the range of the Jacky dragon: Cann River state forest (37°36'25.9" S, 149°09'05.8" E), Yarratt state forest (31°48'17.4" S, 152°25'57.5" E) and Royal National Park (34°04'49.7" S, 151°05'39.6" E), henceforth referred to as Cann River, Yarratt and Royal respectively. Sites were located in three different bioregions (Cann River: South East Corner; Yarratt: NSW North Coast; Royal: Sydney Basin), with differences in thermal regimes and forest composition (Department of the Environment and Water Resources 2007). Sampling was carried out during spring-summer of 2011 and 2012, and we focused only on males as they perform most of the displays in this species.

Habitat characteristics

We quantified the following habitat features at each study site: canopy coverage, number of trees, ground cover, habitat light, and habitat structure. Two measurements were collected at the exact point where undisturbed lizards were first

observed: canopy coverage was measured using a spherical densitometer and habitat light was recorded using a LI-190 quantum sensor connected to a LI-250A light meter (LI-COR Biosciences, USA). Two measurements of light intensity ($\mu\text{mol s}^{-1} \text{ m}^{-2}$) were recorded from the perch site in opposite directions and the average of these was used in subsequent analyses. In a 4-m radius from the perching site of an animal, we counted the number of trees (>5 m in height) and estimated (by eye) the percentage of ground cover using the following categories: 1 = 0-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80% and 5 = 81-100%.

We quantified habitat structure by placing a Panasonic digital camera mounted on a tripod as close as possible to where the lizard perched. We then placed a white screen (1.5 x 1.5 m) 4 m away from this point and took one photograph of the white sheet (see Marsden et al. 2002 for details). Because lizards were observed close to the trails, we only took one photograph per animal placing the screen opposite to the trail and within the bush. Each photograph was then converted to black and white and analyzed using ImageJ 1.46j (National Institutes of Health, USA). We used the percentage of black area (representing the total amount of obstacles between the camera and the white sheet) as a measure of understorey density and also the fractal dimension (Warfe et al. 2008). We also measured the same characteristics at 25 random points (at least 30 m apart) at each site to determine whether lizards were using a particular portion of the habitat available.

Habitat use, morphology and sprint speed

For each undisturbed lizard, we measured the perch height using a measuring tape graduated to the nearest 1 mm and the perch type where it was first observed. Perch types included branches, logs, tree trunks, stumps, rocks and the ground. For animals we captured we recorded the following morphological traits: SVL, tail length,

forelimb length, and hindlimb length. All traits were measured using a ruler (to the nearest 0.5 mm). Thirty-six adult males (12 from each population) were transported to Macquarie University and housed individually in 180 L x 90 W x 88 H cm outdoor enclosures (see Chapter 1 for details). Once the animals were collected from all three populations and had settled in captivity, we measured maximal sprint speed. One individual from Royal and one from Yarratt were not tested because of injuries to their tails. The day before trials commenced, all individuals were moved to indoor enclosures in a temperature-controlled room. During trials, the body temperature of animals was kept between 32 and 34 °C, a range that includes the preferred body temperature of this species (Heatwole et al. 1973). We measured sprint speed using a flat, 175 cm racetrack with a rubber substrate for traction and white lines every 25 cm. Each lizard was tested once a day over three days. A lizard was encouraged to run by gently tapping the tail until it had reached the opposite end of the racetrack. We filmed each trial with a Panasonic video camera mounted above the racetrack and each video was analyzed frame by frame using Final Cut Pro 7.0.3 to extract the maximum sprint speed over the full length of the racetrack. We used only the fastest run because lizards vary considerably in their motivation to run and performance can be highly variable (Losos et al. 2002).

Male display behaviour

We used captive animals to stage male-male contests in a neutral, outdoor arena, in order to characterize the display behaviour of each study population. We filmed interactions for up to 90 min (see Chapter 1 for details) and scored the videos using JWatcher Video version 1.0 (Blumstein & Daniel 2007) to obtain the number of aggressive displays (excluding the tail-flick component) per individual. Because previous studies have shown that the tail-flicks performed during the aggressive

displays by the Jacky dragon function as an introductory signal to attract the attention of conspecifics (Peters & Evans 2003), we counted the number of tail-flicks separately. Finally, because aggressive displays in this species can be performed in volleys (Ord & Evans 2003), we measured the average duration of bouts of aggressive displays.

Statistical analysis

We first examined whether variation in vegetation complexity and habitat light occurs among our study populations. In order to get an index of each habitat's complexity, we performed a Factor Analysis using Principal Component Analysis (PCA) and varimax as the extraction and rotation methods respectively, and the number of trees, percentage of ground cover, understorey density and fractal dimension as variables. We included data for both random and animal points. This analysis produced only one significant principal component (eigenvalue > 1) that explained 58.25% of the variation. We used this PC to detect differences in habitat complexity across sites using a two-way ANOVA, with the PC as the dependent variable and site and data type (i.e. animal and random) as fixed factors. We also included in the model the interaction between these two factors. Habitat light was analyzed using a two-way ANCOVA, with average light as the dependent variable and site and data type as fixed factors. We also included in the model the interaction between these two factors and two co-variables: canopy coverage and solar exposure. Because the data were collected along several days with varying conditions, we included solar exposure as a measure of the total solar energy for a day falling on a horizontal surface. Therefore, low values of solar exposure indicate that the day was cloudy, whereas high values indicate clear sky. We obtained these data from the Australian Bureau of Meteorology

(<http://www.bom.gov.au/climate/data/>) for the days when data on random points and animal points were collected. We log-transformed all variables to improve normality.

Variation in habitat use, morphology and performance along the distribution of the species was also analyzed. We performed a one-way ANOVA to determine whether perch height (dependent variable) varied across populations (fixed factor) and a Correspondence Analysis to detect differences in the use of perch types across populations. Because the number of individuals per perch type was low, we considered logs, tree trunks and stumps as one category called 'tree', and rocks and ground as another category called 'ground'. We left 'branch' as a separate category. We analyzed differences in limb morphology by first regressing both forelimb and hindlimb lengths against SVL to remove the effect of size. We used these relative lengths as the dependent variables in a MANOVA, with population as a fixed factor. Log-transformed maximum sprint speed as a measure of performance was analyzed using a one-way ANOVA, with population as a fixed factor.

Finally, we determined whether differences among populations exist in the characteristics of the signaling behaviour and display morphology of the Jacky dragon. For the latter, we used the relative tail length obtained in a similar way to that for limb morphology. We used this variable in a one-way ANOVA, with population as a fixed factor. Individuals with broken tails were excluded from the analysis. In the case of behavioural traits, we first log-transformed all variables (i.e. number of aggressive displays, number of tail-flicks and duration of bouts) to improve normality and then performed a MANOVA, with population as a fixed factor. We also included in the model as a co-variate, the log-transformed average time that each individual was in sight of the camera during the collection of the footage. All analyses were carried out using SPSS Statistics Version 20 (IBM©).

Results

Habitat variation

Differences in habitat complexity were detected among sites ($F_{2,148} = 65.67$, $P < 0.001$), with Royal being the most complex and Yarratt the least (Fig. 1a). Overall, we observed more trees, less ground cover and a low dense understorey in Yarratt compared to Royal, while the habitat in Cann River was intermediate (Table S1). We did not detect any significant differences in habitat complexity between random points and lizard locations at any site ($F_{2,148} = 1.96$, $P = 0.145$, Fig. 1a). We also found similar average light levels among sites ($F_{2,147} = 0.34$, $P = 0.712$, Fig. 1b) and between random points and lizard locations at all sites ($F_{2,147} = 0.79$, $P = 0.454$, Fig. 1b).

Habitat use, morphology and sprint speed

Perch height was not significantly influenced by population ($F_{2,43} = 0.88$, $P = 0.424$, Fig. 2a), but lizards from the three populations used different perch types ($X^2 = 18.64$, $df = 4$, $P = 0.001$, Fig. 3). Lizards from Yarratt and Cann River were observed more often using wooden perches (i.e. logs, trees, and stumps), whereas animals from Royal perched more often on branches and the ground (Fig. 3). Relative limb length was significantly different between populations (forelimb: $F_{2,82} = 10.36$, $P < 0.001$, hindlimb: $F_{2,82} = 18.37$, $P < 0.001$). In both cases, lizards from Cann River had shorter limbs compared to those from Royal and Yarratt (Fig. 2b). Maximum sprint speed also differed significantly ($F_{2,31} = 3.80$, $P = 0.033$) among sites, with animals from Cann River being slower than lizards from Royal and Yarratt (Fig. 2c).

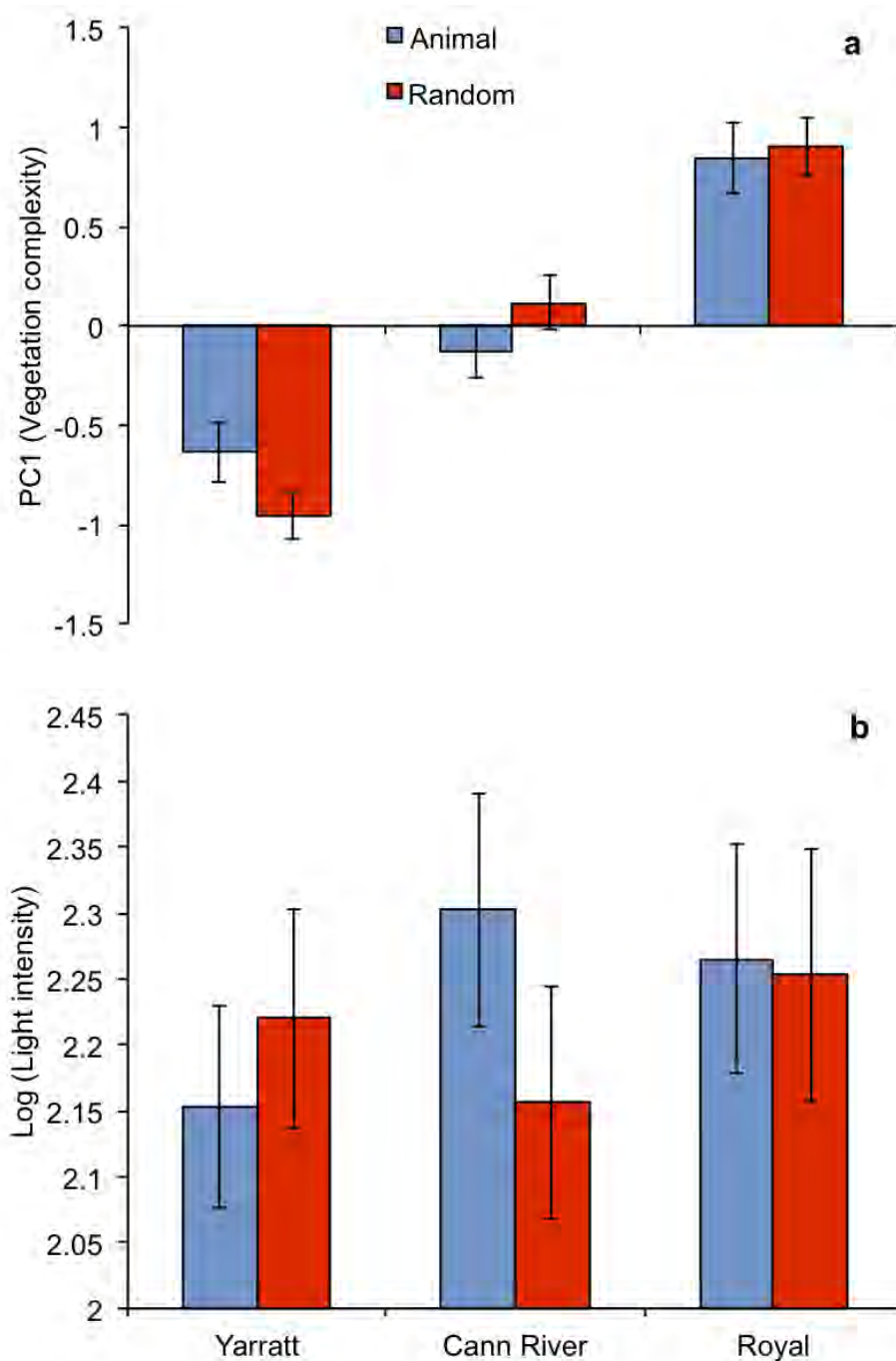


Figure 1. Mean (\pm SE) vegetation complexity (a) and ambient light levels (b) for both random points ($n = 25$ at each site) and lizard locations (see text for an explanation) by population (Taree $n = 29$, Cann River $n = 27$, Royal $n = 24$).

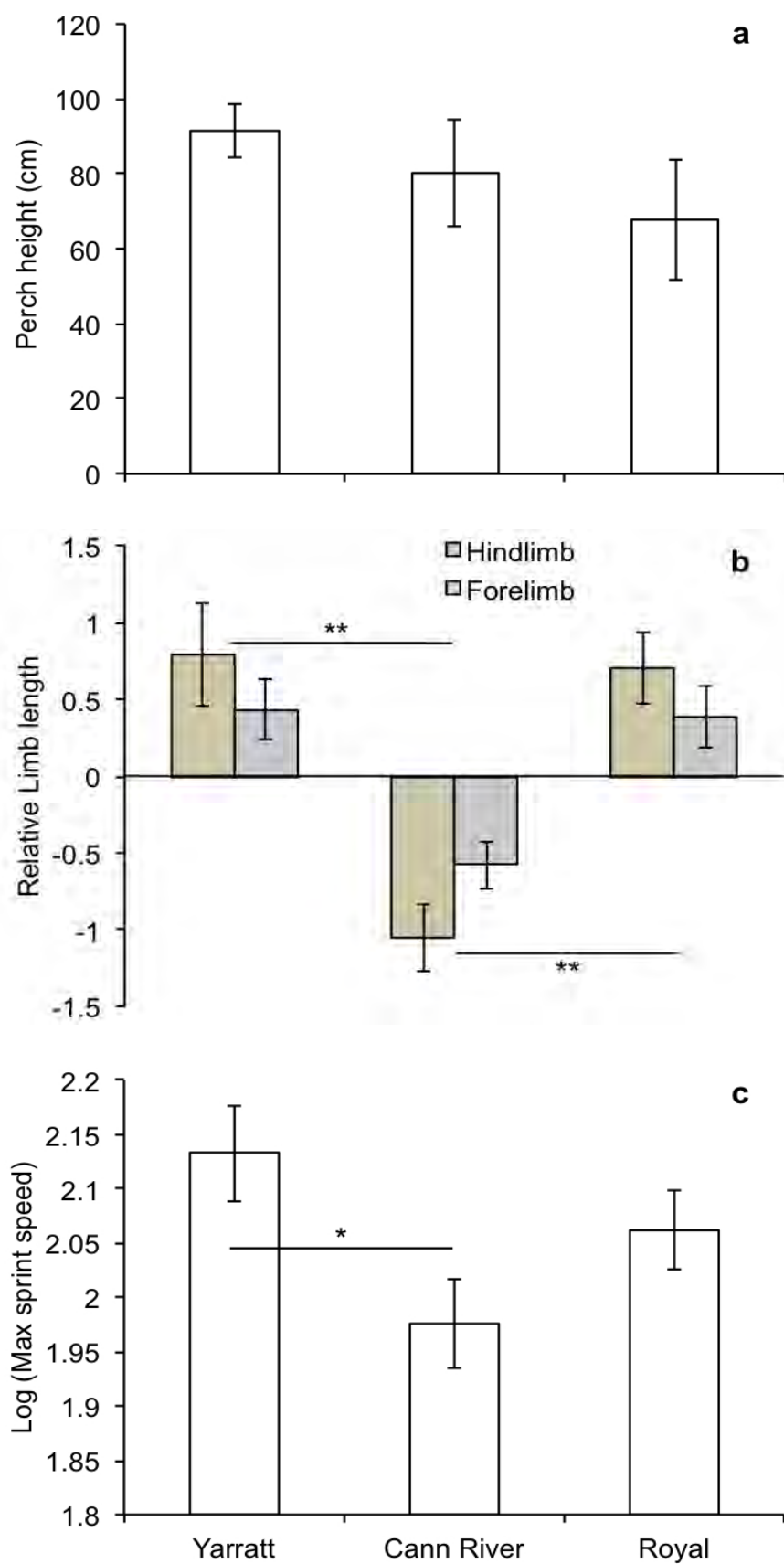


Figure 2. Mean (\pm SE) perch height (a), relative forelimb and hindlimb length of males (b), and maximum sprint speed (c) by population. Significant results among pairwise comparisons are shown (* $P < 0.05$, ** $P < 0.01$).

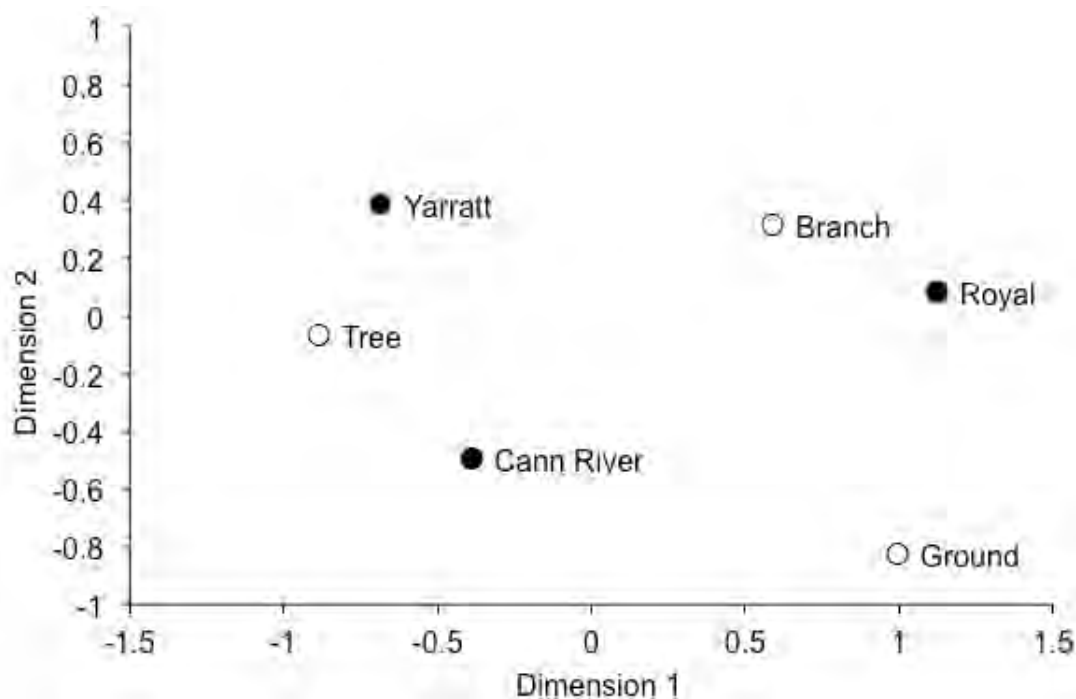


Figure 3. Distribution of perch types and populations of the Jacky dragon, *A. muricatus*, in a 2-dimensional space based on a Correspondence Analysis. Dimension 1 explains 95.5% of the inertia of the data and it contributes strongly (> 0.70) to the inertia of all populations and perch types.

