

EVOLUTIONARY BEHAVIOURAL ECOLOGY  
OF AN AUSTRALIAN LIZARD WITH  
ALTERNATIVE REPRODUCTIVE TACTICS



**EVOLUTIONARY BEHAVIOURAL ECOLOGY OF AN  
AUSTRALIAN LIZARD WITH ALTERNATIVE REPRODUCTIVE  
TACTICS**



**Daniel W.A. Noble, M.Sc.**

Department of Biological Sciences  
Macquarie University

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## SUMMARY

Evolution by natural and sexual selection requires an understanding of trait variability and heritability and the strength and form of selection on phenotypic traits. This thesis is an integrative overview of these important tenants of evolutionary biology using a model lizard system, *Eulamprus quoyii*. I begin by exploring the individual level of variability in spatial cognitive traits among males and test whether individuals are capable of complex spatial learning under semi-natural conditions. Contrary to previous research, I show that lizards are capable of quick and flexible spatial learning as would be predicted given its close link with fitness in the wild. In more controlled conditions, I test for sex differences in spatial learning and whether individual behavioural traits, linked to personality, affect spatial learning ability. As predicted, there were sex-differences in spatial learning, representing the first evidence of cognitive differences between the sexes in a reptile. In contrast to recent theory, I show that behavioural types were not linearly associated with cognitive performance in the spatial task and this may be a result of alternative mating tactics in *E. quoyii*. In the second part of my thesis I explore how selection acts on behavioural and performance traits using a large-scale breeding experiment under semi-natural conditions. Using a genetic pedigree, I test two alternative hypotheses for the prevalence of polyandry in this system and assess the degree to which offspring performance traits are heritable. I show that polyandry may evolve through direct benefits females receive through multiple mating rather than genetics benefits, given that offspring from single and multiple paternity females did not differ in fitness and because the fitness traits showed weak heritability. Finally, I test how natural and sexual selection act on behavioural and performance traits. I show that selection acts on behavioural traits disruptively possibly promoting the evolution of ARTs in *E. quoyii*. Selection on performance also suggests that different ARTs adopt different mechanisms to acquire paternity and that behaviours may be integrated with whole-organism performance traits. Patterns of selection on performance traits also provide a possible explanation for sex-differences in morphology and performance I observed in *E. quoyii*.

## DECLARATION

I certify that the work in this thesis entitled “Evolutionary behavioural ecology of an Australian lizard with alternative reproductive tactics” 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.

Daniel W.A. Noble (Student ID:                      )

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## INTRODUCTION

### **The beginning of a revolution**

“...multiply, vary, let the strongest live and the weakest die”

– Charles Darwin – The origin of species, Chapter VII pg. 244

In 1859, Charles Darwin proposed a revolutionary and controversial theory that would prove to be the genesis of modern evolutionary thinking. His theory of natural selection, proposed that phenotypes of organisms change through time gradually as a result of the differential survival of individuals within a population (Darwin, 1859). However, his theory received some opposition because it was difficult for his antagonists to comprehend how such extravagant displays and ornamentation, so commonly encountered in males of a species, could evolve. How can such structures evolve if they invariably compromise the survival of an individual? Surely such structures are energetically costly to produce and/or would be more likely to capture the attention of predators? To resolve this dilemma it was necessary for Darwin to make a few critical observations. First, Darwin noted that in animal systems many individuals do not mate and that there is fierce competition for access to mating opportunities. Such fierce competition can be resolved through fights and displays, which may involve armaments (Darwin, 1871). Second, males often make ‘showy’ displays or have extravagant ornaments to win over ‘choosy’ females (Darwin, 1871). These fundamental observations led Darwin to propose that seemingly non-adaptive structures, such as horns and extravagant ornamentation, could evolve through the processes of male-male competition for access to mates and/or female mate choice (Darwin, 1871). These two processes generate strong selection by creating variance in reproductive success among males that the reproductive benefit of having these structures outweighs their survival costs. This became known as Darwin’s theory of sexual selection (Darwin, 1871). Fundamentally, both natural and sexual selection are similar and are sometimes considered the same process, however, there is utility in separating these two processes from one another to better distinguish the differences in the mechanisms/or agents of selection.

Darwin's theories of natural and sexual selection have transformed the way biologists understand evolution and have provided an arsenal of testable predictions. Both natural and sexual selection require that individuals within a population vary in their phenotype and that this phenotype leads to the differential survival and/or reproductive success of individuals. Finally, this phenotype must be able to be passed on from one generation to the next (i.e. the phenotype is heritable).

### **Life after Darwin**

Darwin's theories of natural and sexual selection led to an explosion of evolutionary thinking in biology. Evolutionary biologists became increasingly interested in how phenotypes of organisms were selected upon and modified to 'optimally' fit the niche they occupied. Although the idea of 'perfection' and 'optimality' in evolutionary biology persisted for quite some time, it is now recognized that organisms are not optimally designed for their environments and that natural selection is not the only mechanism for evolutionary change, even though this was stressed by Darwin himself (Gould & Lewontin, 1979; Pigliucci & Kaplan, 2000). At the time Darwin conceived his theory he did not have working knowledge of Mendelian inheritance, providing a mechanism by which phenotypes can be passed between generations. Advances in molecular biology later identified that this heredity information is coded in the deoxyribonucleic acid (DNA) of every organism providing a suitable pathway for which phenotypes can be modified over time. The desire to understand selection and heritability of traits paved the way for the development of various statistical techniques for evaluating selection in nature (Lande & Arnold, 1983; Mitchell-Olds & Shaw, 1987; Brodie III *et al.*, 1995) and the degree to which these traits were heritable (Kruuk, 2004; Hadfield, 2010). These statistical developments have shaped the way researchers quantify selection in nature and understand responses to selection. This led to a rapid increase in selection estimates (Kingsolver *et al.*, 2001) and estimates of additive genetic variation (Tsuju *et al.*, 1989; Sorci *et al.*, 1995; Taylor *et al.*, 2012). There is also growing interest in how alternative forms of selection, such as disruptive, stabilizing and correlational selection shape phenotypic variances and covariance's (Brodie III, 1992; Sinervo & Zamudio, 2001; Sinervo & Svensson, 2002; McGlothlin *et al.*, 2005; Calsbeek & Smith, 2007; Miles *et al.*, 2007; Bolnick & Lau, 2008) and how this may lead to integrated phenotypes, such as ARTs and



behavioural syndromes, where correlations between behavior, physiology and morphology exist (Sinervo & Lively, 1996; Sinervo & Zamudio, 2001; Sinervo & Calsbeek, 2006; Miles *et al.*, 2007; Biro & Stamps, 2008; Stamps & Groothuis, 2010; Dingemanse & Wolf, 2012).

An understanding of both natural and sexual selection requires an integrative approach. One must understand the degree of variability in traits, their heritability (if possible), how selection acts on these traits and each traits covariation with other traits, which may or may not be under selection themselves and may or may not be pleiotropically or epistatically linked to the trait of interest (Darwin, 1859; Darwin, 1871; Brodie III, 1989; Lynch & Walsh, 1998). This latter point is important because it is likely commonplace that traits do not evolve on their own (Gould & Lewontin, 1979), but rather in a correlated fashion, where suites of traits are selected for that increase the fitness of an organism, although only a few studies have been able to demonstrate such forms of selection (e.g. Brodie III, 1992; McGlothlin *et al.*, 2005; Calsbeek & Smith, 2007; Hendry *et al.*, 2009).

### **Eastern Water Skinks (*Eulamprus quoyii*) as a model system in ecology and evolution**

My thesis explores predictions of Darwin's theories along with understanding the relationships between traits using a model lizard system. I use a common lizard species, the Eastern Water Skink (*Eulamprus quoyii*) as my model organism. *Eulamprus quoyii* is widely distributed across eastern Australia occupying riparian habitats along rocky, river shores and its commonly found in urban areas. They are live-bearing and give birth to 1- 10 offspring in December and January after a relatively short breeding season in September-October (Head *et al.*, 2005; Schwarzkopf, 2005). This is an ideal candidate for integrative studies for a number of reasons. First, their larger size makes them amenable to manipulation and a wide array of behavioural and physiological measurements. Second, we have a great deal of prior information on *Eulamprus* and different species are ecologically conserved, which allows for the generation of testable hypotheses and predictions (Scott *et al.*, 2001; Morrison *et al.*, 2002; Stapley & Keogh, 2004; Head *et al.*, 2005; Schwarzkopf, 2005; Stapley & Keogh, 2005; Dubey *et al.*, 2011; Keogh *et al.*, 2012). Third, this species responds well to captive and semi-natural conditions, breeds easily and gives birth to reasonably large litters of offspring providing the necessary statistical power to address questions in evolutionary biology. Lastly, there is strong sexual selection in this system leading to the evolution of condition-dependent

alternative reproductive tactics, which are primarily behaviorally driven. This provides a unique opportunity to test predictions about the co-evolution between traits. My thesis is structured into seven empirical chapters and I provide a brief description of each below.

## **CHAPTER 1: Learning outdoors: Male lizards show flexible spatial learning under semi-natural conditions**

Cognitive traits have important bearing on fitness, from finding food and shelter to escaping predatory threats (Shettleworth, 2010). Selection is therefore predicted to act strongly on these traits given their inherent link with fitness. Spatial cognitive abilities have been particularly important in reptiles because of their link with avoiding predatory threats (Cooper Jr. & Wilson, 2007). However, many studies use small sample sizes (see references in Burghardt, 1977) preventing an inadequate understanding of the degree of individual variability in these traits, which forms the necessary raw material for natural selection to act upon. In this chapter, in collaboration with Dr. Pau Carazo (University of Oxford), we use a large number of male lizards and assess the degree to which they can learn a spatial learning and spatial reversal task. Our large sample sizes allowed us to get a good understanding of the level of variability in this trait and allowed us to assess whether *E. quoyii* is capable of flexibly learning such tasks. Both Pau Carazo and Martin Whiting are co-authors on this manuscript (my contributions to experimental design: 50%; data collection: 70%; data analysis: 75%; writing: 85%)

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## **CHAPTER 2: Sex and boldness explain individual differences in spatial learning in a lizard**

Consistent behavioral tendencies of individuals ('personality') and behavioral types (actual behaviours exhibited by individuals) can have important direct effects on fitness (Sih & Bell,

2008; Stamps & Groothuis, 2010; Dingemanse & Wolf, 2012; Sih & Del Guidice, 2012). However, the behavioural types of individuals can also affect fitness through their effects on cognitive traits related to learning fitness related tasks (Cole *et al.*, 2012; Cole & Quinn, 2012). Currently, there are no studies on squamate reptiles assessing the importance of behavioural traits on cognition. Furthermore, theories about how behavioural types affect cognition have primarily been derived from studies on birds, mammals and fishes (Dugatkin & Alfieri, 2003; Cole *et al.*, 2012; Cole & Quinn, 2012) neglecting important diversity in reptiles. In this chapter, I use *E. quoyii* as a model system to understand the role behavioural types play in affecting an individual's ability to learn a spatial task. *Eulamprus* has already been shown to exhibit behavioural syndromes (Stapley & Keogh, 2004) and these behaviours may have evolved in a correlated fashion as they are also associated with alternative reproductive tactics (Stapley & Keogh, 2004; 2005). Although there are predictions about the role behavioural types play in affecting cognition (Dugatkin & Alfieri, 2003; Cole *et al.*, 2012; Cole & Quinn, 2012) these predictions may be made more complex in systems with ARTs because disruptive selection predicts two fitness optima. *Eulamprus quoyii* therefore provides a unique system with which predictions in this growing field can be evaluated. Pau Carazo, Martin Whiting and Dani Chandrasoma are co-authors on this manuscript, which is currently in review. Pau and I contributed equally to the manuscript (my contributions to experimental design: 40%; data collection: 40%; data analysis: 60%; writing 40%).

### **CHAPTER 3: Multiple mating in a lizard increases fecundity, but provides no evidence for genetic benefits**

Understanding why females mate multiply has been a topic of considerable interest for many decades (Bateman, 1948; Andersson, 1994; Jennions & Petrie, 2000; Slatyer *et al.*, 2012) given that females gain little by mating with multiple males and also because mating itself can be costly, increasing predation risk and reducing foraging and basking times (Jennions & Petrie, 2000; Slatyer *et al.*, 2012). However, the incidence of multiple mating and multiple paternity in natural reptile populations remains high (Uller & Olsson, 2008). Water skinks show a high incidence of multiple paternity in natural populations (Morrison *et al.*, 2002; Stapley & Keogh, 2005; Dubey *et al.*, 2011; Keogh *et al.*, 2012; Keogh *et al.*, 2013) and this chapter tests two competing hypotheses of why this may be the case. The lack of parental care



## Introduction

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and the low levels of male harassment in *E. quoyii* make them an ideal candidate species for testing competing hypotheses explaining the evolution of polyandry because the direct benefits females receive through polyandry are more simplistic. In this chapter I test whether females gain direct and/or indirect (genetic) benefits by mating with multiple males using data from a large breeding experiment where I could ascertain paternity and measure a series of fitness proxies on offspring. Scott Keogh and Martin Whiting are co-authors on this paper (my contributions to experimental design: 75%; data collection: 100%; data analysis: 100%; writing: 90%).

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## CHAPTER 4: Offspring fitness traits show strong maternal effects in an Australian lizard

Evolutionary responses to selection require that traits have a genetic basis and are heritable; however, responses to selection also depend on maternal effects which can dampen or accelerate such responses (Sheldon, 2000; Qvarnstrom & Price, 2001). This chapter tests a fundamental prediction of 'good' genes models of sexual selection, that traits are heritable. In chapter 3, I found weak evidence for indirect genetic benefits in explaining patterns of multiple paternity in *E. quoyii*. Using a genetic pedigree and quantitative genetic models (Hadfield, 2010) I assess whether offspring fitness traits show evidence for additive genetic variation, which would suggest that such traits can respond to selection (Kruuk, 2004). I found that these traits are more strongly controlled by maternal effects and there was weak evidence for most traits having a strong heritable basis. This suggests that these traits would respond weakly, if at all, to selection by females and that females have a lot of control over these traits in any case, negating the need to select particular males. These results support the lack of differences I found in our traits in chapter 3. Martin Whiting, Scott Keogh and Eryn McFarlane are co-authors on this manuscript, which is currently in review (my contributions to experimental design: 80%; data collection: 100%; data analysis: 50%; writing: 90%).

## **CHAPTER 5: Behavioral and morphological traits interact to promote the evolution of alternative reproductive tactics in a lizard**

Correlational and disruptive selection are predicted to be major drivers in the evolution of integrated phenotypes such as alternative reproductive tactics (ARTs), yet we have a poor understanding of how selection acts on behavioural and morphological traits to generate suites of correlated phenotypes (Reuffer *et al.*, 2006). Furthermore, very few studies have quantified selection on behavioural traits (Kingsolver *et al.*, 2001). *Eulamprus* is an ideal system to test how selection acts on behavioural traits and to understand how behavioral traits may evolve in a correlated fashion because of the existence of behaviourally driven ARTs in this system (Morrison *et al.*, 2002; Stapley & Keogh, 2004; 2005). The lack of discrete behavioural differences allows for the quantification of continuous behavioural variation and the relationship between intermediate behavioural phenotypes and fitness, allowing us to test whether there is evidence of correlational disruptive selection of suites of traits related to ARTs in this system (Morrison *et al.*, 2002; Stapley & Keogh, 2004; 2005; Keogh *et al.*, 2012). In this chapter, I conducted a controlled mating system experiment where individual lizards were monitored through the course of the breeding season and behavioural traits quantified to test predictions of selection acting on these traits and whether selection was correlational and disruptive. Kerrie Wechmann, Scott Keogh and Martin Whiting are co-authors on this chapter (my contributions to experimental design: 80%; data collection: 60%; data analysis: 90% and writing: 90%).

### **Citation:**

Daniel W.A. Noble, Kerrie Wechmann, J. Scott Keogh and Martin J. Whiting (2013). Behavioral and morphological traits interact to promote the evolution of alternative reproductive tactics in a lizard. *The American Naturalist*, in press.

## **CHAPTER 6: Sex, testosterone and performance in an Australian lizard with sexually dimorphic body shape but not size**

Arnold (1983) proposed a framework to understand the relationship between morphology, performance and fitness, whereby morphology affects individual performance which in turn influences fitness. This framework of cause-and-effect has been expanded to understand the role physiology plays in this relationship with androgens, such as testosterone, affecting morphology-performance-fitness relationships (Sinervo *et al.*, 2000; Miles *et al.*, 2007). Much of our understanding of androgenic affects on morphology and performance have focused on males in the context of alternative reproductive tactics (Miles *et al.*, 2007) and in species with clear sexual dimorphism and dichromatism (Irschick *et al.*, 2006; Whiting *et al.*, 2006; Irschick *et al.*, 2008). We know much less about their role in generating sex specific differences in morphology and performance even though testosterone can affect the sexes similarly or differentially (Cox *et al.*, 2005; Cox *et al.*, 2009). Weakly sexually dimorphic species provide excellent systems to dissect this path of cause-and-effect and to test ‘well-established’ theoretical relationships between physiology-morphology and performance because we can understand how slight differences in shape affect these relationships and whether androgens have similar or differential affects among the sexes. Kerry Fanson and Martin Whiting are co-authors on this manuscript (my contributions to experimental design: 70%; data collection: 60%; data analysis: 100%; writing: 90%).

### **CHAPTER 7: Natural and sexual selection on whole-organism performance traits in an Australian lizard with alternative reproductive tactics**

Evolutionary biologists have expressed great interest in understanding how natural and sexual selection act on traits predicted to be more direct targets of selection, such as whole-organism performance traits [i.e. the ability of individuals to perform an ecologically relevant task such as running or jumping which are important for eluding predators] (Arnold, 1983; Irschick *et al.*, 2008). Understanding selection on functional traits may help elucidate sex-differences in morphology and performance. Although there are numerous studies quantifying viability selection on performance traits only a few have quantified sexual selection on traits predicted to be important for male dominance and mate acquisition (Irschick *et al.*, 2008). In this chapter I explore how natural and sexual selection act on three important whole-organism performance traits (sprint speed, endurance, bite force), while also considering the role ARTs may play in understanding patterns of selection in *E. quoyii*. I show that body size was strongly related to



bite force and endurance in males and females, however, selection only acted on body size, condition and sprint speed. In accordance with predictions, there was directional selection for sprint speed in males, but not females, and there was evidence for non-linear and correlation selection in males that may relate to ARTs in *E. quoyii*. Both fast sprinters and slow sprinters had similar reproductive success among large males while average-condition fast-sprinters and high-condition slow-sprinters were also predicted to have high reproductive success, suggesting that adoption of ARTs in *E. quoyii* is condition-dependent. My advisors, Martin Whiting and Scott Keogh are co-authors on this manuscript (my contributions to experimental design: 80%; data collection: 80%; data analysis: 100%; writing: 95%).