Male display behaviour

We detected significant differences in male tail length among populations ($F_{2,76} = 41.69$, $P < 0.001$); lizards from Cann River had shorter relative tails compared to the other two populations (Fig. 4a). We also detected significant differences in male display behavior among populations (number of aggressive displays: $F_{2,32} = 4.32$, $P = 0.022$; number of tail-flicks: $F_{2,32} = 4.47$, $P = 0.019$; average duration of bouts: $F_{2,32} = 4.12$, $P = 0.026$). Bonferroni post-hoc tests revealed that animals from Cann River displayed more often (Fig. 4b) and for longer (Fig. 4c) than individuals from Yarratt.

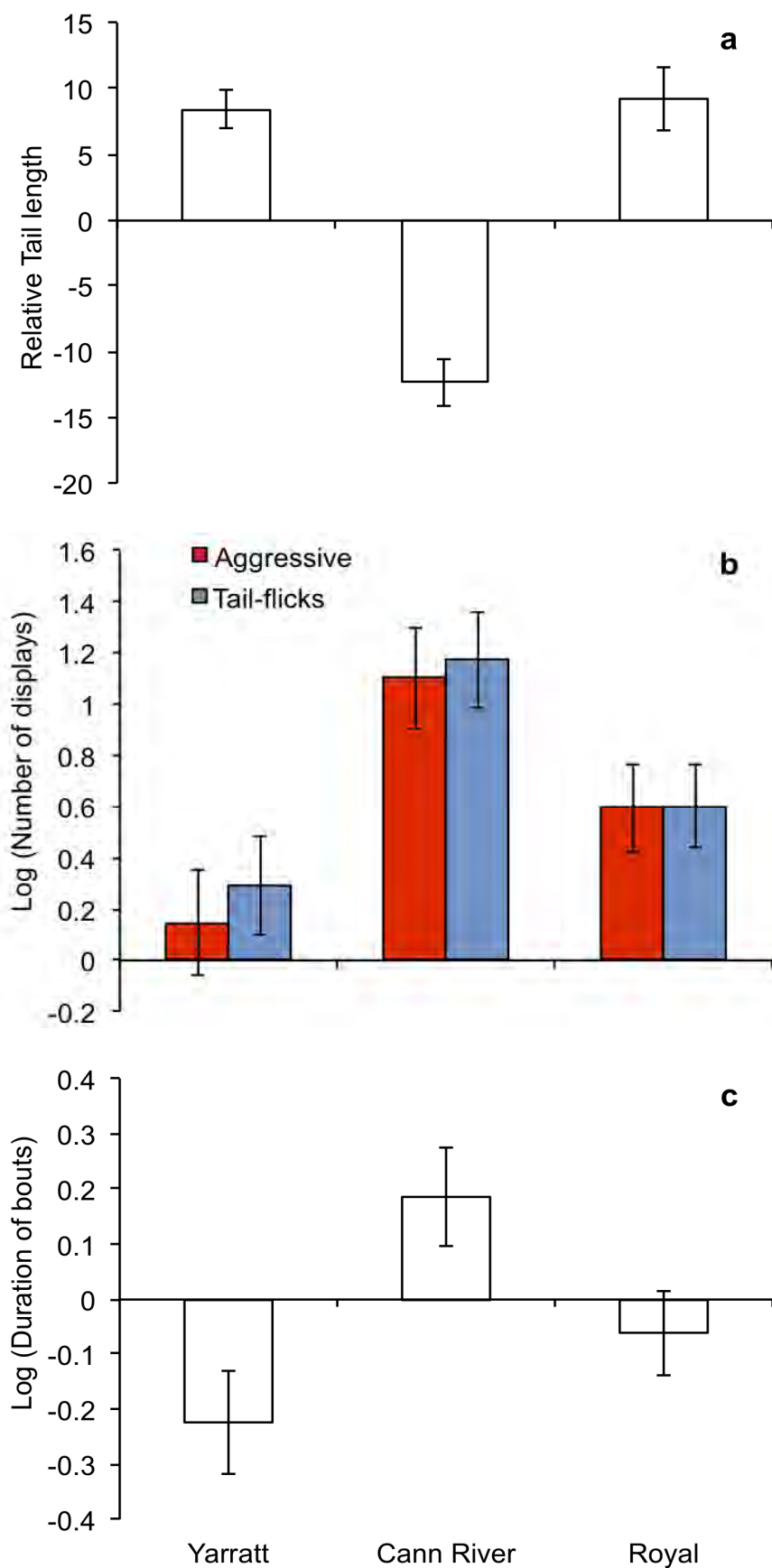


Figure 4. Mean (\pm SE) relative length of the tail of males (a), number of aggressive displays and tail-flicks (b), and duration of aggressive bouts (c) by population.

Discussion

The following are the key findings of our study: 1) the Jacky dragon occupies habitats that vary significantly in vegetation complexity but not in levels of ambient light; 2) a congruent pattern of variation in morphology and performance was found, but not related with the pattern of habitat use or habitat variation; and 3) display behaviour (aggressive displays and duration of bouts) differed across populations of the species but the pattern was not congruent with habitat variation. Therefore, these findings reflect a discordant pattern between habitat variation (complexity and light levels) and variation in both display behaviour (frequency of aggressive signals) and habitat use.

Habitat variation and habitat use

The fact that habitat use across sites was not correlated with ambient light levels or vegetation complexity is intriguing. Jacky dragons have previously been referred as a species able to survive in different habitat types, even those disturbed by human intervention, with a preference for open or relatively dense understorey vegetation (Lunney & Barker 1986; Letnic & Fox 1997; Bragg et al. 2005; Lindenmayer et al. 2008). As many species of lizards often show a shift in habitat use according to changes in habitat characteristics or perch availability (Martín & López 1998; Herrel et al. 2001; Irschick et al. 2005), we were expecting to find a similar pattern in populations of the Jacky dragon occupying habitats with differences in vegetation complexity. Although this was not the case, some insights can be extracted from our results.

First, there is a gradient in vegetation complexity across the study sites. In one extreme of this gradient, the forest in Yarratt had many trees and a comparatively low understorey density (Fig. 1). At the other extreme, there was less trees and high

understorey density in Royal (Fig. 1). Letnic & Fox (1991) mention that the Jacky dragon seems to prefer a habitat type with structurally complex vegetation. Our results, however, show that the species is able to occupy sites with differences in vegetation complexity.

Second, despite their semi-arboreal habits and their ability to climb up to the canopy when disturbed (Lunney & Baker 1986; Barquero pers. obs.), individuals from all populations showed similar preferences for perch heights. Figure 2a shows the variation of perch heights considering all individuals, but when those perching on the ground are removed the average perch height converges to 92 cm at all sites. We expected that animals from less complex environments would select lower perches in order to avoid detection by avian predators. However, the similar perch height observed across populations despite differences in vegetation complexity also indicates that animals are selecting a non-random position in vertical space.

Finally, because of differences in the forest structure across sites, we were expecting to detect variation in the intensity of ambient light that reaches the understorey. However, we found no differences in ambient light among sites, which might indicate that Jacky dragons are selecting habitats and microhabitats with similar illumination levels. Jacky dragons have a large eye compared to other diurnal lizards, with a pupil that responds quickly to changes in the photic environment (New 2012). Therefore, they could perform well under a range of different light conditions, but instead they seem to be actively selecting perches with specific sunlight conditions. This result is in accordance with previous studies that suggest Jacky dragons require relatively high levels of incident sunlight (Letnic & Fox 1991).

Morphology, performance and behaviour

Variation in morphology could be influenced by both ecological and behavioural divergence. In the case of ecological divergence, measured in this study as

differences in habitat use, we were expecting an effect on morphology such that animals perching lower would have longer limbs (Herrel et al. 2001) and hence, would run faster. At the same time, animals using low perches were expected in habitats where vegetation was less complex. Therefore, lizards with large limbs (faster runners) were expected in less complex (or more open) habitats, a pattern that has been reported previously for interspecific comparisons in lizards (Goodman 2009). Limb morphology was indeed a good predictor of sprint speed; however, our results did not support the rest of the predictions. Individuals occurring in the extremes of the complexity gradient (Yarratt and Royal) had similar limb lengths, whereas individuals from the habitat with an intermediate level of vegetation complexity (Cann River) had smaller limbs.

In the case of signal divergence, measured as differences in frequency and duration of signals, we expected that body parts used to perform displays, specifically the tail length for the Jacky dragon, would be more conspicuous in more complex habitats. Therefore, longer tails would be easier to detect in habitats where visibility is a constraint for communication with conspecifics. However, similar to the pattern for limb morphology, individuals from Yarratt and Royal had longer tails compared to those from Cann River. We were also expecting that animals in more complex habitats would display less frequently but for longer periods. Again, this was not the case, since lizards from the less complex habitat (Yarratt) displayed less often and during shorter periods compared to those from the intermediate habitat (Cann River).

The results for both ecological and behavioural divergence suggest that selective pressures other than habitat complexity might be influencing habitat use, communication and the expression of phenotypes. Several possibilities emerge to explain the differences observed between populations of the Jacky dragon. For example, thermoregulation could explain the pattern in habitat use such that animals

are perching at the same height to get enough thermal radiation. However, thermoregulation does not explain morphological differences between populations. Predation could be a factor influencing both variation in signaling behaviour and morphology. Individuals occurring in a less complex, more open habitat, where predators could detect more easily animals performing conspicuous displays, would be constrained in the frequency of displays and at the same would have larger limbs and better sprinting capabilities. However, predation alone might not explain the high display frequency of animals from intermediate complex habitats. Rather than one single factor explaining the patterns observed, perhaps a combination of factors is a more likely scenario.

Conclusions

We aimed to disentangle the effect of habitat complexity and ambient light from communication with conspecifics and habitat use. We asked whether morphological traits and signaling behaviour changed geographically in order to optimize the transmission of signals through habitats with different characteristics. We also tested whether habitat use and associated morphological traits changed as a function of habitat complexity and habitat light. The discordant pattern of habitat variation and both signal design and habitat use posed an interesting dilemma. We attribute these non-congruent patterns to the fact that other factors are acting as strong as or even stronger than habitat variation. Therefore, animals could be trading off effective communication for other aspects that increase survival. Research on the effects of predation, temperature and population density on variation of the signaling behaviour should help to understand the synergisms between all these factors.

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Supplementary material

Table S1. Mean \pm SD of all habitat measurements recorded where lizards were observed and at random points within three populations of the Jacky dragon, *Amphibolurus muricatus*.

Variable	Yarratt	Cann River	Royal
<i>Lizard points</i>			
Habitat Light	263.42 \pm 245.16	331.85 \pm 231.42	368.26 \pm 302.30
Canopy coverage	82.95 \pm 11.38	69.98 \pm 13.47	56.36 \pm 28.93
Number of trees	2.97 \pm 1.61	2.93 \pm 3.00	1.04 \pm 1.83
Ground cover (%)	2.21 \pm 1.40	2.48 \pm 1.16	2.71 \pm 1.16
Understorey density	21.00 \pm 16.02	33.13 \pm 19.72	68.31 \pm 30.69
<i>Random points</i>			
Habitat Light	234.05 \pm 203.01	143.58 \pm 139.02	195.00 \pm 175.74
Canopy coverage	91.08 \pm 6.90	71.15 \pm 12.39	60.57 \pm 30.21
Number of trees	5.08 \pm 2.52	4.04 \pm 3.52	1.40 \pm 2.40
Ground cover (%)	1.60 \pm 0.87	2.76 \pm 1.23	3.00 \pm 1.00
Understorey density	18.86 \pm 19.27	42.19 \pm 20.27	68.99 \pm 21.35

CHAPTER 4

Effect of temperature on the visual displays of the Jacky dragon

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Abstract. In an era of global warming, it is more important than ever to understand the role of temperature on signaling in ectotherms. In lizards, ambient temperature influences their ability to signal and the properties of that signal. We tested whether changes in the thermal environment along the distribution of an agamid lizard, the Jacky dragon (*Amphibolurus muricatus*), modifies the properties of its signaling behaviour. We first collected temperature data from 12 weather stations to understand variation in temperature across the species' range. We then collected field body temperatures for three different populations before measuring preferred body temperatures in the lab. Finally, we directly examined the role of temperature on the frequency and duration of display behavior at a low (28 °C) and high (35 °C) temperature consistent with what they would experience in the wild. We found significant differences in the ambient temperature across the species' range. Field body temperatures also differed among populations, but not preferred body temperatures. We also found that higher ambient temperatures increased the number of displays used during intraspecific communication. We interpret these findings as local adaptation, such that variation of body temperatures mirrors that of the thermal environment and the characteristics of the signaling behaviour are modified accordingly.

Temperature is a major selective pressure that can impact the development, survival and evolutionary pathway of any organism (Gagliano et al. 2007). Several aspects of life history and behavioural ecology, such as physiological performance (Navas et al. 1999; Lailvaux 2007), reproductive activity (McElhinny et al. 1997; Luo et al. 2010), feeding behaviour (Van Damme et al. 1991), and antipredator responses (Crowley & Pietruszka 1983; Amo et al. 2004; Herrel et al. 2007) are all shaped by the thermal environment that a species occupies. Both daily variations and long-term changes in ambient temperature can have equally strong effects on the characteristics and habits of the individuals of a population. For example, individuals can be restricted in their foraging behaviour or breeding activities and modify their microhabitat use during very warm or very cold days (Huey & Pianka 1977; Christian et al. 1983). At the same time, prolonged changes in ambient temperature can cause modifications in spatial distribution and phenology (Parmesan & Yohe 2003; Bradshaw & Holzapfel 2008).

Terrestrial ectotherms are particularly susceptible to changes in ambient temperature (Deutsch et al. 2008). For example, 4% of lizard populations have gone extinct and many more (ca. 20%) could disappear worldwide because of the impacts of climate warming (Sinervo et al. 2010). To counteract the negative effects of increasing temperatures, Kearney et al. (2009) have suggested that tropical ectotherms should attempt to stay cool while temperate ones should avoid attaining high body temperatures. In either case, animals could increase the time spent in thermoregulation and decrease that used for other activities. This could be problematic for species that perform certain activities within a narrow range of thermal tolerance. Communication with conspecifics, which involves interactions within (e.g. social dominance, territoriality) and between (e.g. courtship, parental care) sex/age classes, can be strongly affected by changes in ambient temperature

(Jiao et al. 2009). A well-known example is the effect of temperature on the vocal communication of most species of frogs, for which temperatures outside the preferred thermal range can cause a decrease in the detection of calls and subsequent response of receivers, and a modification in the properties of the calls (Gerhardt 1978; Walkowiak 1980).

Although the effect of temperature on vocal communication with conspecifics has received significant attention (Gerhardt & Huber 2002), the relationship between the thermal environment and visual communicative system has rarely been investigated. In one of the few examples available, Doherty (1982) determined that temperature influenced the waving behaviour of two species of fiddler crabs (*Uca minax* and *U. pugnax*), with wave duration and ascending and descending wave times decreasing as ambient temperature increased. In vertebrates, Denoël et al. (2005) found that courtship displays of males and responses of females to such displays in the Alpine newts (*Triturus alpestris*) were both temperature-dependent, with tail beats of males performed at a lower rate and for a longer time at lower temperatures. In other groups of ectotherms, the effect of temperature on the variation of visual signaling behaviour has never been quantified. Although lizards have commonly been used as model organisms to explore the influence of temperature on physiological, morphological, behavioural and ecological traits (Huey & Pianka 1977; Crowley & Pietruszka 1983; Mautz et al. 1992; Herrel et al. 2007), the effect on visual display behaviour, to our knowledge, has never been directly studied (but see Phillips 1995).

Here, we examine the influence of ambient temperature on the signaling behaviour of an agamid lizard, the Jacky dragon (*Amphibolurus muricatus*). We address the following questions: 1) how variable is the thermal environment of the Jacky dragon across its distribution?; 2) does variation in body temperatures (T_b)

across populations mirror that of the thermal environment?; and 3) are changes in ambient temperature altering the characteristics of visual displays? We begin by comparing historical temperature data from sites across the range of the species. We then use data on T_b collected in the field from three populations of the Jacky dragon with different thermal environments. And finally, we determine whether lizards modify their signaling behaviour in response to ambient temperature.

We used the Jacky dragon as a model because both its thermal biology and signaling behaviour have received substantial attention. Several studies have investigated the T_b of this species in the field (Heatwole et al. 1973), panting thresholds (Chong et al. 1973; Heatwole et al. 1973; Heatwole et al. 1975; Parmenter & Heatwole 1975; Firth & Heatwole 1976) and voluntary maximum temperature (Heatwole & Firth 1982) in the lab. In addition, the effect of temperature on incubation periods of eggs (sex determination in this species is temperature-dependent) and the phenotype of hatchlings has been extensively quantified (Harlow & Taylor 2000; Warner & Shine 2008, 2011).

The signaling behaviour of the species has also been studied and described in some detail (Carpenter et al. 1970; Peters & Ord 2003; Watt & Joss 2003). The signal repertoire includes an aggressive display composed of five movements, two types of submissive displays (i.e. slow circumduction of the forelegs and head bows) and several postures. Recent evidence suggests that variation in the signaling behaviour occurs along the range of the Jacky dragon (see Chapter 1), so that the thermal regime experienced by animals in the wild might contribute to this variation. However, and despite having a distribution spanning over 10° in latitude on the southeastern coast of Australia (Cogger 2000), no study has accounted for variation of the thermal environment of the species or geographic variation of T_b and the effect of ambient temperature on the communicative system.

Methods*Geographic variation of thermal environment*

We examined the degree of variation in the thermal environment of the Jacky dragon by selecting 12 sites within the distribution of the species (Fig. 1). We used the Atlas of Living Australia (<http://bie.ala.org.au>) to explore the distribution of the Jacky dragon and selected sites based on a high number of specimens that were collected in the past. The sites ranged from 29° to 38° in latitude and from 5 to 1079 m in elevation, with half of the sites above 200 m a.s.l. We obtained historical maximum temperatures for each of these sites from the Australian Bureau of Meteorology between spring 1997 and autumn 2013. We chose this period because climatic data were incomplete for most sites before than 1997. We used monthly means to compare among sites, but because the Jacky dragon hibernates from May to August (Harlow & Taylor 2000) we excluded these months from the analysis.

Body temperature

We visited three sites along the range of *A. muricatus* (Fig. 1) during the spring and summer of 2011 and 2012: Yarratt state forest (31°48'17.4" S, 152°25'57.5" E), Royal National Park (34°04'49.7" S, 151°05'39.6" E), and Cann River state forest (37°36'25.9" S, 149°09'05.8" E), henceforth referred to as Yarratt, Royal and Cann River respectively. At each site, we captured adult individuals by noosing and recorded the following data: sex, skin temperature (using a noncontact infrared portable thermometer) and cloacal temperature (using a long-stem thermometer). We excluded animals that ran more than 2 m from the original perch where they were first sighted and we only collected data during sunny, warm days.

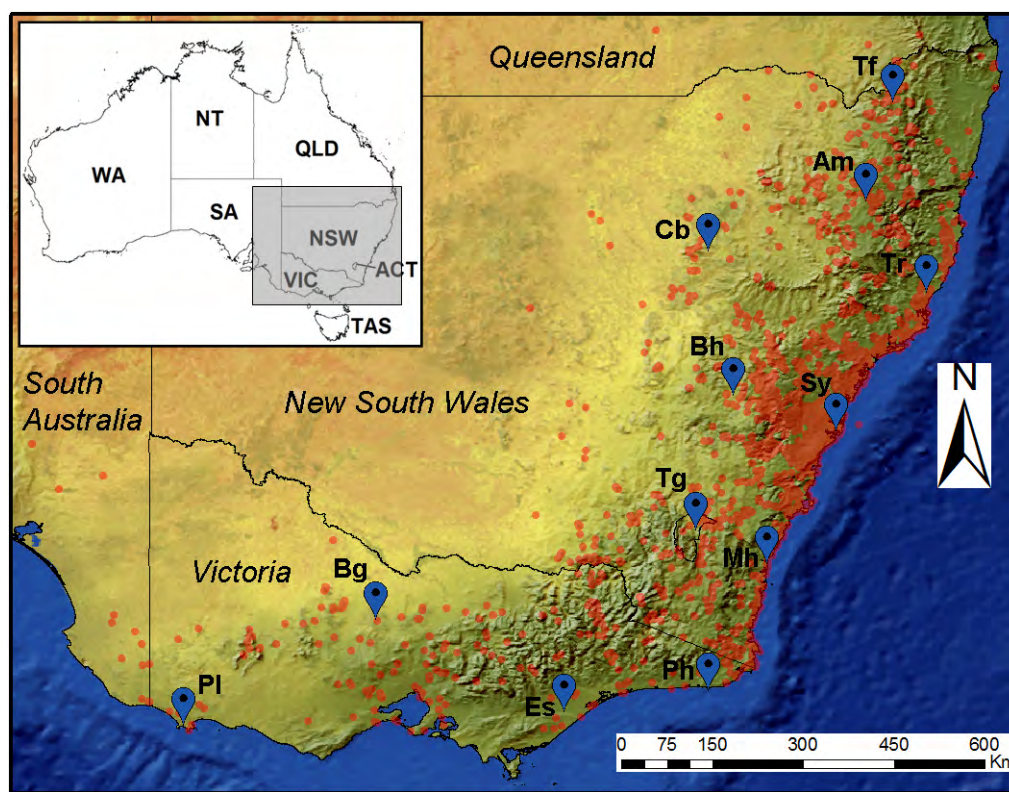


Figure 1. The current distribution of the Jacky dragon across Australia (Atlas of Living Australia) and the weather stations used to extract historical climate data (Australian Bureau of Meteorology). Am = Armidale; Bh = Bathurst; Bg = Bendigo; Cb = Coonabarabran; Es = East Sale; Mh = Moruya Heads; Ph = Point Hicks; Pl = Portland; Sy = Sydney; Tf = Tenterfield; Tg = Tuggeranong; Tr = Taree. Study populations are Yarratt = Taree; Royal = Sydney; and Cann River = Point Hicks.

Additionally, 32 individuals from the same three populations (10 from Cann River; 12 from Royal; 10 from Yarratt) were transported to facilities at Macquarie University where we measured the preferred T_b in a thermal gradient (e.g. Light et al. 1966; Lailvaux et al. 2003). Animals were kept in outdoor enclosures (see Chapter 1 for details) during several months before trials commenced. Lizards were moved

indoors the day before trials commenced and kept in a temperature-controlled room at 24 °C. We constructed a thermal gradient consisting of a wooden enclosure (120 L x 90 W x 80 H cm) divided in four 20-cm-wide runways to perform the trials, with packed sand as substrate. Within the sand we set up a system of parallel copper tubes that extended along the width and to the center of the enclosure. This system of tubes was connected to a water bath (Haake F3) set at -8 °C. At the opposite end of the enclosure we suspended a 150 W heat lamp in each runway, creating a gradient of 4-50 °C. Trials commenced at 10:00 h when four lizards were moved into the thermal gradient, each animal occupying a separate runway. The cloacal temperature was measured every 30 min during a 6-h period. The preferred T_b of each individual was considered as the average of these measurements and we also recorded the maximum T_b attained by each subject.

Influence of temperature on visual displays

In order to determine the effect of temperature on visual displays, we performed two lab experiments. In the first experiment we staged male-male encounters in an outdoor, neutral arena (180 L x 180 W x 88 H cm) (see Chapter 1 for details). We used 39 captive individuals from the three populations (13 lizards per population) and performed both intra- and interpopulation encounters. Each lizard participated in up to five trials and the interactions of the subjects were filmed for up to 90 min using a Panasonic HDC-HS60-K digital video camcorder. The ambient temperature was measured using 3 temperature loggers (DS1921G thermochron iButtons®, Maxim Integrated) placed around and right outside the arena. The mean ambient temperature combining the information of all loggers was obtained for the time of the day that each trial took place.

A second experiment consisted of controlling the ambient temperature. We separated 15 captive lizards from the Cann River area into two temperature-controlled rooms with different ambient temperatures: low (28 °C) and high (35 °C). We chose these temperatures based on two criteria: 1) the range encompasses the preferred T_b (32-36 °C) reported previously for the species (Heatwole et al. 1973; Heatwole & Firth 1982) and 2) historical temperature data shows that this is the range of temperatures experienced by lizards in the wild during their activity period (Fig. 2). Lizards were under each thermal regime for 3 days as a habituation period and trials commenced on the fourth day. A live animal was presented to each focal individual as a stimulus to elicit a behavioural response. The animal was kept in a separate aquarium so that no direct contact was possible between the two individuals. We filmed the behaviour of each focal subject for 35 min using the same equipment mentioned above. At the end of a session, we recorded the skin temperature of the focal lizard using a noncontact infrared portable thermometer. Once all animals from both treatments were tested, we swapped treatments for each group of lizards and replicated the experiment, so that all animals experienced both temperature treatments.

The footage obtained from both experiments was analyzed using JWatcher Video version 1.0 as an event recorder program (Blumstein & Daniel 2007). We quantified the number of aggressive displays and submissive displays for both experiments. Because visual signals can also be accompanied by other behaviours (Ord et al. 2002), we quantified the number of tongue flicks. For the second experiment we also measured the number of locomotion bouts towards the stimulus and the duration of all behaviours.

Statistical analyses

Historical climate data from the Australian Bureau of Meteorology were used to determine the degree of variation of the Jacky dragon's thermal environment. We included site and month as fixed factors in a two-way ANOVA, with mean monthly temperature as the dependent variable. We also used the daily maximum temperatures obtained from the Australian Bureau of Meteorology to compare among our three study sites, using only the days when data collection took place. The log-transformed temperature was included as the dependent variable in a one-way ANOVA, with site as a fixed factor.

As our study sites might have different thermal regimes, we wanted to examine the relationship between lizards' T_b and the ambient temperature. We performed Pearson's correlations for all sites combined and then for each site separately using daily maximum temperature obtained from Australian Bureau of Meteorology, as ambient temperature and both cloacal and skin temperatures as T_b measures. For each site we also did separate correlation analyses for males and females. As both measurements of T_b were highly correlated ($r = 0.95$, $P < 0.001$), we only report the cloacal temperature. We also determined whether cloacal temperatures measured in the field differed across our study populations using a two-way ANOVA, with sex and population as fixed effects. Bonferroni post-hoc tests were used to examine which populations differed among each other. We used a MANOVA to detect differences among populations in the preferred T_b measured in the lab, with population as a fixed effect and the log-transformed average and maximum temperatures as dependent variables.

The effect of ambient temperature on the number of displays, both aggressive and submissive, during male-male contests was examined in R 2.13.0 (R Development Core Team 2011) using generalized linear mixed-effect models

(GLMMs), with Poisson errors for count data. We used population and ambient temperature as fixed effects and subject identity and rival identity as random effects. Separate analyses were performed using the number of tongue flicks and the sum of aggressive and submissive displays as dependent variables. In addition, we used paired t-tests to compare the effect of high- and low-temperature treatments on the number of displays (aggressive and submissive displays combined) and the duration of activity directed to a stimulus (i.e. the total time spent displaying or moving towards the stimulus) during an indoor experiment. Unless otherwise specified, all analyses were carried out using SPSS Statistics version 20.0 (IBM©).

Table 1. Pearson's correlations between the ambient temperature and the cloacal temperature recorded for three populations of the Jacky dragon. An overall correlation for sexes combined, and separate correlations for each sex, are listed for each population. Significant correlations are in bold.

Effect	N	r	P
Cann River	51	0.09	0.520
Females	18	0.12	0.646
Males	33	0.04	0.804
Royal	47	0.27	0.062
Females	16	0.56	0.023
Males	31	0.16	0.393
Yarratt	39	0.36	0.023
Females	19	0.49	0.033
Males	20	0.12	0.606

Results

Thermal environment

Significant variation was detected across sites in the mean maximum temperature over the activity period of the Jacky dragon ($F_{11,1477} = 234.66$, $P < 0.001$) (Fig. 2) during 1997-2013. The maximum difference between sites with the lowest and highest mean maximum temperatures occurred in January (9.4 °C), whereas the minimum occurred in April (5.6 °C). Overall, the warmest site was Coonabarabran (mean temperature = 26.9 °C) and the coldest was Portland (mean temperature = 19.5 °C) (Fig. 2).

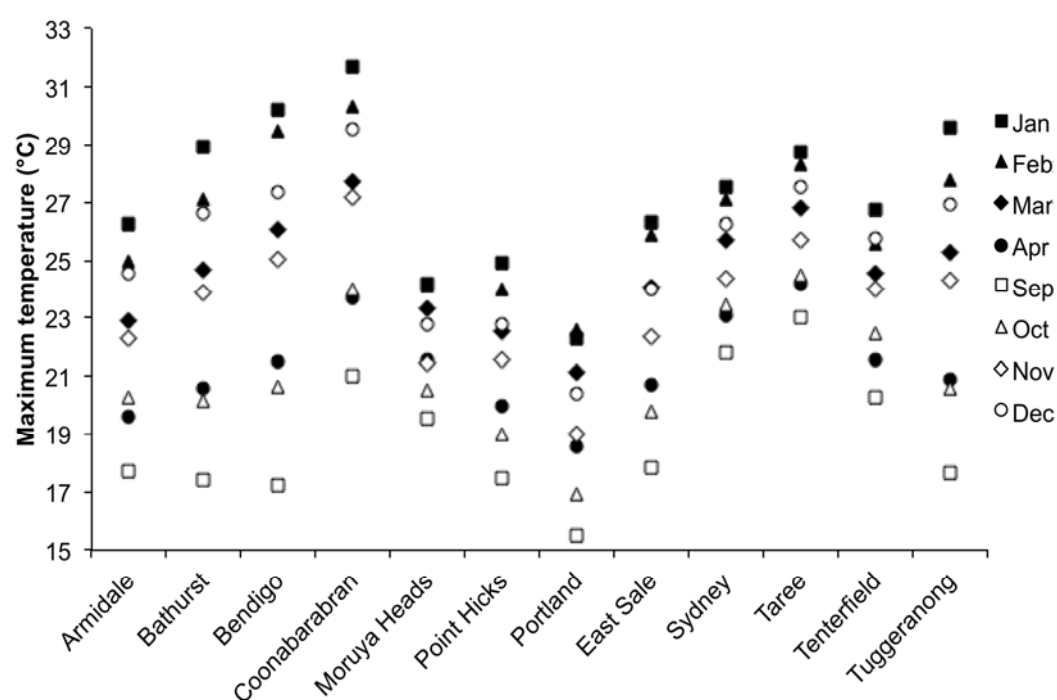


Figure 2. Mean monthly maximum temperatures recorded at 12 sites (Australian Bureau of Meteorology) distributed across the range of the Jacky dragon during a period between 1997 and 2013.

Body temperature

No significant ($F_{2,56} = 0.88$, $P = 0.419$) differences in ambient temperatures were detected across sites during fieldwork (Fig. 3). Overall, T_b in the field was correlated with ambient temperature ($r_P = 0.21$, $P = 0.015$, $N = 137$). However, correlational analyses for each population and sex indicated that only females from Yarrat and Royal showed a positive correlation between ambient and cloacal temperatures (Table 1). Also, T_b varied across populations ($F_{2,130} = 8.77$, $P < 0.001$) but not among sexes ($F_{1,129} = 0.42$, $P = 0.519$) or sex * population ($F_{2,129} = 0.88$, $P = 0.418$). Bonferroni post-hoc tests showed that lizards from Cann River had higher T_b than animals from the other two populations and the temperatures of individuals from Royal and Yarratt did not differ (Fig. 3). We did not find differences in the preferred T_b ($F_{2,29} = 0.17$, $P = 0.848$; Fig. 3) or the maximum T_b ($F_{2,29} = 0.20$, $P = 0.816$) of lizards from all three populations.

Table 2. Main effects of generalized linear mixed models using the number of tongue flicks and the number of visual displays as dependent variables in relation to population and ambient temperature. LRT = likelihood-ratio test.

Effect	Estimate	SE	LRT	df	P
<i>Number of tongue flicks</i>					
Population			10.85	2	0.004
Ambient temperature	0.018	0.006	9.08	1	0.003
<i>Number of visual displays</i>					
Population			15.12	2	<0.001
Ambient temperature	0.042	0.005	73.85	1	<0.001

Visual displays

Lizards from all populations experienced similar ambient temperatures (Mean \pm SD: Cann River = 37.31 ± 3.95 ; Royal = 37.37 ± 5.39 ; Yarratt = 37.46 ± 6.07) in the outdoor encounters. We found a significant effect of ambient temperature on both the number of tongue flicks and the number of displays for all populations (Table 2). Overall, there was an increase in the number of tongue flicks and the number of displays as the ambient temperature rose. Similarly, by controlling the ambient temperature we found that lizards from Cann River exposed at a high-temperature treatment performed more displays ($t = 3.05$, $df = 14$, $P = 0.009$) (Fig. 4) and interacted for longer ($t = 2.82$, $df = 14$, $P = 0.014$) (Fig. 4) with a subject used as stimulus than those at a low-temperature treatment.

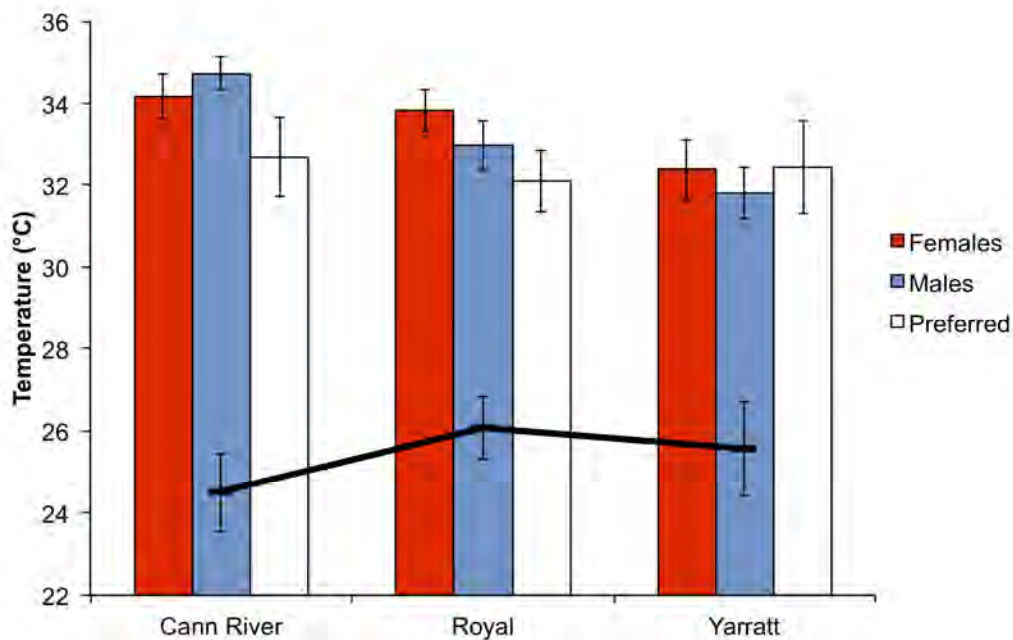


Figure 3. Mean (\pm SE) field body temperatures of males and females and the preferred body temperature of males from three populations of the Jacky dragon. Mean (\pm SE) ambient temperature (Australian Bureau of Meteorology) recorded during the days of data collection in the field is also shown (linked by lines).