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## CHAPTER 2: SEX AND BOLDNESS EXPLAIN INDIVIDUAL DIFFERENCES IN SPATIAL LEARNING IN A LIZARD

Pau Carazo<sup>1, 2,\*, ‡</sup>, Daniel W.A. Noble<sup>2, ‡</sup>, Dani Chandrasoma<sup>2</sup> and Martin J. Whiting<sup>2</sup>

Under Review

### Abstract

Understanding individual differences in cognitive performance is a major challenge to animal behaviour and cognition studies. We used the Eastern Water Skink (*Eulamprus quoyii*) to examine associations between exploration, boldness and individual variability in spatial learning, a dimension of lizard cognition with important bearing on fitness. We show that males perform better than females in a biologically relevant spatial learning task. This is the first evidence for sex differences in learning in a reptile, but we argue it is probably due to sex-specific selective pressures that are widespread in lizards. Across the sexes, we found a clear association between boldness after a simulated predatory attack and the probability of learning the spatial task. In contrast to previous studies, we found a non-linear association between boldness and learning: both ‘bold’ and ‘shy’ behavioural types were more successful learners than intermediate males. Our results do not fit with recent predictions suggesting that individual differences in learning may be linked with behavioural types via high-low risk/reward trade-offs. We discuss the possibility that differences in spatial cognitive performance may arise in lizards as a consequence of the distinct environmental variability and complexity experienced by individuals as a result of their sex and social tactics.

## INTRODUCTION

A fundamental aim in cognitive studies is to understand the factors that might explain the extraordinary levels of individual variability in cognitive performance observed in almost every animal species (Healy et al., 2008; Mery and Kawecki, 2002; Plomin, 2001; Raine and Chittka, 2008; Shettleworth, 2010; Tolman, 1924; Tryon, 1940). Recent research has made some progress in this respect, but we are only just beginning to understand how cognitive performance relates to development and selection at the intraspecific level (Seed et al., 2012; Sih and Del Guidice, 2012; Thornton and Lukas, 2012). In sharp contrast, the study of intraspecific variation in non-cognitive behavioural traits is a thriving area of research. The study of behavioural types or personality traits (i.e. consistent behavioural tendencies across time and context) has driven our understanding of intraspecific behavioural differences during the last decade, generating several hypotheses about the evolution of adaptive behavioural variation at the individual level (Bergmüller and Taborsky, 2010; Dall et al., 2012; Dingemanse and Wolf, 2012; Sih and Bell, 2008; Stamps and Groothuis, 2010; Wolf and Weissing, 2010, 2012). Interestingly, recent hypotheses have proposed that learning and non-cognitive behavioural traits may co-vary as part of the same suite of correlated traits, or because they can both determine the environment that is to be experienced by different individuals and generate feed-back loops that lead to cognitive-behavioural syndromes (Arbilly et al., 2010; Carere and Locurto, 2011; Cole and Quinn, 2012; Dingemanse and Wolf, 2012; Seed et al., 2012; Sih and Bell, 2008; Sih and Del Guidice, 2012; Thornton and Lukas, 2012; Wolf et al., 2007; Wolf and Weissing, 2012).

Sih and Del Guidice (Sih and Del Guidice, 2012) recently proposed that variation in cognition might be functionally related to variation in personality by the existence of a shared risk-reward trade-off between fast-slow behavioural traits and speed-accuracy cognitive styles (Sih and Del Guidice, 2012). Many behavioural traits can be classified into a fast-slow axis (e.g. bold vs. shy, proactive vs. reactive, fast vs. slow exploration), and this variation can be associated with variation in a risk-reward axis because bolder, more aggressive, exploratory and/or proactive individuals have a greater potential to gather resources, but take more risks in doing so (Biro and Stamps, 2008; Réale et al., 2007; Sih and Del Guidice, 2012; Wolf et al.,



2007). Similarly, speed-accuracy trade-offs are bound to affect cognitive styles because animals that learn fast do so at the expense of acquiring inaccurate information (Chittka et al., 2009). The speed-accuracy trade-off is also essentially related to variation in the risk-reward axis because fast learning is inherently risky (i.e. based on inaccurate information) but will tend to draw more resources in the short-term (Sih and Del Giudice, 2012). The overarching idea of the risk-reward hypothesis is that selection for factors leading to the adoption of a more risk-prone lifestyle will result in correlated selection for both faster behavioural traits and faster but less accurate and flexible learning, and *vice versa* (Burns, 2005; Burns and Dyer, 2008). For example, risk-prone individuals may be selected for in stable local habitats, where fast exploration would give them a competitive advantage and the evolution and/or development of learning abilities would aid in the quick formation of routines, while risk-averse individuals with learning abilities that are more flexible and sensitive to environmental change may develop or be selected for in more variable local habitats (Guillette et al., 2011). This hypothesis has found some support in a few bird and fish species, that seems to suggest that proactive individuals tend to be quicker than reactive individuals at operant learning tasks and in avoidance learning tasks fundamentally guided by external environmental cues (Boogert et al., 2006; Exnerová et al., 2010; Guillette et al., 2009; Sih and Del Giudice, 2012), but slower in reversal learning tasks (Guillette et al., 2011; Titulaeur et al., 2012). In short, recent advances suggest that an obvious avenue for understanding adaptive individual variation in learning is to study the existence of co-variation between cognition and non-cognitive behavioural traits; ideally in an ecologically relevant context where learning may directly impinge on individual fitness.

Sex is an equally important factor to understand individual variation in learning and in associated behavioural types. The sexes will frequently experience different environmental complexity and/or variability as a consequence of their different reproductive strategies, which are likely to drive differences in both cognitive and non-cognitive traits, and in the way they co-vary (Jones et al., 2003; Schuett, 2009; Titulaeur et al., 2012). However, while sex differences in learning have been well documented in some taxa (Shettleworth, 2010), it has been completely neglected in others, such as lizards (and reptiles at large). Even less information is available about sex differences in personality traits, which have only been documented in a handful of fish and bird species (Johnsson et al., 2001; Oliveira et al., 2008; Piyapong et al., 2009; Schuett, 2009). Finally, scarcely any attention at all has been paid to

studying sexual differences in the existence and form of cognitive-behavioural types (Titulaeur et al., 2012) despite the fact that there are sound theoretical reasons to expect them. In the context of the risk-reward hypothesis, for example, we suggest that males may be generally forced to adopt more risky reproductive strategies than females due to their different sexual roles, and this could lead to general sex differences in learning, personality traits, and their covariation.

Spatial learning is a cognitive dimension believed to be of utmost importance to lizards. It is believed to be under strong selection in foraging, territorial, and anti-predatory behaviour, which often require quick and flexible learning of territorial boundaries, suitable escape routes, and hides (Cooper and Wilson, 2007; Noble et al., 2012; Pianka and Vitt, 2003). Not surprisingly, lizards have been found to be capable of quick and flexible learning when tested under a biologically realistic learning paradigm (Noble et al., 2012). Furthermore, males and females of many lizard species are generally subject to different spatial demands because of differences in reproductive tactics and behaviour during the reproductive season. These sex-specific tactics and behaviours may have given rise to widespread sexual differences in spatial learning abilities, and to sex-specific associations between spatial learning and other behavioural traits (Jones et al., 2003; Pianka and Vitt, 2003; Titulaeur et al., 2012). An additional dimension in many lizard systems is that exploratory and boldness traits co-vary with alternative reproductive tactics (hereafter ARTs) in males (Calsbeek and Sinervo, 2008). Because lizard ARTs are closely associated with territorial behaviour, these traits represent ecologically significant behavioural variation in a context in which spatial learning is important for lizards (Carazo et al., 2008).

Here, we used an Australian lizard, the Eastern Water Skink (*Eulamprus quoyii*), to explore associations between individual variability in spatial learning performance, sex, and exploratory and boldness traits that have been previously identified as important covariates of ARTs in *E. quoyii* and *Eulamprus heatwolei* (Keogh et al., 2012; Keogh et al., 2013; Noble et al., 2013b; Stapley and Keogh, 2004, 2005). Our main aims in this study were: (i) to examine the existence of sexual differences in learning performance; and (ii) to explore the existence, form, and potential sex differences in associations between spatial learning, exploration and boldness. In order to do so, we assayed behavioural and cognitive traits in four successive experiments in which we quantified exploratory behaviour, boldness in two different contexts

(neophobia towards novel prey and boldness after a predatory attack) and performance in a simple spatial task in 32 male and 32 female *Eulamprus quoyii*.

## MATERIALS AND METHODS

### *Study Species*

The Eastern Water Skink (*Eulamprus quoyii*) is a large [90 – 122 mm snout-vent-length (SVL)], viviparous lizard species that is widely distributed across Eastern Australia, from South Australia and Victoria through to New South Wales and into Queensland. It frequently inhabits rocky water edges in suburban areas and can reach high densities. We collected 216 water skinks in August and September 2010 from five separate sites throughout the Sydney region as part of a separate natural mating experiment that took place during the breeding season (see Noble et al., 2013a for details). After the breeding season, all lizards were transferred to large outdoor bins (3.2 m diameter) with a bark mulch substrate, logs and roof tiles for hides, constant access to water, and were fed crickets every second day.