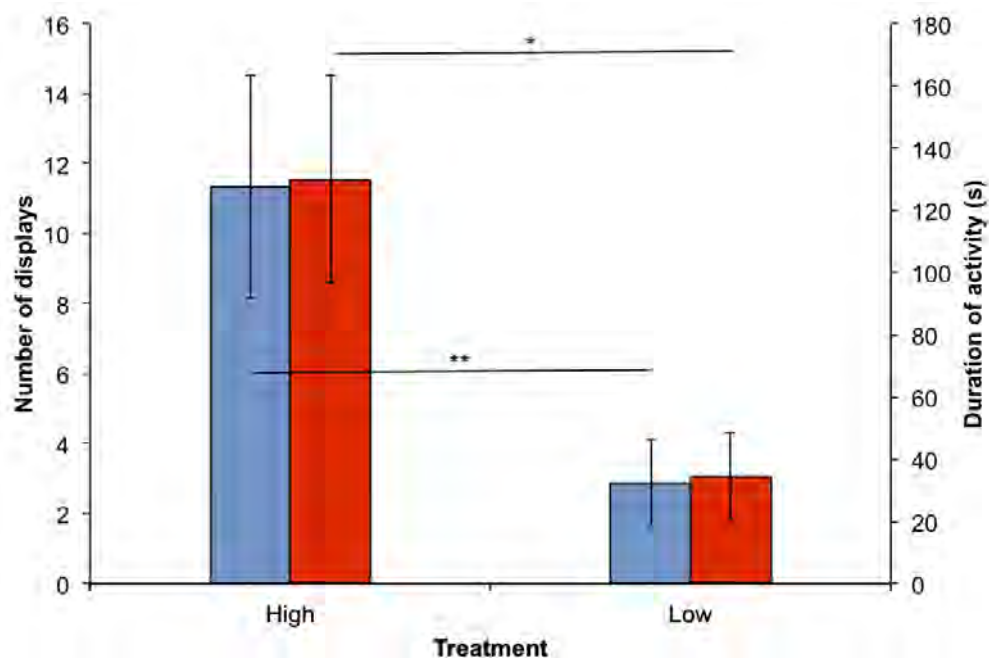


Figure 4. Mean (\pm SE) number of displays (blue bars) and mean (\pm SE) duration of activity (red bars) of Jacky dragons from Cann River across temperature treatments. Significant results among pairwise comparisons are shown (* $P < 0.05$, ** $P < 0.01$).

Discussion

In the present study we have addressed questions to determine the variation of the thermal environment and T_b of Jacky dragons, as well as the effect of ambient temperature on the signaling behaviour of the species. As expected, we found that the thermal environment varies significantly along the distribution of this species, with hotter environments at lower latitudes and inland of the continent. Both the T_b of lizards and its relation with ambient temperature varied across populations, with individuals from one population (Cann River) attaining higher T_b s. Finally, we found

that ambient temperature has a significant effect on the frequency of visual displays performed and the time spent in male-male interactions, so that animals experiencing high temperatures performed more displays and interacted for longer.

The high variation of the mean historical (1997-2013) temperatures found across the distribution of the Jacky dragon during its activity period (Fig. 2) highlights two facts. First, the species as a whole has a wide thermal tolerance, since it occupies habitats that differ widely in their thermal conditions. This finding support previous studies on other terrestrial ectotherms that have found broad thermal tolerances of species from higher latitudes (Hertz et al. 1983; Deutsch et al. 2008). Second, lizards from different populations are confronted with different temperature-related challenges and constraints that might affect their behaviour, physiology and performance capacities. As heliothermic lizards usually maintain a relatively constant T_b , it is possible that geographic variation in ambient temperature has little effect on T_b (Andrews 1998). Therefore, regardless of the differences in thermal regimes along the distribution of the Jacky dragon, geographic variation in T_b can be minimal. This static view assumes that thermoregulatory behaviours might change across populations, but all result in similar T_b (Hertz et al. 1983). On the other hand, differences in thermal regimes can have a strong effect on T_b , so that local adaptation to specific thermal conditions could occur and T_b is labile (Hertz et al. 1983).

The labile view is supported by previous studies of preferred and field T_b s of Jacky dragons. Light et al. (1966) reported a mean preferred T_b of 36 °C (± 1.22 SD) for individuals captured in south Queensland, at the northernmost part of the species range. The mean preferred and field T_b of animals from Armidale, at the north-central part of the species distribution, were between 34.5 °C and 35 °C (Heatwole et al. 1973). Our data support the labile view of T_b . Field T_b of animals from Cann River

(Mean \pm SE = 34.2 ± 0.30 °C) differed significantly from the other populations (Royal: 32.6 ± 0.50 °C; Yarratt: 31.7 ± 0.46 °C), although no difference was found in the preferred T_b across populations (overall Mean \pm SE = 32.4 ± 0.23 °C). Therefore, there is roughly 4 °C difference between the mean preferred body temperature reported by Light et al. (1966) and the one measured in this study and there is a 2.5 °C difference between Cann River and Royal.

Our results also suggest that at least lizards from Cann River are attaining higher T_b s in the field than their preferred T_b . A possible explanation is that ambient temperatures during fieldwork in Cann River were higher compared to the other populations, but no differences in mean ambient temperatures were detected among sites (Fig. 3). Therefore, higher T_b s in this population could be a reflection of an increasing need to remain active for longer, so that daily activities, such as foraging and courtship, are not substantially affected by variation in ambient temperature. Indeed, mean monthly ambient temperatures in the Cann River area (see Point Hicks in Fig. 2) are lower compared to those of Royal (= Sydney in Fig. 2) and Yarratt (= Taree in Fig. 2) during the activity period of the Jacky dragon. Moreover, even though we carried out fieldwork exclusively during sunny, warm days, there was high variation in daily ambient temperatures during the time of our data collection in the Cann River area. This suggests that lizards from Cann River may be more likely to experience thermal stress that might influence their behaviour.

Males from Cann River spend a significant proportion of time interacting with conspecifics compared to other populations (see Chapter 2), so that attaining high T_b s might allow them to sustain a high rate of motion-based signals used during male-male encounters. This idea is supported by the results obtained during our lab experiments. Overall, animals from the three study populations performed more displays used during male-male contests as temperature increased; however, lizards

from Cann River performed a higher number of displays compared to the other two populations. Moreover, during our second experiment individuals from Cann River displayed more, and interacted for longer, when temperatures were high. Therefore, we show not only that ambient temperature has a strong effect on the signaling behaviour of the Jacky dragon, but also that geographic variation in visual displays occurs as a function of variation in thermal regime.

In summary, we have demonstrated that the variation of the thermal environment of the Jacky dragon appears to affect the T_b that animals attain in the field. Also, the frequency of displays increases as temperature increases. Therefore, local adaptation to different thermal regimes might occur, causing a modification of features of the visual displays. Since climate is warming across the globe (Sinervo et al. 2010), the effect of increasing temperatures on the signaling behaviour of the Jacky dragon might have different repercussions depending on where the dragons live. Temperate ectotherms, such as the Jacky dragon, seem to have better chances of survival from increasing ambient temperatures by avoiding high T_b s (Kearney et al. 2009). However, a trade-off could be expected for populations like Cann River that seem to depend on attaining high body temperatures for effective communication.

Behavioural thermoregulation allows mobile animals to avoid critical T_b s, but also imposes a constraint for other activities such as signaling. Social interactions commonly decrease when environmental conditions are adverse, such as ambient temperatures lying outside the thermal tolerance range of a species (Huey et al. 2012). This might interfere with key social behavior such as defending territories or courtship, ultimately impacting their fitness. Although it is well known that sensitivity to thermal heterogeneity is species-specific, our study shows that a population-level effect also occurs. Geographic variation of signaling behaviour, thus, is partly a result of differences in the thermal environment. Animals spanning over large altitudinal

and/or latitudinal gradients are expected to have different thermoregulatory behaviours and modifications in their communicative system. Future research should explore in detail the specific effect of temperature on visual displays and the energetic costs involved in producing these signals.

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CHAPTER 5

Do Jacky dragons trade-off signal efficacy against survival?

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Abstract. Predator avoidance involves the use of multiple strategies that can vary within and between populations of the same species. How animals respond to predators has a strong bearing on fitness. We asked whether antipredator tactics covary with display conspicuousness in the Jacky dragon, *Amphibolurus muricatus*, along the range of the species as a function of predation risk. We quantified predation risk across three populations using four measures: proportion of clay models attacked, number of predators, flight initiation distances and wariness in the lab. We also measured antipredator tactics (active escaping vs. immobility) and changes in the latency and frequency of aggressive displays. We found that predation risk varies among populations. Individuals from populations with higher predation risk tend to be more wary. Furthermore, differences in the latency to display during conspecific signaling among populations were detected in response to the ‘cut-out’ of a raptor used as a stimulus during a lab experiment. We show that predation risk is a good predictor of signaling behavior and this likely represents a trade-off between signal efficacy and survival in a lizard with dynamic visual signals.

Predation risk is considered a major force driving the behaviour of most organisms (Andrén & Nilson 1981; Longland & Price 1991; Rodríguez et al. 2001; Calsbeek & Cox 2010). Individuals face the challenge of performing daily activities without attracting the attention of predators, although most actions involve the risk of being detected by predators (Lima & Dill 1990). Communication in particular, involves increasing an organism's detectability by a predator and therefore, susceptibility to predation (Endler 1992). Animals can use a wide range of signals to convey a message to conspecifics, but those that use visual displays as a communication system are particularly affected by the presence of predators (Zhu et al. 2012). Animals that communicate through visual displays have to expose themselves in areas of their habitat where the transmission of a signal is effective enough to reach the intended receivers (Rodríguez et al. 2001). Therefore, a trade-off between effective communication and survival is inevitable (O'Steen et al. 2010).

Besides a temporal and/or spatial separation as predator avoidance, animals can use a range of strategies to minimize the risk of predation while visually displaying to conspecifics (Lima & Dill 1990; Leal & Rodríguez-Robles 1995). These strategies can be roughly grouped into two categories: modification of signaling behaviour and antipredator responses. In the first case, the properties of the behaviour, either temporal (e.g. the display rate, duration of signals and total time invested to display) or physical (e.g. the amplitude and intensity of the movements), can be altered in order to reduce their conspicuousness to predators (Endler 1987; Simon 2007). In the latter case, animals can react to the presence of predators by actively escaping (i.e. moving away from the threat), relying on crypsis (immobility) or performing a pursuit deterrent behaviour (e.g. signaling to a predator to let it know that it has been detected) (Caro 1995; Stuart-Fox et al. 2004; Fowler-Finn & Hebets 2011). All these strategies impose different physiological and behavioural costs to the

animals that use them, so that choosing them becomes essential for increasing the benefit-cost ratio.

Animals adjust their signaling behaviour or escape tactics based on a multitude of factors including body condition, level of perceived risk, and physiological state and performance capacity (Stankowich & Blumstein 2005), and changes in one or more of these factors can cause a shift in the strategy used. For example, female common lizards, *Zootoca vivipara* (formerly *Lacerta vivipara*), shift their escape tactics from active escape to crypsis when they are gravid (Bauwens & Thoen 1981). Therefore, it is expected that modifications of the signaling behaviour and variation in the antipredator responses occur across populations of wide ranging species, potentially facing different levels of predation risk (Labra & Leonard 1999; Zani et al. 2013). Thus, individuals in a population with high predation risk might increase their chances of survival by reducing their display rates to reduce their detection probability. Conversely, individuals from populations with less predation risk can invest more in sexual displays. While this relationship is a fundamental prediction of signaling theory, it has been rarely explored or tested because of the inherent difficulty in measuring predation risk.

Traditionally, predation risk in the wild has been quantified using both direct and indirect measures. For example, when comparing across populations some studies have: 1) exposed prey in direct contact with predators to measure mortality rates (Longland & Price 1991); 2) measured the diversity and density of predators (Clark et al. 2013); 3) measured strike rates on stationary models of prey (Andrén & Nilson 1981); and 4) quantified behavioural traits of prey as a function of predation risk (Bulova 1994). Most commonly, one or a few of these metrics are used in isolation with limited explanatory power.

Here, we test whether different predator avoidance strategies occur across populations of an agamid lizard, the Jacky dragon (*Amphibolurus muricatus*), as a function of variation in the risk of predation and whether male display behavior is traded-off against predation risk. We first ask whether predation risk is geographically variable and significantly different between populations. Based on these results, we made predictions about individual responses at the population-level in relation to modification of signaling behaviour and antipredator responses (Table 1). Because antipredator behaviour and signal conspicuousness are both likely under selection, we predicted that geographical variation in the risk of predation will have a strong effect on the strategies used by individuals from different populations (Table 1). We also tested whether differences in body condition among populations can explain differences in antipredator strategies.

Table 1. Predictions associated with variation among populations in predation risk, antipredator behaviour and signaling behaviour.

Predation risk	Antipredator behaviour	Signaling behaviour
1. Proportion of attacked models: high proportion indicates higher predation risk	1. Escape tactic: higher proportion of animals using crypsis in populations with higher predation risk	1. Latency to resume visual displays after a 'predatory' attack: higher latency in populations with higher predation risk
2. Number of predator species: more predators represents higher predation risk	2. Flight distance: shorter distances in populations with higher predation risk	2. Proportion of displaying males: lower proportion in populations with higher predation risk
3. Wariness in the wild: larger flight initiation distances indicates higher predation risk	3. Distance to the closest refuge: shorter distances in populations with higher predation risk	
4. Wariness in the lab: stronger response to a simulated predatory attack denotes higher predation risk		

We used the Jacky dragon because is an abundant, wide-ranging species and its signaling behaviour has been described in detail (Peters & Ord 2003; Watt & Joss 2003). The aggressive display is performed only by males and is composed of five movements performed in a ritualistic fashion, including an introductory tail-flick, two arm-waves, push-ups and a body-rock. Furthermore, signaling behaviour appears to be geographically variable (see Chapter 1) and could be affected by population differences in predation risk as a function of differences in predator type and density. The behavioural response of Jacky dragons to looming bird predators has been quantified previously, although only under artificial conditions and with individuals from one population (Carlile et al. 2006). Anecdotal references mention that Jacky dragons appear to rely on crypsis as an anti-predator tactic but flee when detected (Cogger 2000). Little is known about predation of Jacky dragons although potential predators include other lizards (Warner 2007), snakes (Shine 1977) and birds (Marchant & Higgins 1993; Allen et al. 2009). It has been suggested that the biggest threat is from birds (Carlile et al. 2006).

Methods

Study sites

We sampled three sites, separated by over 280 km, along the range of the species: Yarratt state forest (31°48'17.4" S, 152°25'57.5" E), Royal National Park (34°04'49.7" S, 151°05'39.6" E), and Cann River state forest (37°36'25.9" S, 149°09'05.8" E), henceforth referred to as Yarratt, Royal and Cann River respectively. Differences in thermal regimes and forest composition occur across sites (Department of the Environment and Water Resources 2007) and there is significant variation in habitat structure (see Chapter 3), with Yarratt and Cann River

having a deciduous eucalypt woodland and Royal a mixed heathland and eucalypt forest. Sampling was carried out during spring-summer of 2011 and 2012, corresponding with the breeding season of the species (Harlow & Taylor 2000).

Morphology and escape behaviour

When lizards were encountered in the field we quantified wariness and escape behavior (details below) before capturing them. All trials were filmed using a Panasonic HDC-HS60-K digital video camcorder. For each captured lizard, we determined the sex and measured snout-vent length (SVL) with a ruler (to the nearest 0.5 mm) and the body mass with a digital balance (to the nearest 0.1 g). We also determined the sex of individuals that escaped by examining the video of each animal. Male and female Jacky dragons differ in morphology: males are darker and have bigger heads relative to the body than females (Cogger 2000; Harlow & Taylor 2000). We extracted snapshots from videos to compare colouration and, whenever possible, head size between individuals of verified and unverified sex. We then determined an index of body condition by regressing mass against SVL and then using the residuals as a variable. Because several females from all sites were gravid at the moment of data collection, we decided to use data on males only. We included the residuals in an ANOVA as the dependent variable, with population as a fixed factor.

Predation risk and signaling behaviour

We designed an experiment to measure whether males adjust their display rates under predation risk. Ten males were collected from each site and transported to Macquarie University, where they were housed individually in outdoor pens. Each enclosure (180 L x 90 W x 88 H cm) consisted of a sand substrate, branches suitable

for basking, screen for sun cover, water containers, and two refuges (one plastic container and one tile). Trials commenced after lizards were kept under these conditions for at least seven months. During a trial, we first placed a Panasonic HDC-HS60-K digital video camcorder mounted on tripod just outside the enclosure of the focal animal (considered the resident). We then removed one lizard (considered the intruder) from its enclosure and placed it inside the enclosure of the resident behind transparent plexiglass, so they were never in direct contact. We only used two intruders per population and each intruder experienced 4 trials. In order to maximize the probability of the resident displaying, we chose the smallest individuals of each population to be intruders (Mean SVL in mm for Cann River = 82.00, Royal = 85.75, Yarratt = 84.25). Intruders and residents were always from the same population.

We filmed the behaviour of the resident animal following the sequence explained in Figure 1. A session consisted of an initial period that varied in length until the subject performed an aggressive display. Two minutes after this first display, we presented a 'cut-out' of a raptor in flight, attached to a 120 cm pole, to the resident male, to simulate a looming attack. The stimulus was presented for 30 s, followed by a pause of 120 s, and presented again for another 30 s. We call this 3-min period the 'scaring' period. The fake predator was presented only if the resident male displayed aggressively to the intruder. Each session terminated if the resident did not perform any aggressive displays after 35 min, either before or after the 'scaring' period, or as soon as an aggressive display was recorded after the 'scaring' period.