We used 64 of these lizards (32 males, 32 females) in our experiments. Experiments commenced immediately after capture for 32 lizards (16 males, 16 females) while the remaining 32 lizards (16 males, 16 females) were temporarily held in small holding bins until we finished the first batch of lizards; due to space constraints we were only able to process 32 lizards at a time. All lizards were provided with a middle refuge, had constant access to water and UV lighting during the experiments. Heat cord was used to create a thermal gradient in each enclosure so that lizards had ample opportunity to thermoregulate. Crickets were fed *ad libitum* every second day except during the first six days of experiments because we did not want lizards to be satiated during feeding trials with novel prey (details below).

### *Exploratory Behaviour (Day 1)*

We removed lizards from their holding tubs and transferred them to the lab, where they were released into a novel enclosure measuring 68.3 (L) x 48.3 (W) x 38.5 (H) cm. Two weighted black hide boxes with three entrances each were situated in the lateral walls at each end of the

enclosure, facing opposite each other. Lizards were given 3 minutes under a central acclimation box before lifting it and initiating trials, which lasted for 30 minutes and were recorded using a mounted security camera (Swann® security system). We used JWatcher (<http://www.jwatcher.ucla.edu/>) to score the following behaviours: (i) time spent moving (TL), and (ii) time taken to enter the two opaque hides in the enclosure (T2ER). Lizards that did not visit both hides within the 30 min were assigned a latency of 1800 seconds. We included the latency to visit both hides as a measure of quickness to explore in a novel environment, and locomotion as a measure of the amount of exploration. At the end of this trial we introduced a third refuge in the middle of one of the long walls of the terrarium; all three refuges were left in each lizard's holding tub for the next experiments.

### *Measures of Boldness*

Boldness is most often interpreted as the tendency to take risks, especially in novel situations, and is usually measured experimentally in relation to anti-predatory behaviours or individual response to novel cues (Carter et al., 2012). In this study, we used two experiments to assay boldness separately in an anti-predatory and in a neophobic context.

### *Assay I – Neophilia (Days 2-7)*

We first quantified boldness in relation to neophobia/neophilia to a novel prey item. To obtain a neophobia/neophilia estimate, we examined individual lizard responses when presented with a completely novel food item (i.e. a dead silkworm pupae). Immediately preceding trials, we moved the focal lizard's holding tub to the recording area and, after a 2 - 5 minutes acclimation period, we presented the pupa by gently dangling it in front of the lizard (or the entrance of the hide it was in) for 3 minutes. Pupae were presented at ca. 15 cm (i.e. three 5 cm squares) from the lizard's snout (or entrance of the hide) for the first minute and this distance was decreased by 5 cm each minute thereafter. We repeated this procedure once a day for five consecutive days. On day 6 we presented each lizard with a pupa left hanging ca. 1 - 2 cm from the centre of the tub for one hour, and in the absence of observers. We recorded at which stage (during these trials) each lizard first attacked or ate the novel prey item. Only 25 of 64 (39%) lizards actually consumed the larva, although lizards that ate did so repeatedly



throughout the feeding trials. We therefore divided lizards in two categories (NEO): neophilic (lizards that ate the novel prey at some point during trials) and neophobic (lizards that did not eat the novel prey at all).

### *Assay II – Anti-predatory trial (Day 8)*

On day 8 we staged an anti-predatory trial designed to gauge individual boldness against predators. As in assay I, lizards were tested in their own holding tub. The assay began by gently chasing each lizard into the middle hide. We then randomly selected one of the two remaining lateral hides and designated it as the ‘hot’ hide by suspending a 60/100W incandescent bulb ca. 25 cm above this hide, effectively transforming the top surface into a basking platform. Finally, we packed a box with ice and placed it beneath the other lateral hide, under the tub (i.e. the ‘cold’ hide). We then removed the central hide, left lizards to acclimate for five minutes, switched the basking light on and allowed lizards 15 minutes to reach the basking platform and initiate basking. Once the 15-minute period had elapsed, we simulated a predatory attack by chasing each lizard off the basking ‘hot’ hide until it entered into the ‘cold’ hide situated at the opposite end of the tub. Researchers simulating a predatory attack wore blue nitrile gloves and used their fingers to chase lizards around in a standardized way. Simulated predation by an approaching human has been intensively used in the past for lizards because they are known to respond to an approaching human as a predator (Font et al., 2012). After simulated attacks, lizards were allowed 45 minutes to emerge from the ‘cold’ hide and return to the basking platform, during which time we measured the time to return to their basking sites (LATB). Lateral refuges were removed after the end of this trial.

### *Spatial learning trials (days 9-28)*

To measure spatial learning ability, we set up a simple spatial essay using an anti-predatory paradigm successfully used in previous studies (Burghardt, 1977; Noble et al., 2012). Lizards were tested in their own holding terrariums. We initiated trials by re-introducing the two lateral hides (same positions as the ‘hot’ and ‘cold’ hides in the anti-predatory trial; the hide positioned where the ‘cold’ hide had been in the anti-predatory trial was selected to act as the ‘safe’ hide across the spatial learning trials) and removing the middle hide, after which lizards

were given a variable amount of exploration time (30 – 45 minutes). After this time, we used the scaring protocol used in the anti-predatory trial to scare lizards around the tub until they entered into the ‘safe’ hide. Each lizard was tested once a day for an overall period of 20 days (i.e. 20 trials), during which we measured whether it chose the correct ‘safe’ hide or not, the number of incorrect choices (i.e. number of times it ran into the ‘unsafe’ hide), and the overall latency to enter the ‘safe’ hide. We could film a maximum of 8 trials simultaneously so cognition trials were conducted at four different time slots each day: morning (10:00 – 11:30), midday (11:30 – 13:00), afternoon (13:00 – 14:30) and early evening (14:30 – 16:00). We divided the 32 lizards in each batch into four groups of eight lizards and scheduled trials so that we counter-balanced the time of the day in which lizards were tested. Results were hence blocked every 4 days (i.e. 4 trials) into a single learning block. We considered a lizard to have learnt the spatial cognition task when: a) it accumulated a significant correct/incorrect tally according to a binomial distribution (e.g. 5/5, 7/8, etc.); and b) when, from this point on, its overall correct/incorrect tally until the end of trials remained significant. Lizards for which their overall correct/incorrect tally did not include at least 10 trials (e.g. because their first significant tally happened towards the end of the experiment) were also considered as non-learners (see OSM for more details about behavioural trials).

### *Statistical analyses*

We categorized 62 of 64 lizards as learners and non-learners and excluded two ambiguous learners. Also, due to an unexpected contingency (camera malfunction) we missed considerable data from 3 males and 2 females that we excluded from analyses. We used generalized estimating equations (GEEs) to analyse learning curves for (i) the number of incorrect choices; and (ii) the latency to enter the safe hide for males and females and learners and non-learners. We rounded latency to the nearest whole number and modelled both latency and incorrect choices using a Poisson error distribution. We included lizard ID as the grouping variable and used an autoregressive 1 correlation structure (AR1). The GEEs estimate a scale parameter and account for over-dispersion in the models. We compared models using Wald tests to test for significant block, sex and sex\*block effects as well as significant effects of learning, block, and learning\*block effects.

To analyse the relationship between behavioural traits and learning we used generalised linear models (GLMs) with a binomial error distribution and ‘logit’ link function, in R v 2.14.0 (R Development Core Team, 2010). Learning (binary: ‘Learn’ or ‘No learn’) was modelled as a function of sex and body condition, exploratory behaviour [i.e. time to explore two hides (T2ER) and time moving in novel environment (TL)] and boldness [i.e. latency to bask after simulated predatory attack (LATB), and whether an individual ate a novel prey item (1 = ate; 0 = no eat) (NEO)]. Body condition was calculated by taking the residuals from a linear regression between log mass and log body size (SVL) (Schulte-Hostedde et al., 2005). Using the latter independent variables, we generated a series of candidate models based on *a priori* hypotheses about the role behavioural types play in affecting the probability of learning based on previous results from birds and fish (Arbilly et al., 2010; Carere and Locurto, 2011; Cole et al., 2012; Cole and Quinn, 2012). In all models we controlled for sex differences in learning and in some models we also controlled for body condition because there is evidence that behaviours exhibited can be condition-dependent (Leary et al., 2004; Selonen and Hanski, 2010). Graphical inspection of variables suggested that LATB was not necessarily linearly related to the probability of learning and had two clear peaks at each end of its distribution. Given that previous work has shown evidence for the existence of ARTs in this system and that these ARTs are linked to behavioural syndromes along a ‘bold-shy’ continuum (Keogh et al., 2012; Keogh et al., 2013; Noble et al., 2013b; Stapley and Keogh, 2004, 2005) we included a quadratic parameter in models with LATB to estimate whether there was evidence for non-linearity. Given that our sample size is small (logistical and practical difficulties in collecting these data precluded large sample sizes) we limited the number of parameters to be estimated to a maximum of five. In addition, because our exploratory behaviours were taken from the same assay, we did not include both measures of exploration in the same model to avoid possible autocorrelation between these variables. Prior to analysis we standardized our independent (input) variables (mean = 0, sd = 2) because this permits interpretation of main effects in the presence of higher order parameters and also eases comparisons among model estimates (Gelman, 2008; Schielzeth, 2010).

Alternative models were evaluated using the second order information criteria, AIC<sub>C</sub>, which is most appropriate when the ratio of parameters to sample size is less than 40 (Burnham and Anderson, 2002). Since there was no clear ‘best’ model in our candidate set (i.e. Akaike model weight > 90%) we adopted a model averaging approach in addition to

presenting our top ranked model (Symonds and Moussalli, 2011). We chose to present model-averaged coefficients in addition to the top model as opposed to one or the other on their own for two reasons. First, we made predictions about the direction of individual parameter estimates for behavioural traits and because the hypothesised role of behavioural types on learning is still in its infancy we feel that it is important to present effect sizes for all hypothesized parameters to guide future research (Grueber et al., 2011; Symonds and Moussalli, 2011). Second, we used our top model and the estimated parameters to make predictions about the probability of learning given that the coefficients in this top model explain the greatest amount of variation in our data. Although it has been suggested to exclude models in the candidate set that are more complex versions of reduced models during model-averaging (Burnham and Anderson, 2002) we decided not to do this because this can exclude biologically relevant and important effects in more complex models (Grueber et al., 2011). Due to our limited set of models and hypothesized relationships between learning and our behavioural traits we choose natural model averaging using the models that had a cumulative model weight of 95% (Burnham and Anderson, 2002). We inspected the fit of all top models (2  $\Delta$ IC) by looking for influential points (Cook's distance and hat values) and testing for collinearity using variance inflation factors. We found weak evidence for over-dispersion (residual deviance/residual degrees of freedom  $\leq 1.3$ ). The model with the highest dispersion was the top model; however, re-fitting this model with a quasi-binomial error distribution, where a dispersion parameter is estimated, did not affect the results.

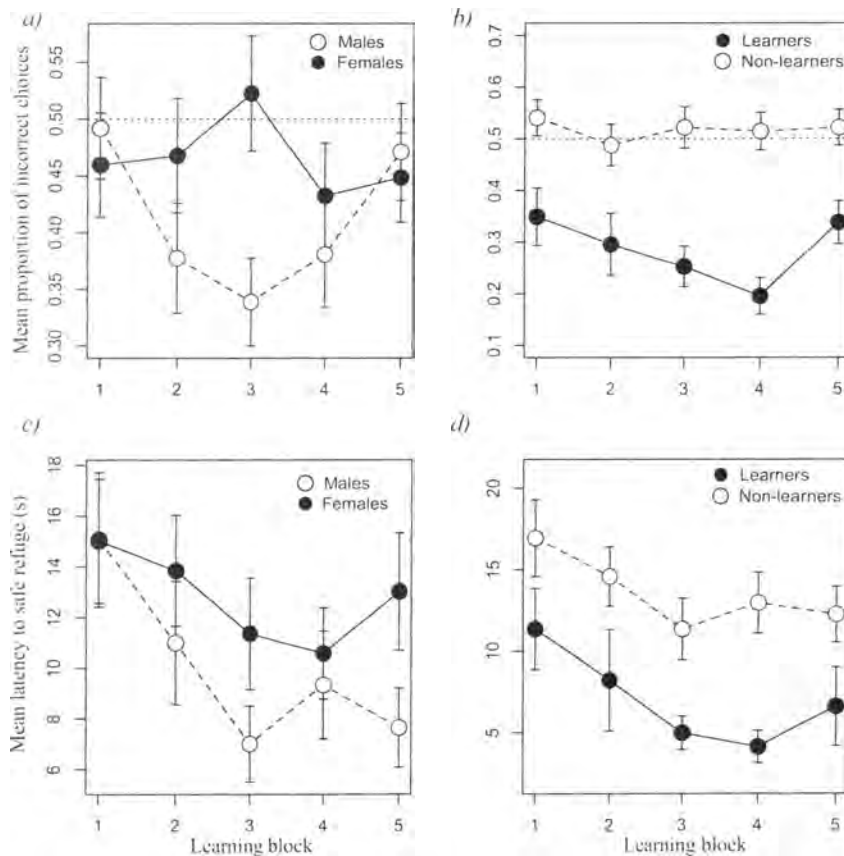
## RESULTS

### *Sex differences in spatial learning*

Twice the number of males learnt the spatial task within 20 trials compared to females [14 of 31 (45.2%) males and 7 of 31 (23.3%) females]. The analysis of learning curves revealed differences in learning between males and females. The number of incorrect choices across blocks decreased significantly (Wald  $\chi^2 = 4.73$ ,  $P = 0.03$ ; Fig. 1a) and females made significantly more incorrect choices than males (Wald  $\chi^2 = 25.1$ ,  $P < 0.001$ ; Fig. 1a) with no evidence for a significant sex\*block interaction (Wald  $\chi^2 = 2.92$ ,  $P = 0.57$ ). Males and females did not, however, differ significantly in their latency to enter the safe hide (LAT)



across blocks (Sex: Wald  $\chi^2 = 3.07$ ,  $P = 0.55$ ; block: Wald  $\chi^2 = 0.32$ ,  $P = 0.57$ ; Fig. 1c). Although there was a tendency for both learners and non-learning lizards to decrease their latency to enter the correct hide across blocks this was not significant (Wald  $\chi^2 = 3.55$ ,  $P = 0.47$ ; Fig. 1d); however, learners had significantly lower LAT than non-learners (Wald  $\chi^2 = 3.96$ ,  $P = 0.047$ ; Fig. 1d). Learners also made significantly fewer incorrect choices than non-learners across blocks (Learn: Wald  $\chi^2 = 25$ ,  $P < 0.001$ ; Block: Wald  $\chi^2 = 14.8$ ,  $P < 0.001$ ; Block\*Learn: Wald  $\chi^2 = 4.71$ ,  $P = 0.32$ ; Fig. 1b). Non-learners always exhibited a proportion of wrong choices close to 0.5 (i.e. the probability of entering into the correct hide by chance). In contrast, the proportion of wrong choices for learners was already well below that expected by chance ( $p = 0.5$ ) within the first learning block (4 trials).



**Fig. 1** - Learning curves for males and females (a & c); and learning vs. non-learning lizards (sexes pooled) (b & d). The dashed line in b) and c) marks the probability of entering into the correct hide by chance given the experimental setup. Note that instances in which lizards were

already encountered in the ‘safe’ hide at the time of the simulated attack (see methods) were not considered for the analysis of latencies.

**Table 1.** Models evaluated testing the effects of sex, body condition (condition) and exploratory (TL and T2ER) and boldness (LATB and NEO) variables on the probability of learning a spatial task. The sample size (N), the number of parameters estimated (K), sample size corrected Akaike information criteria (AIC<sub>C</sub>), the ΔAIC<sub>C</sub> between the top model (ModelA2.8) and each candidate model, the probability of the model in the candidate set, Akaike’s weight (*w*), the evidence ratio (ER) and the model formula are provided. Note continuous independent variables are standardized prior to analysis.

Model	N	K	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	Akaike <i>w</i>	ER	Model Formula
ModelA2.8	56	4	64.89	0	0.372	1	Learning ~ Sex + LATB + LATB <sup>2</sup>
ModelA2.9	56	5	65.97	1.08	0.217	1.72	Learning ~ Sex + condition + LATB + LATB <sup>2</sup>
ModelA2.10	56	5	66.70	1.81	0.150	2.47	Learning ~ Sex + Neo + LATB + LATB <sup>2</sup>
ModelA2.11	56	5	67.21	2.32	0.117	3.19	Learning ~ Sex + T2ER + LATB + LATB <sup>2</sup>
ModelA2.12	56	5	67.31	2.42	0.111	3.35	Learning ~ Sex + TL + LATB + LATB <sup>2</sup>
ModelA2.2	56	2	72.00	7.11	0.011	34.99	Learning ~ Sex
ModelA2.3	56	3	72.72	7.83	0.007	50.15	Learning ~ Sex + condition
ModelA2.1	56	1	73.82	8.93	0.004	86.92	Learning ~ 1
ModelA2.6	56	4	74.28	9.39	0.003	109.40	Learning ~ Sex + condition + Neo
ModelA2.4	56	4	74.58	9.69	0.003	127.10	Learning ~ Sex + condition + LATB
ModelA2.5	56	4	74.78	9.89	0.003	140.47	Learning ~ Sex + condition + TL
ModelA2.7	56	4	74.84	9.95	0.003	144.75	Learning ~ Sex + condition + T2ER

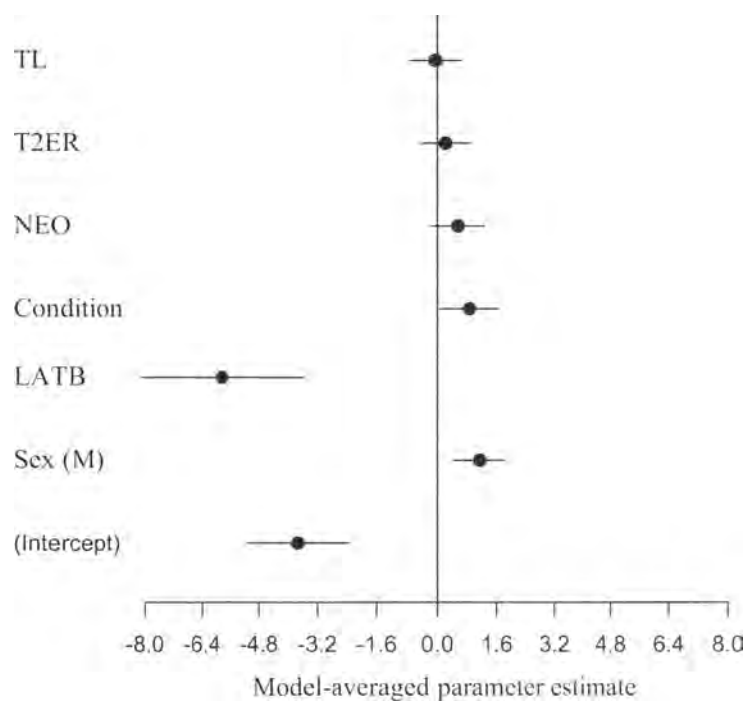
**Table 2** – Parameter estimates and 95% confidence interval around estimates for the top supported model (ModelA2.8; Table 1). Note that coefficients are standardized  $[(x_i - u_i)/(2\sigma_i)]$ .