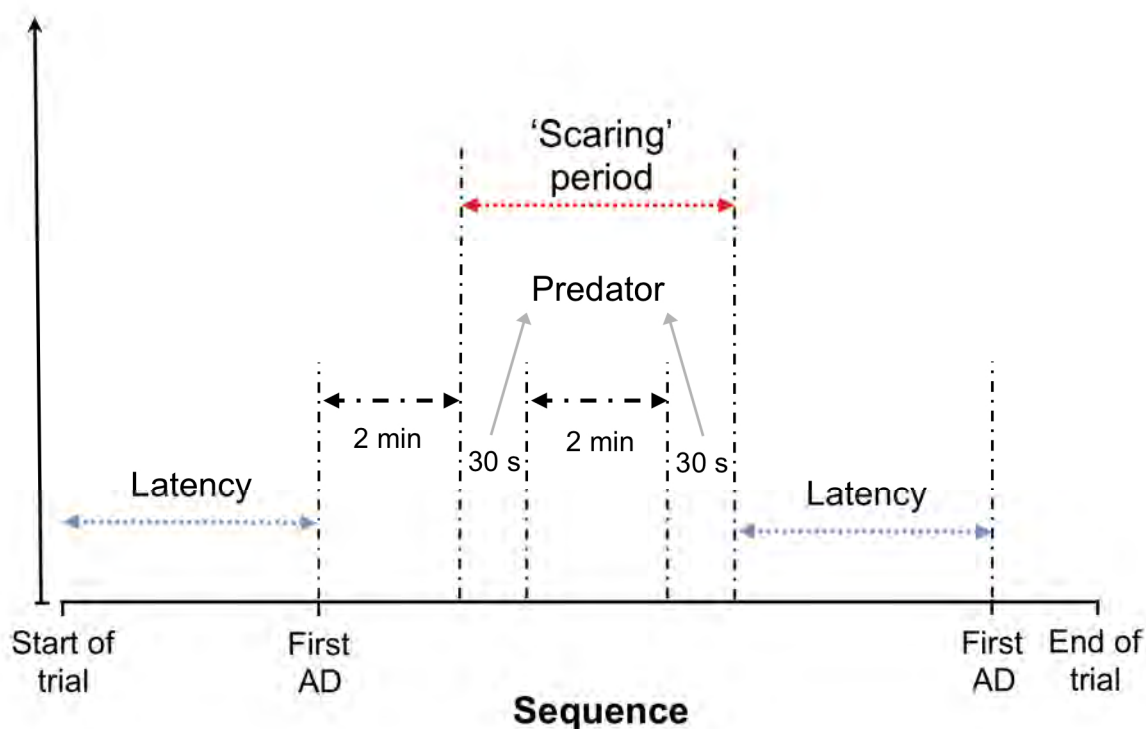


Figure 1. Sequence used during a lab experiment to test the response of Jacky dragons from different populations towards a fake predator while confronted with a conspecific. The ‘scaring’ period is considered from the first introduction of the predator until its final removal from the view of the focal subject.

Variation in predation risk

To quantify variation in predation risk and its relation to population, we used four different measures and, based on the ranks obtained by each population at each measure, we classified our populations as experiencing high or low predation risk. We used a particular classification when it was supported by the results of at least two measures. The following are the measures used:

a) Clay models: We constructed a total of 120 models using Sculpey® III clay by first creating a master template cast from a recently preserved lizard (78 mm SVL). We

used black clay and white, non-toxic paint, mixed to resemble the dark dorsal colouration of male Jacky dragons during the breeding season. We also added the two light stripes to the dorsum. We placed four batches of 10 models at each site (40 models per site), each batch 1 km apart with 20 m between each model. Each model was attached to branches, logs or rocks using adhesive putty (Blu Tack®, Bostik), and all models were left in the bush for 96 h. Each model was photographed at the start and end of each trial. Using these images, we then scored the number and type (bird, mammal or unidentified) of marks imprinted in the model.

b) Predator abundance: We collected information on the number of predator species occurring at each site from the Atlas of Living Australia. This is a digital database that compiles information of all flora and fauna of the country (Belbin 2011). The database allows the user to collect information on the species present in a region enclosed in a circle of up to 10 km in radius. We used this measure to extract the number of species of birds, mammals and reptiles occurring at our study sites, locating the center of the circle as close as possible to the center of the forest patch explored. We then selected species that feed on lizards by consulting multiple field guides (birds: Birdlife Australia; Marchant & Higgins 1993; Higgins 1999; Higgins et al. 2001, 2006; Higgins & Peter 2002, mammals: Van Dyke & Strahan 2008; Menkhorst & Knight 2011, reptiles: Cogger 2000; Cronin 2009). Once a final species list was obtained (Table S1), we estimated species richness for each study site and calculated the Sørensen similarity index, which quantifies of the level of similarity of two species assemblages.

c) Wariness in the wild: In order to quantify wariness and escape behavior in the wild, one of us (MB) walked through each study site until a stationary lizard was detected. The lizard was then approached at a constant speed of ca. 1.33 m/s until the individual moved >10 cm from its perch. We measured flight initiation distance (FID)

defined as the distance at which an animal will permit a (potential) predator to approach before it flees (Bauwens & Thoen 1981; Cooper et al. 2009). Lizards had to move at least 10 cm to avoid including postural adjustments and bouts of movement were considered distinct when separated by at least 2 s. We used Kruskal-Wallis tests for data on males only and both sexes combined to analyze the approaching distance among populations.

d) Wariness in the lab: When lizards were presented with a cut-out of a raptor (details above), we classified the response of the resident male during the ‘scaring’ period as weak (including immobility or minimal position shift) or strong (including running or escaping towards a refuge). We analyzed the proportion of individuals per population showing a particular response by using a Fisher’s exact test.

Variation in antipredator behaviour

We measured the response of lizards in the field to an approaching ‘predator’ (see wariness in the wild) in 2012. We classified the response of each individual as ‘active escaping’ and ‘immobility’ and then counted the number of individuals per population that performed each response. In addition, we measured the ‘flight distance’ defined as the distance an animal flees (to the nearest 1 cm) (Bauwens & Thoen 1981; Cooper et al. 2009), and the distance to the closest refuge, defined as the distance between the original perch where the animal was observed and the closest place or object to hide (e.g. a log, rock, leaf litter). We used a Chi-square test of independence to detect differences across populations in the response of lizards to an approaching ‘predator’. Flight distance was analyzed by using Kruskal-Wallis tests to compare both sexes and populations. The distance to the closest refuge was first log-transformed to improve normality and then included in ANOVAs as the dependent variable, with population and sex as fixed factors.

Variation in signaling behaviour

From the footage obtained during the lab experiment, we quantified latency to the first tail-flick and the first aggressive display before and after the 'scaring' period, and number of displaying males before, during and after the 'scaring' period. Data on tail-flick latencies were not enough to do population comparisons, so that we only present data on the latencies of aggressive displays. Latencies were first log-transformed to improve normality. We used these log-transformed variables as the dependent variable in one-way ANOVAs, with population as a fixed factor. The proportion of displaying males during each period was analyzed using Fisher's exact tests. All statistical analyses were carried out on SPSS Statistics version 20.0 (IBM©).

Results*Predation risk*

The proportion of clay models attacked by predators was not significantly ($X^2 = 3.67$, $df = 2$, $P = 0.160$) different for the three populations. The proportion of individuals 'strongly' responding to the cut-out bird predator was also not significantly (Fisher's exact test, $P = 0.509$) different (Table 2). We did detect differences in the number of predatory species and the flight initiation distance. In the first case, higher numbers of predators were found in Royal and Yarratt compared to Cann River (Table 2). The results from the Sørensen similarity index indicate that all sites share similar assemblages (Cann River vs Royal = 0.660, Cann River vs Yarratt = 0.660, Royal vs Yarratt = 0.875) and therefore, the low number of species observed in Cann River is not likely to be significant. Lizards from Cann River and Yarratt had significantly longer flight initiation distances and were therefore more wary compared

to those from Royal (Kruskal-Wallis including both sexes $H = 11.54$, $df = 2$, $P = 0.003$; Kruskal-Wallis for males only $H = 9.03$, $df = 2$, $P = 0.011$) (Table 2). Therefore, we classified Yarratt as a population with high predation risk and Royal and Cann River as populations with low predation risk, as at least two data sets support this classification.

Antipredator behaviour

We detected variation in the escape tactic used at each population ($X^2 = 10.10$, $df = 2$, $P = 0.006$). Overall, more animals from Royal and Yarratt used immobility compared to those from Cann River (Fig. 2a). Flight distance did not change neither among sexes (Kruskal-Wallis $H = 0.41$, $df = 1$, $P = 0.840$) or populations (Kruskal-Wallis $H = 1.33$, $df = 2$, $P = 0.514$) (Fig. 2b). This result could have been affected by animals that remained immobile and for which we recorded a flight distance of zero. However, after excluding individuals that did not escape from the analysis, we still found no significant (Kruskal-Wallis $H = 3.48$, $df = 2$, $P = 0.176$) differences among populations. Finally, we found that animals from Yarratt were closer to refuges compared to those from the other two populations ($F_{2,81} = 3.83$, $P = 0.026$), although we did not find differences for sexes ($F_{1,81} = 2.28$, $P = 0.135$) or the interaction sex * population ($F_{2,81} = 0.704$, $P = 0.498$) (Fig. 2c).

Table 2. Predation risk experienced by three populations of the Jacky dragon. The significance (Sig.) of the statistical test (NS = non significant, S = significant) used to compare the data among populations is also mentioned. Sample size is included in parentheses for flight initiation distance.

Variable	Population			Sig.
	Cann River	Royal	Yarratt	
Proportion of models attacked	0.35	0.50	0.30	NS
Number of predators	33	67	61	S
Mean rank for flight initiation distance				
Both sexes	55.70 (30)	32.75 (28)	47.09 (32)	S
Males only	31.63 (20)	16.73 (15)	27.66 (16)	S
Proportion of lizards showing a strong response towards a fake predator	0.25 (2 out of 8)	0.50 (3 out of 6)	0.60 (3 out of 5)	NS

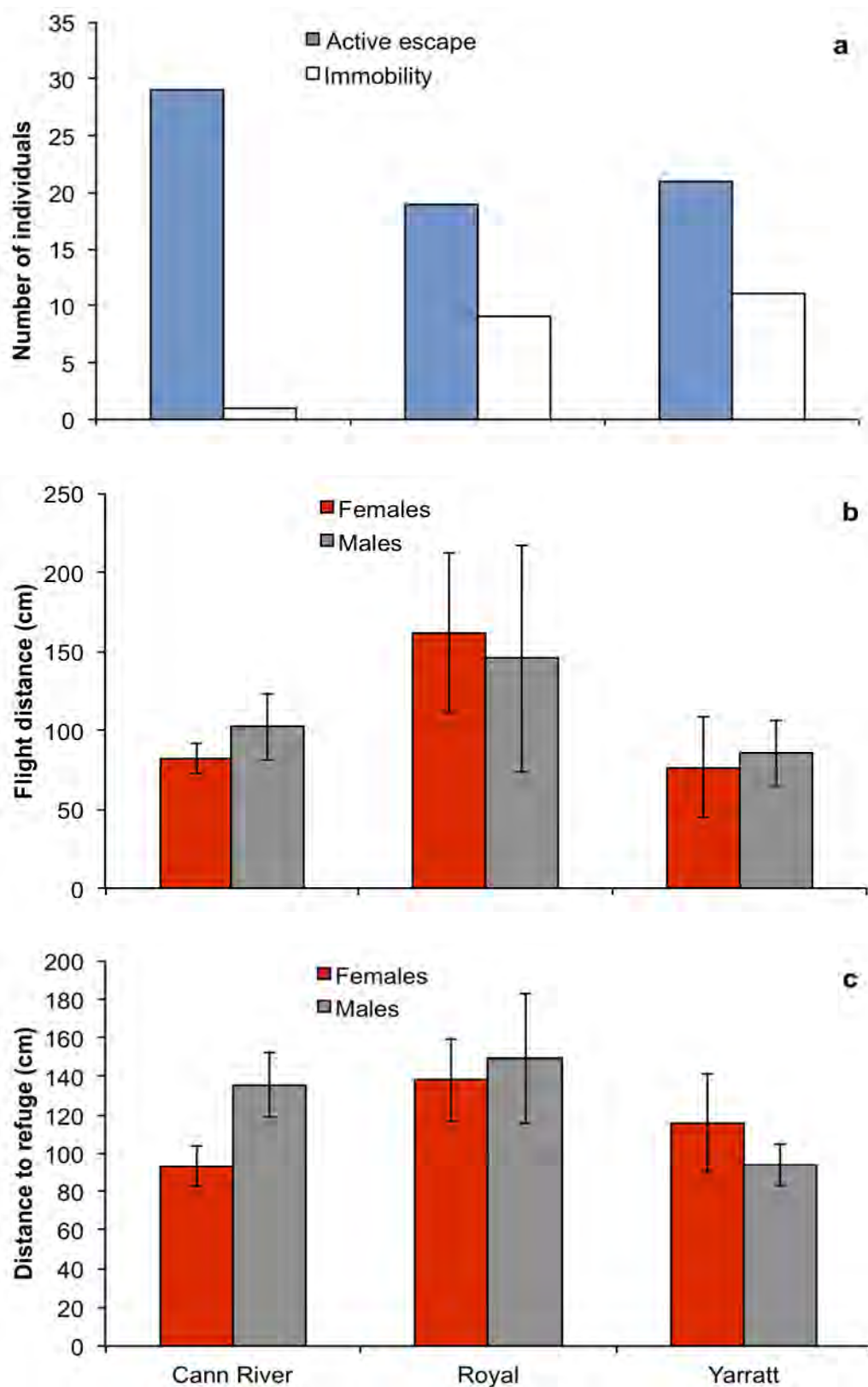


Figure 2. Measurements of antipredator responses towards an approaching predator (human) across populations of the Jacky dragon. Measurements include the number of animals performing an escape tactic (a), mean (\pm SE) distance covered after fleeing (b), and mean (\pm SE) distance to the nearest refuge (c).

Signaling behaviour

Latency to first aggressive display differed significantly among populations after the 'scaring' period ($F_{2,14} = 4.439$, $P = 0.032$) but not before ($F_{2,16} = 1.91$, $P = 0.180$). Lizards from Cann River displayed significantly earlier than those from Yarratt (Fig. 3a). We did not find differences among populations in the proportion of males displaying before (Fisher's exact test, $P = 0.120$) and after (Fisher's exact test, $P = 0.474$) the 'scaring' period. However, we did observe differences in these proportions during the 'scaring' period (Fisher's exact test, $P = 0.004$), with more animals from Cann River performing displays compared to the other two populations (Fig. 3b).

Body condition

Differences across populations were detected in the body condition of males ($F_{2,84} = 6.02$, $P = 0.004$), with animals from Cann River having better condition than those from Yarratt (Fig. 4). No relationship was found between body condition and log-transformed flight initiation distance of males from Yarratt ($r = -0.67$, $P = 0.099$, $N = 7$) and Royal ($r = -0.01$, $P = 0.997$, $N = 11$), although a positive relationship was found for those from Cann River ($r = 0.75$, $P = 0.013$, $N = 10$).

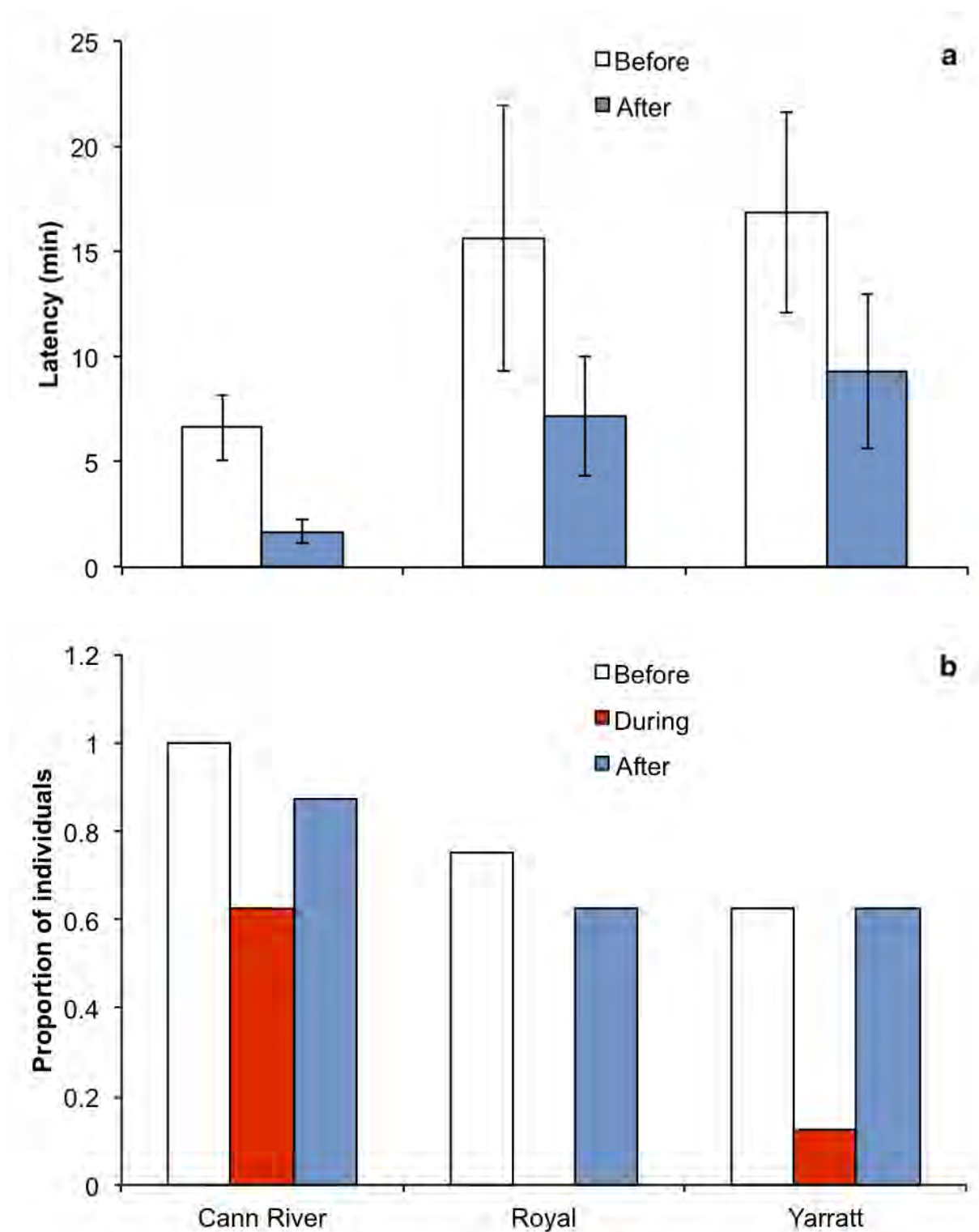


Figure 3. Differences among populations of the Jacky dragon in mean (\pm SE) latency to the first aggressive display (a) and the proportion of males performing aggressive displays (b) before, during and after the presentation of a fake predator.