Coefficient	Estimate	Lower 95% CI	Upper 95% CI
Intercept	-3.79	-6.70	-1.67
Sex (M)	1.20	-0.09	2.63
LATB	-5.82	-10.71	-2.07
LATB <sup>2</sup>	10.98	4.02	20.18

*Individual behavioural type and the probability of spatial learning*

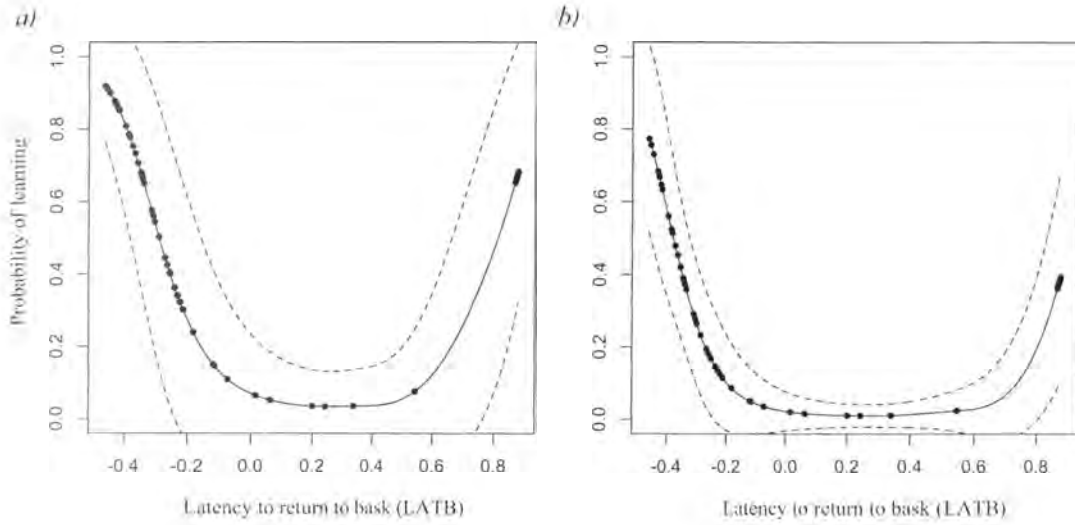
Our two measures of exploratory behaviour, time in locomotion (TL) and time to explore the two hides (T2ER), explained little variation in the probability of learning with all models containing these two variables being greater than 2  $\Delta AIC_c$  units from the top model (Table 1; ModelA2.11 and ModelA2.12). This was also evident in the model-averaged estimates with these two variables having small effect sizes; T2ER had a positive estimate while TL had a slightly negative estimate (Fig. 2).

Whether a lizard ate a novel food item or not (NEO) did not contribute to much variation in the probability of learning (Model A2.10; Table 1) and the model-averaged estimate showed a small positive effect on the probability of learning (Fig. 2). Models containing only the main effect of the latency to return to the basking hide after a predatory attack (LATB) poorly explained variation in the probability of learning (Table 1), however, there was strong evidence that this relationship was non-linear (Table 1 & 2) with models containing a quadratic parameter for LATB being superior than competing models (Table 1 & 2). The predicted probabilities of learning showed that there were two groups of individuals with a high probability of learning located at the extremes of this distribution (Fig. 3). Individuals with short latencies to return to the basking hide ('bold') had a high probability of learning the task and there was a sharp decline in this probability of learning to individuals with intermediate latencies (Fig. 3). The probability of learning the spatial task increased again for individuals taking a long time to return to the basking hide ('shy') (Fig. 3). 'Bold' males (i.e. individuals at -0.4 units from the mean) were predicted to have an 82% probability of learning while 'bold' females had a 57% probability of learning the spatial task (Fig. 3a & b). In contrast, 'shy' males (individuals at 0.8 units from the mean) are predicted to have a 45% probability of learning the spatial task where as 'shy' females are predicted to have a 20% probability of learning (Fig. 3a & b).



**Fig. 2** - Model-averaged parameter estimates for the behavioural traits (LATB, NEO, TL and T2ER) and covariates (sex and condition) hypothesized to influence the probability of learning. Model averaged estimates are based on standardized input variables  $[(x_{LATB_i} - u_{LATB})/(2\sigma_{LATB})]$  using the natural averaging method (Burnham and Anderson, 2002).





**Fig. 3** - The predicted probability of learning a spatial task for *a)* males and *b)* females as a function of the latency to return to a basking hide after a simulated predatory attack (LATB). Predicted probabilities are based on our top-supported model (ModelA2.8; Table 1) using standardized input variables  $[(x_{LATB_i} - u_{LATB}) / (2\sigma_{LATB})]$ . We used our standardized input LATB variable to predict probabilities and fit a smoothed cubic spline function to the data. Dashed lines above and below fitted lines are the 95% confidence intervals [predicted (p)  $\pm 1.96 \cdot se$  of fit and smoothed with a cubic spline].

## DISCUSSION

We show that male *Eulamprus quoyii* were significantly better at spatial learning than females. We also found significant variation among individuals in boldness (latency to exit a hide and return to a basking platform after a simulated attack) that shows clear differences in behavioural types ('shy', intermediate, 'bold') and show a significant association between variation in boldness and the probability of learning a spatial task: 'bold' and 'shy' behavioural types were both more likely to learn the spatial task than intermediate individuals.

### *Sex differences in spatial cognition*

In mammals and other taxa, sex differences in cognitive abilities have been best studied in relation to spatial cognition, where males have been reported to exhibit better spatial cognitive abilities than females (Du Toit et al., 2012; Geary, 1995; Healy and Rowe, 2010; Healy et al., 2008; Hodgson and Healy, 2005; Jozet-Alves et al., 2008; Sovrano et al., 2003; Vallortigara, 1996). Sex differences in spatial cognition have been hypothesized to arise from differential selective pressures in relation to sex-specific dispersal, mobility during reproduction, intra-sexual competition, female choice, and differences in home range size (i.e. the range-size hypothesis; (Gaulin and Fitzgerald, 1989)). Although the latter hypothesis seems to have the most support (Geary, 1995; Jones et al., 2003), most hypotheses actually link spatial ability to space use and differ only in their explanations as to why the sexes differ in their use of space.

In many lizard species, reproduction seems to pose higher spatial challenges to males compared to females. In particular, males generally possess larger home ranges and/or need to process more complex spatial information than females in order to achieve copulations (e.g. home-range boundaries, location of rivals, location of females within their home range; (Calsbeek and Sinervo, 2008; Carazo et al., 2008; Jones et al., 2003)). As predicted by such differences between male and female social roles (Pianka and Vitt, 2003; Stamps, 1977), we found that male *E. quoyii* have better spatial learning abilities than females. More males successfully learnt the spatial learning task than females (45.2% male learners vs. only 23.3% female learners), and females generally made more incorrect choices than males across the experiment (Fig. 1). To the best of our knowledge, sex differences in learning have never been reported in a reptile, which is particularly striking in the case of lizards (and in the context of spatial cognition) because sexual differences in spatial demands seem widespread in lizards (Pianka and Vitt, 2003; Stamps, 1977). Our study is therefore the first evidence to date for sexually dimorphic cognitive performance in a reptile, but we suggest that the home-range size hypothesis put forward to explain sexual differences in spatial cognition in mammals is likely to apply to many lizard species too (Jones et al., 2003).

### *Boldness, spatial learning and ARTs in Eulamprus quoyii*

In this study, we found considerable variation in individual latency to return to basking after a simulated predatory attack, and a clear association between variation in this boldness measure and variation in individual spatial learning performance. ‘Bold’ individuals (quick to return to

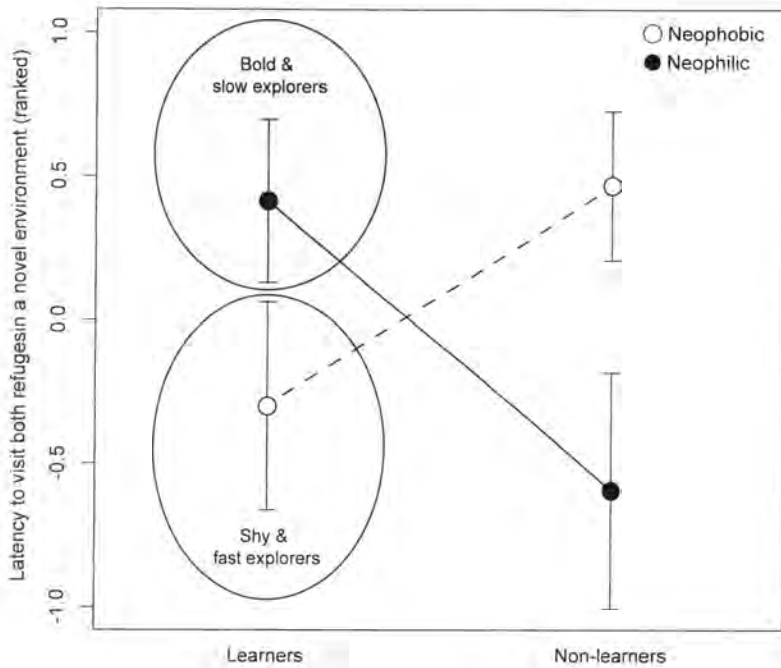
bask after attack) had the highest probability of learning the spatial task. However, ‘shy’ behavioural types also learnt the spatial task with a higher probability than individuals with intermediate behavioural types. This is the first evidence linking cognition and behavioural types in a reptile, and it is inconsistent with the risk-reward hypothesis and with previous studies ((Boogert et al., 2006; Dugatkin and Alfieri, 2003; Overli et al., 2006; Sih and Del Guidice, 2012), where the relationship between boldness and learning was always reported to be linear.

We suggest an interesting possibility is that ‘bold’ and ‘shy’ behavioural types may both have enhanced spatial learning because of their association with alternative reproductive tactics. In *Eulamprus quoyii*, like in other lizards, males exhibit ARTs that include a ‘territorial’ and a ‘floater’ tactic (Noble et al., 2013b). Territorial lizards actively defend core areas that tend to overlap the home range of several resident females against other males, while ‘floater’ males instead navigate their way over longer distances, traversing several different territories in their search for copulations with females (Calsbeek and Sinervo, 2008). Alternative reproductive tactics in *E. quoyii* are associated with divergent selection for different behavioural types (Noble et al. 2013) that seem to be part of a behavioural syndrome relating to boldness, activity and exploratory behaviour, as has been shown in a closely related species: *E. heatwolei* (Stapley and Keogh, 2004, 2005). Territorial male *Eulamprus* are bolder, more active, and slower explorers than floater males, which makes sense from the point of view of their ARTs but does not seem to conform to the typical fast-slow, high-low risk-reward trade-off (Noble et al., 2013b; Stapley and Keogh, 2004, 2005). Interestingly, the reproductive success of each of these tactics crucially depends on spatial cognition. Territorial individuals need to process and memorize detailed spatial information within their territory (e.g. the position of females within a territory, territory boundaries and the degree of overlap with neighbouring males), and such information is bound to be highly variable due to frequent changes in the social status and/or condition of territorial males throughout the reproductive season (Carazo et al., 2008; Stamps and Krishnan, 1998). Conversely, spatial learning is also likely to be crucial for floater males because they need to navigate their way over large home ranges consisting of varied habitat, and where the location of rival males and potential mates is important for both reduced conflict and reproduction (Calsbeek and Sinervo, 2008; Noble et al., 2013b). Under this scenario, we hypothesise that correlational selection of divergent behavioural types may have given rise to enhanced spatial learning for both these male

reproductive tactics. Similarly, ARTs have also been suggested in female *Eulamprus* in relation to territory residency, anti-predatory behaviour and exploration (Stapley and Keogh, 2005), although to date there is no direct evidence of a clear link between these behavioural traits in *Eulamprus* females. Alternatively, strong selection for divergent cognitive-behavioural types in males may have also lead to correlated selection in females (Fortsmeier et al., 2011). Indeed there does appear to be strong sexual selection on the behavioural traits associated with each of the ARTs (Noble et al., 2013b).

As a word of caution, and while the above is certainly compelling, our results cannot be taken as direct support for this hypothesis. First, while ARTs have been documented in this species we did not know the ARTs of the individuals used in our study. Second, because we did not find any evidence that exploration and/or boldness in the neophilia experiment were significantly associated with spatial learning, which is something that we would have expected if ARTs were shaping cognitive-behavioural syndromes. In particular, we would have expected neophilia/boldness and slow exploration (i.e. characteristic of territorial males) and neophobia/shyness and fast exploration (i.e. characteristic of floater males) to be positively associated with spatial learning in male lizards. Plotting these two variables separately for male learners vs. non-learners (*post hoc*) does hint at such a relationship in males (Fig. 4), but this was not picked up in our analysis (perhaps due to the small sample sizes and low statistical power at this level of analysis). We suggest that future studies should measure ARTs (i.e. territorial behaviour) directly in the wild, and then relate this to spatial learning in the laboratory.





**Fig. 4-** Interaction plot looking at male differences in exploration (T2ER) depending on whether male lizards learnt or not (learners vs. non-learners), and ate or not in the food neophobia experiment (NEO). T2ER shows the ranks converted to normal quantiles to normalize the rank data. Existing evidence suggests that territorial males are bold and slow explorers in a novel environment while floater males are shy and fast explorers in a novel environment (Stapley & Keogh 2004, 2005; Noble et al. 2013).

### Conclusions

To conclude, we provide the first evidence of sex differences in learning in a reptile, but suggest that sexual dimorphism in spatial learning is inherently linked to the different social roles (and ensuing spatial demands) experienced by males and females in territorial lizards, and that consequently this phenomenon is probably widespread. We also show that ‘bold’ and ‘shy’ behavioural types both have enhanced spatial learning and suggest that this may be because of their association with alternative reproductive tactics in *Eulamprus*. This is also the first evidence that behavioural traits such as boldness are associated with learning in a reptile

and, along with recent studies in birds, highlights the importance of considering cognitive traits in the study of behavioural syndromes, and *vice versa*. We suggest that future studies consider different social roles and tactics as an important factor in the evolution and/or development of specific behavioural-cognitive syndromes. In lizards, a first step to test this hypothesis would be to examine the link between territorial behaviour (i.e. ARTs), spatial learning, and fitness. Characterizing whether spatial learning is under strong selection and, if so, examining its relative intensity across different social roles and tactics is bound to provide crucial insight into our understanding of how intraspecific variability in spatial cognition arises.

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## SUPPLEMENTARY MATERIAL

### Materials and Methods

#### *Exploratory Behaviour (Day 1)*

We removed lizards from their holding tubs and transferred them to the lab, where they were released into a novel enclosure measuring 68.3 (L) x 48.3 (W) x 38.5 (H) cm. Each enclosure had paper substrate that was gridded with 5 x 5 cm squares. Two weighted black hide boxes with three entrances each were situated at each end of the enclosure (Fig. S1). Immediately before trials, we placed lizards under a central acclimation box that we lifted from behind a blind after 3 minutes. Trials were initiated at this moment, lasted for 30 minutes, and were recorded using a mounted security camera (Swann® security system). All recordings/trials were conducted at a room temperature of 28°C, which is the preferred body temperature for *E. quoyii* (Law & Bradley 1990). Video footage was scored blind to whether individuals had learnt the spatial test. In addition, subsets of data (e.g. trials within each experiment) were scored by the same individual to avoid inter-observer bias (Burghardt *et al.*, 2012). We used JWatcher (<http://www.jwatcher.ucla.edu/>) to score the following behaviours: (i) time spent moving (TL), and (ii) time taken to enter the two opaque hides in the enclosure (T2ER). Lizards that did not visit both hides within the 30 min were assigned a latency of 1800 seconds. We included the latency to visit both hides as a measure of quickness to explore in a novel environment, and locomotion as a measure of the amount of exploration.

#### *Measures of Boldness*

Boldness is most often interpreted as the tendency to take risks, especially in novel situations, and is usually measured experimentally in relation to anti-predatory behaviours



or individual response to novel cues (Carter *et al.*, 2012). In this study, we used two experiments to assay boldness separately in an anti-predatory and in a neophobic context.

### *Assay I – Neophilia (Days 2-7)*

We first quantified boldness in relation to neophobia/neophilia to a novel prey item. Immediately following an exploratory trial, we inserted a third hide against the side of one of the long-sided walls of the tub, in a central position, with the entrances facing inwards (Fig. S1). To obtain a neophobia/neophilia estimate, we examined individual lizard responses when presented with a completely novel food item. We used commercially available dead silkworm pupae, which are often used as a supplement food for reptiles but are unavailable in their natural habitat. We selected silkworm pupae not only because they constitute a novel visual and chemical stimulus, but also because we could standardize their movement during presentation. We suspended a single pupa from the end of a transparent fishing line threaded through the body cavity. The fishing line was tied to a wooden dowel, which the researcher controlled from behind a blind. Immediately preceding trials, we moved the focal lizard tub to the recording area and made note of the location of each lizard within its enclosure. Lizards were then given between 2 - 5 minutes to acclimatize before starting trials. During trials, we presented the pupa by gently dangling it in front of the lizard for 3 minutes. Pupae were presented at ca. 15 cm (i.e. three 5 cm squares) from the lizard's snout for the first minute and this distance was decreased by 5 cm each minute thereafter. All trials were recorded. After the first neophobia trial, we repeated this procedure once a day for four more consecutive days (five days total). Finally, on day 6 we presented each lizard with a pupa that was suspended in the same manner as in prior feeding trials, but which we left hanging ca. 1 - 2 cm from the centre of the tub for one hour, and in the absence of observers. We recorded at which stage (during these trials) each lizard first attacked or ate the novel prey item. Only 25 of 64 (39%) lizards actually consumed the larva, although lizards that ate did so repeatedly throughout the feeding trials. We therefore divided lizards in two categories

(NEO): neophilic (lizards that ate the novel prey at some point during trials) and neophobic (lizards that did not eat the novel prey at all).