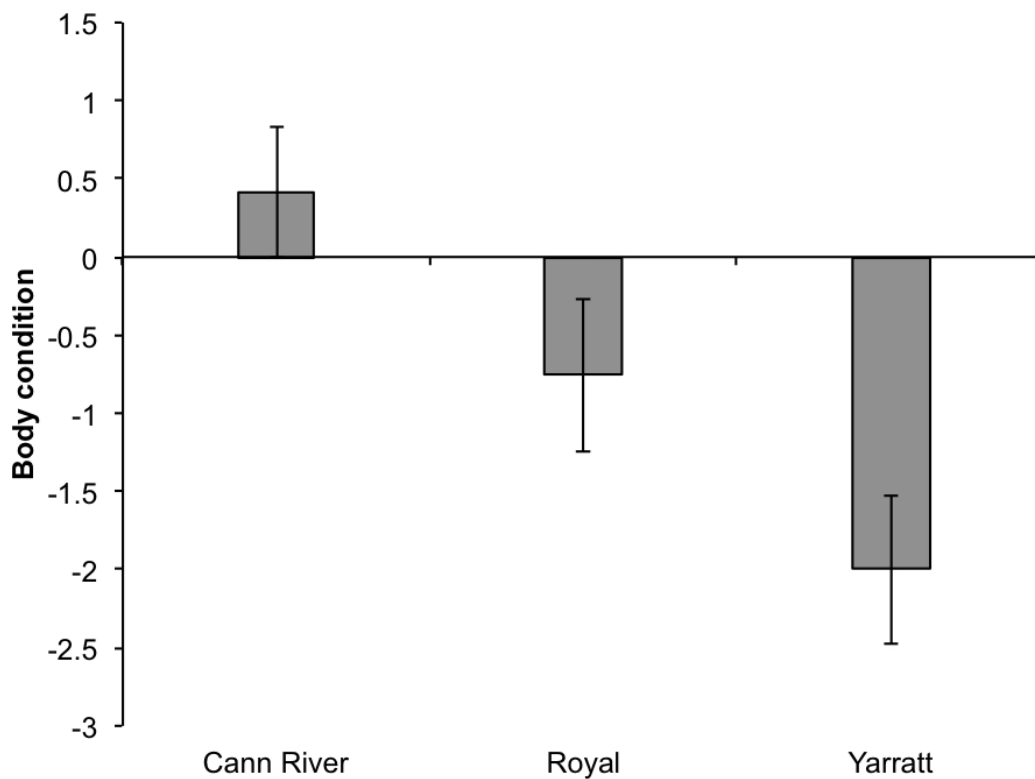


Figure 4. Variation in the relative body condition of males from three populations of *Amphibolurus muricatus*.

Discussion

The way animals respond to predation risk varies both within and between populations. Our study not only confirms that this variation occurs among populations of the Jacky dragon, but also shows that different strategies are used to reduce the detectability and the probability of capture. We show that individuals respond to differences in predation risk by changing the anti-predator strategies, with lizards from populations experiencing higher risk using crypsis more often than those at lower risk. Predation also impacts the properties of the signaling behaviour towards

conspecifics, with less males displaying at the presence of a predator and an increase in the time to resume displaying behaviour.

Interpreting variation in predation risk

Lizards from Yarratt seem to experience higher predation risk compared to those of the other two populations. Predator richness and flight initiation distance, two of our four measures of risk assessment, were consistent with this interpretation. Additionally, a higher proportion of animals from this population reacted more strongly to the presence of the 'cut-out' of a raptor (Table 2). Although the statistical test was not significant in this case, this could be due to the small sample size. Our data also suggest that lizards from Royal could be experiencing higher predation risk compared to those from Cann River. We found more predators and a relatively higher proportion of individuals reacting strongly to the 'cut-out' of a raptor for this population (Table 2). Our results highlight two points. First, the use of multiple measures allowed us to assess more robustly the degree of predation risk experienced by our study populations. Most previous studies have used only one measure to establish predation risk and this might result in ambiguous risk assessments. Therefore, a multi-measure approach to determine the risk of predation is more recommended. Second, it appears that Jacky dragons might experience a "landscape of fear" (i.e. relative levels of predation risk across the range of a species [Hernández & Laundré 2005]), which might decrease the time spent foraging and interacting with conspecifics at areas where the fear is high.

Antipredator strategies and predation risk

We asked whether animals would change their escape and anti-predator tactics according to variation in predation risk. We found that lizards from all populations

were more likely to flee than remain immobile (Fig. 2). For this analysis, we considered immobile animals those that did not move at all when approached by a researcher. This could suggest that active escape is preferred over crypsis in the Jacky dragon. However, in two populations (Royal and Yarratt) we found a significantly high proportion of lizards using immobility, one of which was classified as high predation risk (Yarratt). Moreover, Bauwens & Thoen (1981) suggested that the approach distance (= flight initiation distance) could be used to classify animals as cryptic or fleeing (referred here as active escaping) strategists, with the latter escaping sooner. Thus, our populations could be classified as 'active escaping only' for Cann River (high proportion of active escaping lizards and high approaching distances), 'tendency to crypsis' for Royal (significant proportion of immobile lizards and low approaching distances) and 'mixed strategies' for Yarratt (significant proportion of immobile lizards and high approaching distances).

Schwarzkopf & Shine (1992) also suggest that animals can be assigned to escape categories considering the flight distance and the distance to a refuge and so they added a new category called 'known escape-route'. Animals in this category should flee short distances and/or remain closer to a refuge. Our results show that animals, especially males, from Yarratt remained closer to a refuge compared to the other two populations (Fig. 2) and, even though we did not find significant differences among populations, individuals from Yarratt tended to flee shorter distances (Fig. 2). All these results demonstrate that variation occurs among populations in the escape tactics and that, to some degree, this variation parallels that for predation risk. Thus, in Yarratt, where the risk to be predated seems to be high, animals can remain motionless in the presence of a predator or be very wary by escaping sooner to nearby refuges.

Effects of predators on signaling behaviour and body condition

We were interested in determining whether lizards could reduce the frequency and latency of their displays when confronted with a predator and whether variation in this ability occurs across populations. We found that displaying males from all populations tended to resume signaling to conspecifics soon after predator cues disappeared. However, lizards experiencing high predation risk (Yarratt) took longer to resume signaling than those with lower predation risk (Cann River) (Fig. 3). This pattern of latency after the predator stimulus does not match that of latency before the stimulus, since we did not detect differences among populations for the latter. This indicates that differences among populations occur according to the perceived predation risk. Further corroboration of this result is that we observed more males from Cann River displaying when the fake predator was presented compared to the other two populations (Fig. 3). These results explain the pattern of geographical variation in the characteristics of the signaling behaviour of the Jacky dragon, for which animals from Yarratt were less willing to display during staged male-male encounters (see Chapter 1).

Predators can affect the time that prey allocate to daily activities such as basking and food searching (Lima & Dill 1990). Therefore, we expected to find variation in body condition across populations with different levels of predation risk. Indeed, we found that lizards experiencing high predation risk (Yarratt) were in relative poorer condition compared to those with low predation risk (Cann River) (Fig. 4). Animals with lower body condition must allocate energy to increase survival at the expense of other important activities, such as communication with conspecifics. However, other factors that can affect body condition, such as food availability at each site, must be determined before fully accepting the link between body condition and predation risk.

Conclusions

In this study we have analyzed the variation in predator avoidance of the Jacky dragon, including the antipredator tactics and modification of the signaling behaviour. However, other factors need further analysis, like the role of the colouration of the species as a disruptive patterning when animals are on the ground, especially the leaf litter, so that they can avoid aerial predators. Also, the costs and benefits of perching above the ground, where lizards could be able to avoid terrestrial predators, especially snakes. For example, we witnessed an episode where a lizard jumped to the ground and fled when we approached it, but almost immediately returned at full speed chased by an Eastern Brown Snake (*Pseudonaja textilis*). Our presence probably deterred the snake from continuing the pursuit and the lizard perched on a tree trunk for a few minutes after finally escaping into the bush. Based on the results obtained in this study, we suspect that variation in other factors related with predator avoidance occurs across the distribution of the species.

Natural selection favours different strategies to deal with predators that increase the benefit-cost ratio, so that by adopting the best strategy animals can maximize their future fitness. This study demonstrates that differences in predation risk at the population level significantly affect not only the tactics used to avoid predators but also the communication system and physiological condition of animals. Since population is the basic unit of evolution, all these effects combined can produce different evolutionary pathways for each population.

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Supplementary material

Table S1. List of potential predators of the Jacky dragon occurring at three sites along the distribution of the species.

Taxonomic classification	Cann River	Royal	Taree
Aves			
Caprimulgiformes			
Podargidae			
<i>Podargus strigoides</i>	X	X	X
Charadriiformes			
Burhinidae			
<i>Burhinus grallarius</i>		X	X
Coraciiformes			
Alcedinidae			
<i>Dacelo novaeguineae</i>	X	X	X
<i>Todiramphus pyrrhopygius</i>		X	
<i>Todiramphus macleayi</i>		X	X
<i>Todiramphus sanctus</i>	X	X	X
Cuculiformes			
Cuculidae			
<i>Cacomantis flabelliformis</i>	X	X	X
Centropodidae			
<i>Centropus phasianinus</i>		X	X
Falconiformes			
Accipitridae			
<i>Accipiter fasciatus</i>	X	X	X
<i>Accipiter novaehollandiae</i>		X	X
<i>Accipiter cirrocephalus</i>	X	X	X
<i>Aquila audax</i>	X	X	X
<i>Aviceda subcristata</i>		X	X
<i>Circus approximans</i>	X	X	X
<i>Circus assimilis</i>		X	
<i>Elanus axillaris</i>	X	X	X
<i>Haliaeetus leucogaster</i>	X	X	X
<i>Haliastur sphenurus</i>	X	X	X
<i>Hieraaetus morphnoides</i>	X	X	X
<i>Lophoictinia isura</i>		X	X
<i>Milvus migrans</i>	X	X	
Falconidae			
<i>Falco berigora</i>	X	X	X
<i>Falco cenchroides</i>	X	X	X
<i>Falco longipennis</i>	X	X	X
<i>Falco peregrinus</i>		X	X
<i>Falco subniger</i>		X	

Passeriformes			
Artamidae			
<i>Artamus cyanopterus</i>	X	X	X
<i>Cracticus nigrogularis</i>		X	X
<i>Cracticus tibicen</i>	X	X	X
<i>Cracticus torquatus</i>	X	X	X
<i>Strepera versicolor</i>	X	X	
<i>Strepera graculina</i>	X	X	X
Corvidae			
<i>Corvus coronoides</i>	X	X	X
<i>Corvus mellori</i>	X	X	X
<i>Corvus orru</i>			X
Meliphagidae			
<i>Acanthagenys rufogularis</i>		X	X
Pachycephalidae			
<i>Colluricincla harmonica</i>	X	X	X
Ptilonorhynchidae			
<i>Ailuroedus crassirostris</i>		X	X
Sturnidae			
<i>Sturnus tristis</i>		X	X
Strigiformes			
Strigidae			
<i>Ninox connivens</i>		X	
<i>Ninox novaeseelandiae</i>	X	X	X
<i>Ninox strenua</i>	X	X	X
Tytonidae			
<i>Tyto alba</i>		X	
<i>Tyto javanica</i>		X	X
<i>Tyto novaehollandiae</i>		X	X
<i>Tyto tenebricosa</i>		X	X
Mammalia			
Carnivora			
Canidae			
<i>Canis lupus</i>		X	X
<i>Vulpes vulpes</i>	X	X	X
Felidae			
<i>Felis catus</i>	X	X	X
Dasyuridae			
<i>Dasyurus maculatus</i>	X	X	X
<i>Phascogale tapoatafa</i>			X
Muridae			
<i>Rattus rattus</i>		X	X
Reptilia			
Squamata			
Boidae			
<i>Morelia spilota</i>	X	X	X

Colubridae			
<i>Boiga irregularis</i>		X	X
<i>Dendrelaphis punctulatus</i>		X	X
Elapidae			
<i>Acanthophis antarcticus</i>		X	X
<i>Cacophis krefftii</i>			X
<i>Cacophis squamulosus</i>		X	
<i>Cryptophis nigrescens</i>	X	X	X
<i>Demansia psammophis</i>		X	X
<i>Furina diadema</i>		X	X
<i>Hemiaspis signata</i>		X	X
<i>Hoplocephalus bitorquatus</i>			X
<i>Hoplocephalus bungaroides</i>		X	
<i>Hoplocephalus stephensii</i>			X
<i>Notechis scutatus</i>		X	
<i>Pseudechis porphyriacus</i>	X	X	X
<i>Pseudonaja textilis</i>	X	X	X
Pygopodidae			
<i>Lialis burtonis</i>		X	X
<i>Pygopus lepidopodus</i>		X	X
Varanidae			
<i>Varanus rosenbergi</i>		X	
<i>Varanus varius</i>		X	X

CONCLUSIONS

The major theme of my thesis centers on geographic variation in the signaling behaviour of the Jacky dragon, *Amphibolurus muricatus*, and the potential causes and implications. Although the Jacky dragon has been a model system commonly used to answer questions in disciplines such as behavioural ecology, development and evolutionary biology (Harlow & Taylor 2000; Peters 2008; Warner & Shine 2008), my focus on the ecology and evolution of display behaviour at a population-level is novel for the species and can be generalised to other systems.

One of the most important results obtained from my thesis is the lability of certain features of the Jacky dragon' signaling system. Previous studies on the visual displays of another lizard species (*Anolis carolinensis*), have found both lability and stability (Lovern et al. 1999; Bloch & Irschick 2006), so the results of my study contribute important data for future comparative analyses. I also collected tissue samples and measured morphological traits from both live animals and museum specimens across the distribution of the species. This information was used to analyze the phylogenetic structure of the species (Pepper et al. 2014). I was able to use the genetic information from this study to examine the relatedness of three populations that I studied in some detail. I found that the properties of the visual displays varied across populations regardless of the degree of genetic relatedness, suggesting that signal lability could be a result of behavioural plasticity. This means that an individual could produce different behavioural responses depending on the environmental conditions in which it lives.

My results also suggest that complexity and structure of habitat does not explain the variation in signaling behaviour. I found that the characteristics of the habitats occupied by my study populations differed among each other, although in a

discordant pattern with respect to that of the visual displays. Other factors, however, showed a significant effect on the properties of the signaling behaviour. Populations with high predation risk showed a decrease in display rate and an increase in the time to resume signaling after a fake predatory attack. In addition, an increase in ambient temperature appears to increase the number of visual displays. I consider that multiple factors could explain the lability detected in the display behaviour of the Jacky dragon.

Despite several properties of the signaling behaviour of *Amphibolurus muricatus* varying across populations, the core sequence of ritualized movements performed during aggressive displays remained unchanged. This was supported by my finding of similar levels of aggression within and between populations, suggesting that the species retains a cohesive communication system that is recognized by individuals from dissimilar populations. Nevertheless, dominance varied among populations with at least one population being dominant over the others. This poses a problem for dispersing animals from subordinate populations in the face of dominant individuals. However, more research is required to understand the specific effects on fitness for animals within and between populations

Future directions

The results generated during this project have answered a few questions about signal divergence in the Jacky dragon. However, not all aspects that potentially modify the communicative system of this lizard have been investigated and more in-depth analysis for the factors quantified here are still required. For example, the recent phylogeny of the species has identified five distinct clades (Pepper et al. 2014), although we only sampled populations of two of those clades. Furthermore, our study populations, although far apart from each other, are located in the central

and southeastern areas of the species distribution and all are from lowlands. Therefore, to fully understand the degree of variation in the signaling behaviour of the Jacky dragon is necessary to sample locations all across the range of the species that include all the phylogenetic clades. Sites strikingly different in habitat structure and thermal conditions would be of great interest to compare with the results from my study.

Several other factors might also be influencing the lability of Jacky dragon visual displays. Some factors that I did not include in my study are:

1. Sexual selection: Female preferences for specific traits of males can change both spatially and temporarily. Female lizards use several cues to choose between males, such as size, stamina, colouration and visual displays. Therefore, when variation in these traits occurs among populations, it could be predicted that female preferences will also change. Several questions can be asked; for example: 1) do females show a population-specific mate preference for behavioural cues?; 2) to what extent female mate choice is influenced by signaling of males?; and 3) are visual displays more important than other cues to choose among males?
2. Species recognition: Signal design could diverge in order to avoid useless and potentially costly interactions with heterospecifics, such as hybridization and agonistic encounters. More divergent signal designs are expected when closely-related species occur in sympatry (i.e. character displacement; Losos 2000). The Jacky dragon and a close relative, the Nobbi dragon (*Diporiphora nobbi*), are sympatric in some areas of northern New South Wales, Australia, so that their visual displays are expected to differ among allopatric and sympatric populations.
3. Population density: the number of individuals and the spatial distribution of resources can differ between habitats. A high number of individuals and a clumped distribution of resources will decrease the distance between individuals and increase

their interactions. Conversely, low numbers of animals and evenly distributed resources will have the opposite effect. It is expected, hence, that characteristics of the signaling behaviour, such as display rate and latency to display, vary across populations as a function of population density.

In summary, my research has improved our knowledge on signal divergence at a population-level and has identified factors influencing this divergence. At the same time, this study opens up a whole new set of questions that remain to be answered.

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APPENDIX I

The following is a paper that I collaborated with during my PhD:

Pepper, M., M.D. Barquero, M.J. Whiting & J.S. Keogh. 2014. A multi-locus molecular phylogeny for Australia's iconic Jacky Dragon (Agamidae: *Amphibolurus muricatus*): Phylogeographic structure along the Great Dividing Range of south-eastern Australia. *Molecular Phylogenetics and Evolution* 71: 149–156.



Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

A multi-locus molecular phylogeny for Australia's iconic Jacky Dragon (Agamidae: *Amphibolurus muricatus*): Phylogeographic structure along the Great Dividing Range of south-eastern Australia

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ARTICLE INFO

Article history:

Received 25 June 2013

Revised 14 November 2013

Accepted 20 November 2013

Available online 6 December 2013

Keywords:

Great Dividing Range

Phylogeography

Reptile

Agamidae

Eastern Australia

*BEAST

ABSTRACT

Jacky dragons (*Amphibolurus muricatus*) are ubiquitous in south-eastern Australia and were one of the first Australian reptiles to be formally described. Because they are so common, Jacky dragons are widely used as a model system for research in evolutionary biology and ecology. In addition, their distribution along the Great Dividing Range of eastern Australia provides an opportunity to examine the influence of past biogeographical processes, particularly the expansion and contraction of forest habitats, on the diversification of this iconic agamid lizard. We generated sequence data for two mitochondrial and three nuclear DNA loci (4251 base pairs) for 62 Jacky dragons sampled from throughout their distribution. Phylogenetic analyses based on maximum likelihood and Bayesian species-tree methods revealed five geographically structured clades separated by up to 6% mitochondrial and 0.7% nuclear sequence divergence. We also quantified body proportion variation within and between these genetic clades for more than 500 specimens and found no evidence of any significant differentiation in body proportions across their range. Based on body proportion homogeneity and lack of resolution in the nuclear loci, we do not support taxonomic recognition of any of the mitochondrial clades. Instead, *A. muricatus* is best thought of as a single species with phylogeographic structure. The genetic patterns observed in the Jacky dragon are consistent with fragmented populations reduced to multiple refugia during cold, arid phases when forested habitats were greatly restricted. Consequently, the inferred biogeographic barriers for this taxon appear to be in line with lowland breaks in the mountain ranges. Our results are congruent with studies of other reptiles, frogs, mammals, birds and invertebrates, and together highlight the overarching effects of widespread climatic and habitat fluctuations along the Great Dividing Range since the Pliocene.

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1. Introduction

The Jacky dragon, *Amphibolurus muricatus* White, ex Shaw 1790, is a widespread and abundant agamid lizard found throughout the temperate and coastal habitats of south-eastern Australia (Cogger, 2000). Its distribution includes the location of the first Australian settlement in Port Jackson (Sydney Harbour) in 1788, and the Jacky dragon (along with five other lizards) was among the first Australian reptiles to be formally described in a published diary of Surgeon General John White (White, 1790).

Because they are so common and easy to rear in captivity, Jacky dragons have become a popular model system for exploring diverse evolutionary questions relating to complex visual signals (Peters and Ord, 2003; Peters and Evans, 2007; Van Dyk and Evans,

2007; Hoese et al., 2008; Peters, 2008; Woo et al., 2009; Woo and Rieucou, 2013), temperature-dependent sex determination (Harlow and Taylor, 2000; Warner and Shine, 2008, 2011) and other aspects of reproductive ecology (Warner et al., 2007, 2010; Radder et al., 2007a; Shine et al., 2007), physiology (Heatwole et al., 1973; Heatwole and Firth, 1982; Watt et al., 2003; Warner and Shine, 2006; Radder et al., 2007b), and habitat fragmentation (Bragg et al., 2005; Hitchen et al., 2011). Despite this body of work, no phylogenetic hypothesis of relationships among populations from throughout their range has been available for interpreting these data in an evolutionary context.

In addition, the broad distribution of Jacky dragons down the eastern and south-eastern margins of Australia, encompassing vast differences in climate and habitat, makes them an ideal system for examining emerging patterns concerning the biogeography of the Great Dividing Range – a chain of mountain systems forming the dominant topographic feature in eastern Australia. It is well known that climatic conditions during glacial cycles were colder and drier

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in the tropics (Hewitt, 2000), leading to the expansion of deserts and savannah while reducing rain forests. Along the humid margins of eastern Australia, geological and palaeontological records show cycles of rainforest expansion and contraction in line with glacial and interglacial oscillations (Nix, 1982; Markgraf et al., 1995). Evidence of extensive sand dune activity also has been discovered beneath these presently forested regions, indicating substantially drier conditions during the Last Glacial Maximum (LGM) (Thom et al., 1994; Hesse et al., 2003). Genetic studies of numerous taxa along the Great Dividing Range show congruent phylogenetic patterns indicative of persistence in higher elevation mesic refugia during more arid phases, with deep genetic breaks across lowland areas inferred to be historically dry biogeographic barriers to dispersal (eg. Horton, 1972; Schauble and Moritz, 2001; Chapple et al., 2005; Bell et al., 2010; Milner et al., 2012). In addition, topographic barriers such as mountain ranges, river valleys or disjunct upland regions also are thought to have played an important role in shaping the evolutionary history of modern biota in this region (Chapple et al., 2011; Pavlova et al., 2013).

Understanding the genetic structure and relationships between populations of Jacky dragons is long overdue. Recent phylogenetic studies have placed *A. muricatus* within the *Amphibolurus* group of the Australian agamid radiation *Amphibolurinae* (Schulte et al., 2003; Hugall et al., 2008). This clade comprises its sister taxon *Amphibolurus norrisi*, in addition to *Amphibolurus gilberti* and the iconic frill-necked lizard *Chlamydosaurus kingii*. More recently, Melville et al. (2011) sequenced a number of *A. muricatus* from the northern and southern parts of their range, and found structure in both the *nd2* mtDNA and the nDNA *rag1* loci, highlighting the need for further study and increased sampling. Here we present a comprehensive phylogeny based on five loci and a detailed morphological data set for the Jacky dragon. In addition we compare our results to other published studies to assess the history of species diversification and historical biogeography along the Great Dividing Range of Eastern Australia.

2. Materials and methods

2.1. Taxonomic sampling

We obtained sequence data from 62 *A. muricatus* (Table 1). We collected specimens from Victoria and New South Wales between 2011 and 2012, and the remaining samples were obtained for all unique localities that were available from the collections of Museum Victoria (MV), the South Australian Museum (SAM) and the Australian Museum (AM). We were unable to obtain material from Queensland, where, except on the Granite Belt along the southern border where it is a common lizard, *A. muricatus* is otherwise a rare species in the remainder of the State (R. Hobson, Queensland Parks & Wildlife Service, pers. comm., and see Atlas of Living Australia records). We also included Genbank data from previous studies including *nd2* sequences for nine *A. norrisi* (Melville et al., 2011), as well as for twelve outgroup taxa for four of the five genes used in this study (*nd2*, *nd4*, *bdnf*, *rag1*) (see Appendix Table 1 for all Genbank material used in this study).

2.2. DNA amplification and sequencing

We collected new sequence data for five loci: two mitochondrial (*nd2* and *nd4*) and three protein-coding nuclear (*rag1*, *bdnf* and *prlr*). The data matrix for *A. muricatus* samples is 98% complete, and of the twelve outgroup taxa, all are missing *prlr*, and 10 are missing *nd4*. All new sequences from this study are deposited on Genbank (KF871470–KF871767).

Genomic DNA was extracted from approximately 1 mm³ of tissue (either a tail tip or liver sample stored in 100% ethanol) using the EDNA HiSpEx tissue kit (Chaga), following the manufacturers protocols. PCR amplification was performed using a Corbett PC-960C cooled thermal cycler and negative controls were run for all amplifications. Amplification of all loci was conducted with an activation step at 94 °C for 3 min followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s, with a further 25 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 90 s, followed by a final extension step at 72 °C for 5 min. PRC amplification was performed in a 25 µl reaction mix consisting of 12.5 µl GoTaq (Promega), 10.5 µl double-distilled water, 1.0 µl for both forward and reverse primers (10 pmol) and 1 µl of template DNA. We purified successful PCR amplifications using 0.4 µl Exonuclease 1 (New England BioLabs), 1.6 µl Shrimp Alkaline Phosphatase (GE Healthcare) and 3 µl double-distilled water along with 5 µl undiluted PCR product. For cycle-sequencing reactions and purification see the protocols of Pepper et al. (2006). The PCR/Sequencing primer combinations used in this study are listed in Table 2.

Sequences were edited and contigs were assembled in SEQUENCHER v. 4.10.1 (Gene Codes Corporation). Alignment of sequences was first performed automatically using the software MUSCLE (Edgar, 2004), then refined by eye in Se-Al (Rambaut, 1996). The few heterozygotes found in the nDNA genes were coded as such in the alignments prior to analyses. Protein-coding regions were translated into amino acid sequences and were checked for internal stop codons and frame-shift mutations.

2.3. Analysis of sequence data

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian methods. Initially, ML analyses of individual gene trees were performed in RAxML-VI-HPC v7.0.4 (Stamatakis, 2006). We then concatenated the data and partitioned the combined dataset by gene. Each analysis implemented the general time-reversible substitution model with gamma-distributed rates among sites (GTR + G), with the best ML tree determined using 20 distinct randomized Maximum Parsimony (MP) starting trees. Bootstrap support was determined using 1000 replicates.

In addition to our concatenated ML approach, we used the hierarchical model implemented in *BEAST v. 1.7.5 (Heled and Drummond, 2010), that co-estimates the species tree and all gene trees in one Bayesian MCMC analysis, to estimate the species tree phylogeny. This method requires *a priori* designation of species or groups, so we divided our data based on the five genetic clades identified in our concatenated likelihood analysis, along with the outgroups that were all coded as separate species. Models for each gene were inferred using PartitionFinder (Lanfear et al., 2012) under the Bayesian Information Criterion. For *nd2* and *nd4*, the TVM + I + G substitution model was chosen, while for *rag1*, *bdnf* and *prlr* the K81uf (equivalent to K3Puf) + I substitution model was chosen. The TVM and K3Puf substitution models are not available in the BEAST package so we modified the xml code for the GTR substitution model to accommodate changes to the model parameters (http://beast.bio.ed.ac.uk/Substitution_model_code). We unlinked loci and substitution models, employed a relaxed-clock model, and used a Yule tree prior. For the mean rate priors for the relaxed clock model, we specified a normal distribution with a lower bound of 1e-6 for the mtDNA genes, and 1e-3 for the nDNA loci, both with an upper bound of 1. We conducted four separate runs, with samples drawn every 10,000 steps over a total of 100,000,000 steps, with the first 10% discarded as burn-in. Acceptable convergence to the stationary distribution was checked by inspecting the posterior samples using the diagnostic software Tracer v1.5 (Rambaut and Drummond, 2007). Effective sample sizes

Table 1

Museum accession numbers and collection locality information for all individuals sampled in this study. MB = Marco Barquero, LS = Lisa Schwanz.

CLADE	LAB#	Registration Number	Institution	Scientific name	State/territory	Locality	Latitude	Longitude
A	J03	R167627	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Tenterfield region	−29.07333	151.91278
A	J06	R148385	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Gibraltar Range National Park	−29.51389	152.34333
A	J07	R148375	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Gibraltar Range National Park	−29.53306	152.32361
A	J09	R153806	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Werrikimbe National Park	−31.10000	152.23333
A	J11	R156613	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Limbri	−31.00278	151.19500
A	J12	R157019	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Yarrowick Area	−30.47250	151.37139
A	J13	R157211	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Forestlands State Forest	−29.23944	152.10306
A	J14	R157215	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Tenterfield, Gunyah road	−29.11556	151.88694
A	J15	R157220	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Bolivia	−29.33722	151.89444
A	J16	R157222	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	33.5 Km E Glen Innes	−29.51111	152.04944
A	J18	R159678	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Glen Innes	−29.51111	152.04944
A	J32	SAMAR34730	South Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Lily Creek	−30.80000	151.30000
A	J33	SAMAR39022	South Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Retreat	−30.68333	150.23333
B	J10	R.156047.001	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Warrumbungle National Park	−31.32889	148.99667
B	J25	SAMAR34771	South Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Coonabarabran	−31.25000	149.13333
B	J27	SAMAR34769	South Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Coonabarabran	−31.25000	149.13333
B	J31	SAMAR39147	South Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Brayton	−34.56667	149.98333
B	J34	A1	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Mt. Nowa Nowa	−37.69157	148.09131
B	J35	A2	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Mt. Nowa Nowa	−37.69157	148.09131
B	J36	A3	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Mt. Nowa Nowa	−37.69157	148.09131
B	J37	A4	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cape Conran	−37.81180	148.72918
B	J38	A5	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cann River area	−37.61691	149.14502
B	J39	A6	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cann River area	−37.62601	149.13962
B	J40	A7	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cann River area	−37.59713	149.16162
B	J41	A8	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cann River area	−37.60382	149.15379
B	J42	A9	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Genoa Peak	−37.48377	149.57635
B	J48	B6	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Mt. Nowa Nowa	−37.69157	148.09131
B	J53	C2	MB collection	<i>Amphibolurus muricatus</i>	Victoria	Cann River area	−37.59471	149.16295
C	J01	R172498	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Faulconbridge	−33.69250	150.53306
C	J02	R171161	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Singleton area	−32.55972	151.35194
C	J04	R146138	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Holsworthy Training Area	−34.00250	150.93667
C	J05	R147368	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Bird Island Nature Reserve	−33.21667	151.60000
C	J08	R150447	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Chifley	−33.95000	151.23333
C	J17	R157299	Australian Museum	<i>Amphibolurus muricatus</i>	New South Wales	Scheyville Road	−33.60278	150.85333
C	J43	B1	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Royal National Park	−34.08327	151.02509
C	J44	B2	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Royal National Park	−34.08172	151.02557
C	J45	B3	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Botany Bay	−33.99244	151.23925
C	J46	B4	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Botany Bay	−33.99294	151.23916
C	J47	B5	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Botany Bay	−33.99509	151.24012
C	J49	B7	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Royal National Park	−34.08038	151.09471
C	J50	B8	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Yarratt Taree	−31.82128	152.42461
C	J51	B9	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Yarratt Taree	−31.80483	152.43264
C	J52	C1	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Yarratt Taree	−31.82108	152.42464
C	J54	C3	MB collection	<i>Amphibolurus muricatus</i>	New South Wales	Yarratt Taree	−31.80764	152.42611
C	J55	AMC1	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Wirreanda Rd, Wamboin	−35.252914	149.342941
C	J56	AMA5C2	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Wirreanda Rd, Wamboin	−35.224514	149.35199
C	J57	AMD2	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Kestral Place, Bywong	−35.221008	149.336731
C	J58	AMA4C5	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Burra Rd, Burra	−35.647289	149.215408
C	J59	AMA3B4	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Kioloa Headland	−35.55994722	150.3837083
C	J60	AMA4C2	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Burra Rd, Burra	−35.660873	149.209305
C	J61	AM4	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Pony Club, Birriwa Road, Bywong	−35.18155278	149.3375528
C	J62	AMD5	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Myrtle Beach	−35.70282778	150.279875
C	J63	AMB4	LS collection	<i>Amphibolurus muricatus</i>	New South Wales	Denley & Kestral, Bywong	−35.221176	149.336411
D	J28	SAMAR49467	South Australian Museum	<i>Amphibolurus muricatus</i>	South Australia	Nangwarry	−37.45444	140.87528
D	J29	SAMAR49376	South Australian Museum	<i>Amphibolurus muricatus</i>	South Australia	Donovans	−38.00778	140.96250
D	J30	SAMAR49374	South Australian Museum	<i>Amphibolurus muricatus</i>	South Australia	Donovans	−38.00778	140.96250
E	J19	Z11557	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Steels Creek	−37.57972	145.37000
E	J20	Z18832	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Kinglake National Park North	−37.53417	145.22528

(continued on next page)

Table 1 (continued)

CLADE	LAB#	Registration Number	Institution	Scientific name	State/territory	Locality	Latitude	Longitude
E	J21	Z7821	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Brisbane Ranges National Park	–37.82000	144.27000
E	J22	Z7823	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Brisbane Ranges National Park	–37.82000	144.27000
E	J23	Z7861	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Steels Creek	–37.57972	145.37000
E	J24	Z7866	Museum Victoria	<i>Amphibolurus muricatus</i>	Victoria	Brisbane Ranges National Park	–37.82000	144.27000
F	J26	SAMAR49532	South Australian Museum	<i>Amphibolurus norrisi</i>	South Australia	Padthaway	–36.71778	140.44417

Table 2

Marker information used in this study.

Gene	Abbreviation	Primer name	Size (bp)	TA (°C)	Primers (5'–3')	Primer source
NADH dehydrogenase subunit 2	<i>nd2</i> <i>nd2</i>	Metf-1 CO1r.aga	1016	55–50	AAGCAGTTGGGCCCATRCC ACRGTTCCRAATCTCTTTTGTGRTT	Macey et al. (2000) Modified from H5934 (Macey et al. (1997)) This study
NADH dehydrogenase subunit 4	<i>nd2</i> <i>nd4</i> <i>nd4</i>	nd2Jacky-F1 ^a ND4-1 LEU	732	55–50	TTTCAAGCACATTAATGC TGACTACCAAAAGCTCATGTAGAAGC TACTTTTACTTGGAATTGCACCA	Arevalo et al. (1994) Modified from Leu (Arevalo et al. (1994))
Recombination activating gene 1	<i>rag1</i> <i>rag1</i> <i>rag1</i>	rag1-Faga rag1-Raga JackyIntF ^b	1205	55–50	CAAAGTGAGACSACTTGGAAAGCC CATTTTTCAGGGTGGTTTCCACTC ATCCTGGCCAGATCTCCTTGC	Shoo et al. (2008) Shoo et al. (2008) This study
Prolactin receptor	<i>prlr</i> <i>prlr</i>	prlr-F1 prlr-R3	580	55–50	GACARYGARGACCAGCAACTRATGCC GACYTTGTGRACCTCYACRTAATCCAT	Townsend et al. (2008) Townsend et al. (2008)
Brain-derived neurotrophin factor	<i>bdnf</i> <i>bdnf</i>	bdnf-F bdnf-R	718	55–50	GACCATCCTTTTCTKACTATGGTTATTCATCTT CTATCTCCCTTTTAAATGGTCAGTGACAAAC	Townsend et al. (2008) Townsend et al. (2008)

TA: Temperature of annealing in PCR reaction.

^a Used as an internal sequencing primer.

were >200 for all parameters. All runs produced the same topology with very similar posterior probabilities, so we combined runs to generate a single consensus tree.

2.4. Analysis of body size and shape data

We evaluated body proportion data from 537 specimens collected as part of ongoing behavioral work by MDB and from specimens in the collections of the AM and MV. The following body size measurements were taken with electronic calipers to the nearest 0.1 mm: SVL – snout-vent length; TailL – tail length; InterL – inter-limb length; HeadL – head length; HeadW – head width; HeadH – head height; ForeL – forelimb length; HindL – hindlimb length.

Most of our genotyped animals also formed part of our morphological data set and for the remaining specimens used in the morphological analysis we had to assign them to genetic clades to evaluate morphological differences (if any) between them. We did this by overlaying the locality information of all genotyped and non-genotyped specimens (see Appendix Fig. 1) and we only assigned them to a genetic clade if their locality unambiguously fell in the geographic range of a genetic clade. This allowed us to assign 437 specimens in the morphological data set to genetic clades, and we excluded 101 specimens that were distributed close to the geographic boundaries of genetic clades.

Our primary interest was the evaluation of body shape differences (if any) between well-supported genetic clades. We evaluated this in two ways on natural log transformed data. We used Principal Components Analysis (PCA) with variance-covariance, which does not identify groups *a priori*, and Discriminant Function Analysis (DFA), where the groups were specified *a priori*, to examine the patterns of relationship and discriminating power of the

body proportion characters (natural log transformed) with the statistics software JMP v. 8.0. The first PC represented variation in body size and the second PC summarized shape differences. We performed DFA on the ln-transformed data to examine if body shape differences would be sufficient to distinguish genetic clades when they were specified *a priori*.

Where possible, individuals were scored for sex by the presence of inverted hemipenes, eggs, or by dissection. Jacky dragons are sexually dimorphic in relative head size (Harlow and Taylor, 2000) but for several of the genetic clades we did not have sufficient sample sizes of sexed animals to perform separate analyses of males and females. Therefore, we performed each analysis described above both with and without head length, width and height data. These analyses gave the same results so we only present analyses based on all the data.

3. Results

3.1. Phylogenetic analyses

Following the removal of ambiguously aligned nucleotide sites, the final *nd2* dataset consisted of 1016 base-pairs (bp), *nd4* consisted of 732 bp, *rag1* consisted of 1205 bp, *bdnf* consisted of 718 bp, and *prlr* consisted of 580 bp, totaling 4251 bps for the concatenated dataset. The gene trees for *nd2* and *nd4* exhibited phylogeographic structure with well supported clades but with unresolved relationships among them. The individual gene trees for the nuclear loci showed extremely low variability, with *prlr* and *bdnf* appearing as a single or a series of shallow combs. *Rag1* exhibited more structure, but this was not always consistent with the structure identified in the mtDNA loci. The maximum

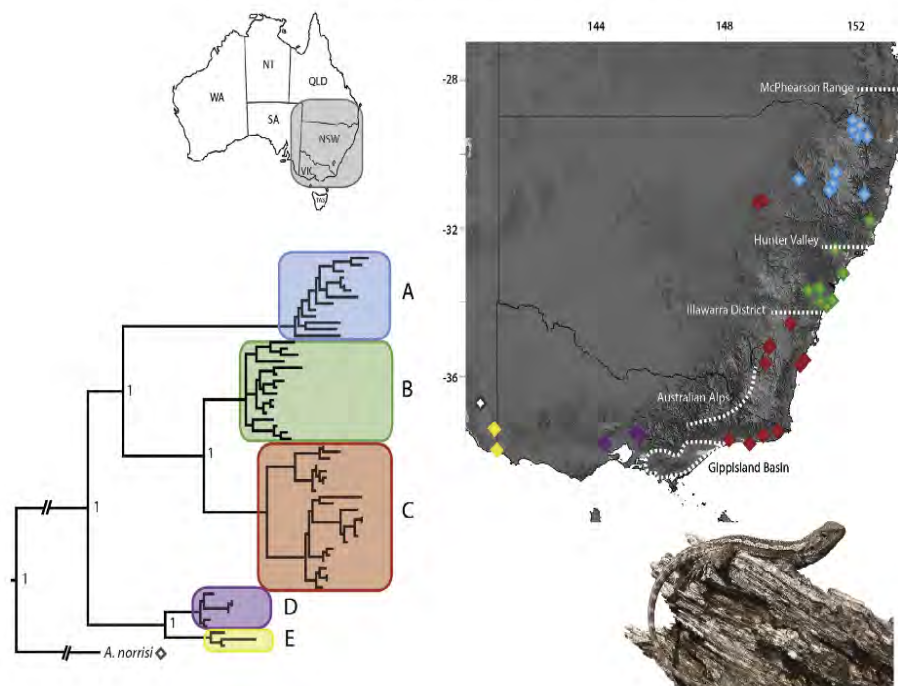


Fig. 1. Phylogeny of 62 *Amphibolurus muricatus* samples and outgroups based on the combined *nd2*, *nd4*, *rag1*, *bdnf* and *prrl* data, and their distribution across eastern Australia. Here we show the relationships among the five clades (A–E) based on the *BEAST species-tree analysis. Numbers beside nodes refer to posterior probabilities. Dark lines on the map represent State boundaries. WA = Western Australia, NT = Northern Territory, QLD = Queensland, SA = South Australia, NSW = New South Wales, ACT = Australian Capital Territory, TAS = Tasmania. White stippled lines indicate biogeographic barriers mentioned in the text. Colored diamonds indicate our sampling for each *A. muricatus* clade, with the white diamond representing our sample of *A. norrisi*. For the Maximum likelihood phylogeny showing individual details of each *A. muricatus* sample see Appendix Fig. 2. Photo: Ben Twist.

uncorrected pairwise genetic distances between clades is 6% for the mtDNA data and 0.7% for the nDNA loci.

Our results from the partitioned ML analyses strongly support five clades within *A. muricatus* (Appendix Fig. 2). While the relationships between clades are poorly supported in the ML analysis, our *BEAST species tree analysis strongly supports the grouping of the three eastern clades (blue, green and red in Fig. 1) as the sister group to the two southern clades (purple and yellow in Fig. 1). A sample identified as *A. muricatus* from the South Australian Museum (J26; SAMAR49532) is highly divergent from other *A. muricatus* samples and instead falls well within *A. norrisi* samples from Genbank for *nd2* (not shown). Both our ML and Bayesian analyses strongly support *A. norrisi* to be the sister taxon to *A. muricatus* (pp = 1, bs = 99).

3.2. Morphological analyses

Appendix Table 2 summarizes the variation in body proportion data for each genetic clade. There was virtually complete overlap between clades in each of the variables examined and this result persisted following multi-variate analysis. We summarize the results of our PCA analysis in Fig. 2, which demonstrates that all five genetic clades overlap entirely in both body size (PC1) and body shape (PC2). PC1 explained 90% of the variation and, as expected, was highly correlated with SVL ($r^2 = 0.99$, $P < 0.0001$). PC2 (body shape) explained 4.4% of the variation with inter-limb length having the highest loading. The remaining PCs explained negligible amounts of the variation. Similarly, DFA, where clades were specified *a priori*, demonstrated that it is not possible to easily allocate specimens to clade based on body proportion variables. Only 52% of the specimens were correctly assigned to their genetic clade.

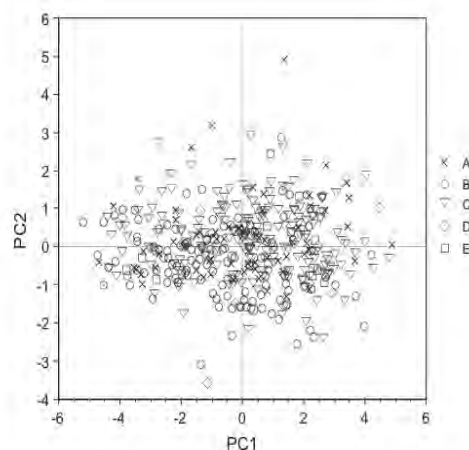


Fig. 2. Summary of results for the PCAs of the morphological data for 437 *A. muricatus* specimens which we assigned to 5 clades (A–E) based on genetic and geographic data.

4. Discussion

We have produced the first comprehensive phylogeny for *A. muricatus*. While the nuclear loci *bdnf* and *prrl* were largely uninformative on their own, the combined data, using information from all five loci, show strong support for five genetic groups with

allopatric distributions down the east coast of Australia. The most northern clade occupies northern NSW on the western side of the Great Dividing Range. South of this a second clade has a largely coastal distribution on the eastern side of the Great Dividing Range. A third clade has a number of divergent populations, the first well inland in NSW around a small cluster of mountains, a population around the ACT and the NSW south coast, and another population around coastal eastern Victoria. A fourth clade occurs around the regions north of Melbourne, with a fifth clade represented by just a few samples located around the Victorian/South Australian border. The Great Dividing Range provides both a topographic and climate-induced barrier for low dispersal taxa, and expansion along either side of this mountain system has long been thought of as an important mechanism leading to population differentiation (eg. *Horton*, 1972). Below we discuss the taxonomic implications of our study, as well as the historical processes and biogeographic barriers that may have influenced the evolutionary history of the Jacky dragon.

4.1. Monophyly and taxonomy of *A. muricatus*

In a mtDNA based study of amphibulurine taxa, *Schulte et al.* (2003) inferred *A. muricatus* to be the sister taxon to *A. norrisi*, in a clade also containing *Lophognathus gilberti* and *C. kingii*. A recent study by *Melville et al.* (2011) included a nuclear gene (*rag1*) in their phylogeny of *Amphibolurus* and *Lophognathus*, and recovered the same sister relationship between *A. muricatus* and *A. norrisi*, but with lower support in the nuclear locus (pp = 70, bs = 86). We only had a single individual of *A. norrisi* for our nDNA loci and *nd4*. However, *nd2* data from nine additional *A. norrisi* samples from Genbank unambiguously place our sample J26 within this taxon. Our combined analysis based on two mtDNA and three nDNA loci strongly support this sister relationship between *A. muricatus* and *A. norrisi* sample J26. Morphologically the two species are very similar in scutellation, with the most conspicuous difference relating to the presence of a dark canthal stripe in *A. norrisi* that extends from the tip of the snout, through the nostril and orbit and to the tympanum (*Witten and Coventry*, 1984; *Cogger*, 2000). The two species have distinct and parapatric distributions that appear to be limited by topography and associated habitats. *A. muricatus* has a distribution closely associated with the uplands of the Great Dividing Range (see below) from south-east Queensland to where the highlands dissipate around the Victorian/South Australian border. In contrast, the distribution of *A. norrisi* is restricted to the topographically low-lying coastal zone along the southern continental margin, from the Victorian/South Australian border to well into Western Australia (Atlas of Living Australia records).

Despite the phylogeographic structure we documented in *A. muricatus*, we found no evidence of body proportion differences between the major mtDNA clades. Based on body proportion homogeneity and lack of resolution in the nuclear loci, we therefore do not support taxonomic recognition of any of the mtDNA clades, and instead suggest that *A. muricatus* is best thought of as a single species with phylogeographic structure. While our morphological analyses were based only on body shape characters, detailed analysis of geographic variation in color and scalation patterns as they relate to phylogeographic structure would be fruitful areas for additional research.

4.2. Biogeography of *A. muricatus* populations

In a detailed study of the genetic structure of *Lampropholis* skinks, *Chapple et al.* (2011) reviewed a number of recurring biogeographic barriers along the eastern Australian margin that correspond to major genetic breaks in this and other disparate taxa. The patterns seen in *A. muricatus* are largely concordant with these

studies, with the numerous allopatric clades along the east coast consistent with the hypothesis of multiple isolated forest refugia during the extensive arid periods of the Plio-Pleistocene (*Thorn et al.*, 1994).

While relationships between lineages based on our concatenated analysis are largely unresolved, our species-tree analysis groups the three eastern clades (northern NSW, central NSW, south-eastern NSW/eastern VIC) together, to the exclusion of the two southern clades (central VIC and western VIC), with strong support. This major phylogeographic division between southern NSW and central VIC is seen in a number of other taxa, including skinks (*Chapple et al.*, 2005), assassin spiders (*Rix and Harvey*, 2012), myobatrachid frogs (*Symula et al.*, 2008), bower birds (*Nicholls and Austin*, 2005) and glow-worms (*Baker et al.*, 2008). In a study on the *Lomatia* genus in the plant family Proteaceae, *Milner et al.* (2012) identified the 'Southern Transition Zone' in southern NSW, and suggested that rather than defining a strict position, this area represented a transitional, climate-dependant barrier that varies over time and influences species distributions differently. In our study, this break in *A. muricatus* populations appears to be associated with the Eastern Highlands (the northeast to southwest trending Australian Alps, which run from NSW to VIC) that include the highest elevation regions of the Great Dividing Range. This region experienced episodes of glaciation during the Pleistocene (*Barrows et al.*, 2002), which would have eliminated suitable habitat and isolated populations. Eastern and central VIC also are separated by the Gippsland Basin and the Strzelecki Ranges (*Dickinson et al.*, 2002), that could provide topographic and habitat induced barriers to dispersal for taxa in this region.

Within the three eastern clades, our northern NSW clade has a distribution that also is seen in other taxa (eg. *Schäuble and Moritz*, 2001; *Chapple et al.*, 2011). To the north it is bound by the Macpherson Range or potentially some other geological barrier, such as a geological break in the uplands of the New England Fold Belt as it grades into the adjacent Clarence/Moreton Basin (*Packham*, 1969). Another well known barrier, the Hunter Valley, generally separates the northern NSW clade from the central NSW clade to the south, however a number of individuals are found just north of this region, suggesting this barrier may not have been as effective in isolating *A. muricatus* populations north and south of this divide.

We also identified a genetic division between the central NSW population and the southern NSW populations (which includes a population well inland to the northwest). This corresponds geographically to the Illawarra district, where there is a break in the sandstone cliffs near Robertson, and where the low-lying coastal plain reaches its widest point at Macquarie Pass. This region, like other low-lying coastal zones was intensely arid during the last glacial maximum, with a dry and windy coastline that extended up to 100 km offshore than the present coastline (*Thorn et al.*, 1994). This same phylogeographic break is also present in broad-headed snakes (*Sumner et al.*, 2010), velvet geckos (*Dubey et al.*, 2012) and assassin spiders (*Rix and Harvey*, 2012).

The timing of tectonic uplift in the south-eastern highlands is contentious, with various hypotheses ranging from the Palaeozoic to the Cenozoic (*Van der Beek et al.*, 1999). Despite this uncertain tectonic history, substantial uplift along the Great Dividing Range is undoubtedly related to the rifting of the Tasman Sea during the Cretaceous ~94 Ma (*O'Sullivan et al.*, 2000), and its formation would have had a profound influence on the evolution of taxa on the east coast of Australia (*Dubey et al.*, 2010). In addition, the non-uniform relief along its great length of more than 3500 km, with a width of over 300 km in some sections, would have provided multiple microclimatic refuges during Plio-Pleistocene cycles of aridification, dividing formerly continuous distributions and allowing the development of genetically distinct local populations. Despite its modest elevation compared to mountain systems

of other continents, the height of the Great Dividing Range particularly around the NSW and Victorian Alps creates winter temperatures too cold for survival for many temperate taxa, and would currently restrict their distribution to lowlands and coastal plains either side of the divide. Our results of population subdivision in *A. muricatus* and the correlation to topography of the Great Dividing Range has been found across a wide variety of organisms, including invertebrates (Garrick et al., 2004), amphibians, reptiles, birds, mammals and plants (reviewed in Chapple et al., 2011). While the nature and location of barriers to dispersal varies among taxa, the diversification of south-eastern Australian biota appears to reflect a history where the Great Dividing Range has played a central role.

5. Conclusions

More than 200 years after its original description in the journal "Voyage to New South Wales" (White, 1790), modern molecular methods have confirmed the distinctiveness of the Jacky dragon within the Australian amphibolurine radiation. Our study, sampling from across their distribution, has highlighted geographically structured diversity and represents a valuable phylogenetic framework for studies of behavior, evolution and ecology, that have led to the recognition of the Jacky dragon as a model species. Remarkably, a number of common and widespread Australian reptiles also mentioned in White's journal (the Eastern water dragon *Intellagama lesueurii*, blue-tongue skink *Tiliqua scincoides* and lace monitor *Varanus varius*) and even the first endemic Australian reptile to be named, the shingle-back skink *Tiliqua rugosa* (Dampier, 1729) are still lacking intraspecific genetic studies detailing population structure across their range. With the rise in discovery of cryptic species of terrestrial vertebrates, in particular squamate reptiles (Oliver et al., 2009), elucidating this unrecognised diversity will require integrated data from genetics, morphology, and ecology. In addition, as comparative phylogeographic datasets increase, along with more accurate reconstructions of past landscapes and palaeoclimates, so too will our understanding of the overarching environmental forces that shaped the diversity and distribution of the Australian biota.

Acknowledgments

We thank the Australian Museum, South Australian Museum and Museum Victoria staff, and Lisa Schwanz, for access to tissue samples. We also thank Brian Harrold for help with mapping software. Ben Twist kindly provided the photograph of *A. muricatus* for the figure. Tissue samples were collected with the approval of the Australian National University Ethics Committee (A2012/12) and the Macquarie University Ethics Committee (ARA 2010/034) under the following permits: NSW: SL101003, S13197; VIC: 10005522. M.J.W. and J.S.K. thank the Australian Research Council for ongoing support.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.11.012>.

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ETHICS APPROVAL



ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2010/034

Date of expiry: 30 June 2011

Full Approval Duration: 1 July 2010 to 30 June 2013 (36 months)

Principal Investigator:

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Associate Investigator(s):

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The above-named are authorised by:
MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Evolution of signal structure in a widely ranging lizard species complex

Type of animal research and description of project: Wildlife Research. The study aims to understand how different factors drive divergence of visual displays in order to improve the fundamental knowledge of speciation. The study will examine how habitat characteristics, predation risk, species recognition and sexual selection influence the divergence of visual displays of lizards in the genus *Amphibolurus*.

All procedures must be performed in accordance with the AEC approved protocol.

Species	Sex	Age	Numbers approved				Supplier/ Source
			Year 1	Year 2	Year 3	Total	
Jacky dragon <i>Amphibolurus muricatus</i>	M/F	Adult	135	225	105	465	Wild capture
Norris's dragon <i>Amphibolurus norrisi</i>	M/F	Adult	20	30	35	85	Wild capture
TOTAL			155	255	140	550	

Location of research: Captive studies will be carried out at BB&E, Macquarie University, NSW.

Approval is subject to the following conditions:

1. Once received, copies of all permits must be forwarded to the Animal Ethics Secretariat. The project cannot commence until permits have been approved.
2. Lizards will be marked using a xylene-free paint pen that is non-toxic and routinely used to mark lizards, instead of with PIT tags (as outlined in the response to Q.3 of the pre-meeting Questions)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **1 July 2010 to 30 June 2011**, unless suspended, cancelled or surrendered, and will only be renewed upon receipt of a **PROGRESS REPORT** before the end of this period.

Prof Michael Gillings
Chair, Animal Ethics Committee

Date: 28th June 2010