### *Assay II – Anti-predatory trial (Day 8)*

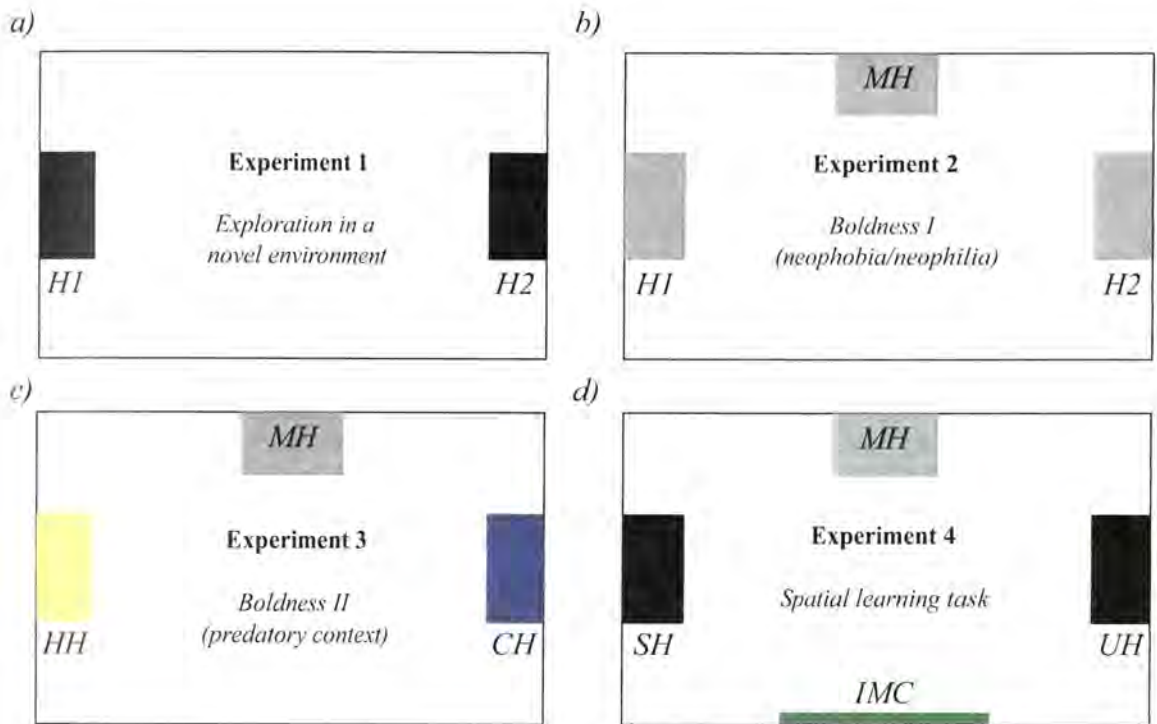
On day 8 we staged an anti-predatory trial designed to gauge individual boldness against predators. Lizards were tested in their own holding tub, which was no longer a ‘novel’ environment. The assay began by gently chasing each lizard into the central hide (Fig. S1). We then randomly selected one of the two remaining hides and designated one as the ‘hot’ hide by suspending a 60/100W incandescent bulb ca. 25 cm above this hide, effectively transforming the top surface into a basking platform. Finally, we packed a box with ice and placed it beneath the second hide, under the tub (i.e. the ‘cold’ hide; Fig. S1). During anti-predatory trials, ambient room temperatures were cooled down (22 – 24°C) to promote basking by lizards. Once the assay had started, we removed the central hide and left lizards for five minutes to acclimate, after which time we switched the basking light on and allowed lizards 15 minutes to reach the basking platform and initiate basking. Once the 15-minute period had elapsed, we simulated a predatory attack by chasing each lizard off the basking ‘hot’ hide until it entered into the ‘cold’ hide situated at the opposite end of the tub. Researchers simulating a predatory attack wore blue nitrile gloves and used their fingers to chase lizards around in a standardized way. Simulated predation by an approaching human has been intensively used in the past for lizards because they are known to respond to an approaching human as a predator (Font et al. 2012). After simulated attacks, lizards were allowed 45 minutes to emerge from the ‘cold’ hide and return to the basking platform, during which time we measured the time to return to their basking sites (LATB).

*Spatial learning trials (days 9-28)*

To measure spatial learning ability, we set up a simple spatial essay using an anti-predatory paradigm successfully used in previous studies (Burghardt, 1977; Noble *et al.*, 2012). Following anti-predatory trials on day 8 we: i) reintroduced the middle hide (Fig. S1); ii) removed the ‘hot’ and ‘cold’ hides along with the basking bulb and ice-box; and iii) introduced a distinct ‘intra-maze’ cue consisting of a piece of green rectangular cardboard pasted to the wall opposite to the middle hide; this served to disambiguate spatial orientation during trials. Lizards were tested in their own holding tubs, which were transferred to the filming area just before the beginning of trials. Each lizard tub was positioned in the same position and orientation within the room to keep extra-maze cues constant across trials. When moved to the filming area, lizards invariably entered the middle hide (the only one available at this time). We took advantage of this circumstance to suspend an incandescent bulb from the middle of the tub and introduce two hides in the same positions as the ‘hot’ and ‘cold’ hides used in the anti-predatory trial (Fig. S1). For each lizard, the hide positioned where the ‘cold’ hide had been in the anti-predatory trial was selected to act as the ‘safe’ hide in the spatial cognition trials. Hence, the position of the ‘safe’ hide was randomly determined for each individual, but was kept constant across trials. We initiated trials by removing the middle hide, after which lizards were given a variable amount of time to explore the tub with the ‘safe’ and ‘unsafe’ hides in place. We randomly varied the time (30 – 45 minutes) lizards were given to explore before simulated predatory attacks to prevent them from learning to anticipate attacks. After this time, we followed the scaring protocol used in the anti-predatory trial to simulate a predatory attack. We scared lizards around the tub and, when necessary, out of the ‘unsafe’ hide until they entered into the ‘safe’ hide. Once a lizard entered the ‘safe’ hide, we removed the ‘unsafe’ hide and we put the central hide back into the tub. ‘Safe’ hides were left inside the tub until dusk, at which time they were gently removed from the tub taking care not to startle lizards that might be inside them. Each lizard was tested once a day for an overall period of 20 days (i.e. 20 trials). For each lizard, we measured whether it chose the correct ‘safe’

hide or not, the number of incorrect choices (i.e. number of times it ran into the 'unsafe' hide), and the overall latency to enter the 'safe' hide. A lizard was considered to have chosen correctly when it was already found inside the 'safe' hide at scaring time or when the first hide it ran into in response to the simulated predatory attack was the 'safe' hide. Cognition trials were conducted at four different time slots each day: morning (10:00 – 11:30), midday (11:30 – 13:00), afternoon (13:00 – 14:30) and early evening (14:30 – 16:00). We divided the 32 lizards in each batch into four groups of eight lizards and scheduled trials so that we counter-balanced the time of the day in which lizards were tested. Hence, each lizard was tested at a different time each day and went through all the time slots every four days; results were blocked every 4 days (i.e. 4 trials) into a single learning block so that each lizard was subject to 5 learning blocks overall. We considered a lizard to have learnt the spatial cognition task when: a) it accumulated a significant correct/incorrect tally according to a binomial distribution (e.g. 5/5, 7/8, etc.); and b) when, from this point on, its overall correct/incorrect tally until the end of trials remained significant. Lizards for which their overall correct/incorrect tally did not include at least 10 trials (e.g. because their first significant tally happened towards the end of the experiment) were also considered as non-learners.





**Fig. S1** - Layout of lizards' experimental/holding tubs during each of the four experiments.

a) Experiment 1 consisted of a single 30 min trial (day 1). Immediately before this trial, two hides (*H1* & *H2*) were introduced in the tub. At the end of this experiment, we added a third hide (Fig. 1b; *MH*), which was to remain as a permanent 'home hide' throughout the rest of the experiments (except during some trials, see below). b) Experiment 2 consisted of 5 trials (days 2-6). Lizards had constant access to the three hides (*H1*, *H2*, *MH*) throughout this experiment, but *H1* and *H2* were removed when it ended. c) Experiment 3 consisted of a single 45 min trial (day 8). Immediately before starting, we introduced a 'hot hide' (*HH*) under a basking light and a 'cold hide' (*CH*). 'Cold hides' were cooled down by sliding a packet of ice underneath the tub (see methods). Note that at this time all lizards were inside their home hide (*MH*). Trials began by removing *MH*, after which lizards were left alone for 15 min. After the 15 min had elapsed, all lizards were basking on top of the *HH* and we immediately chased them into *CH*. We terminated trials once a lizard resumed basking, or after 30 min. At the end of experiment 3, we removed the *HH* and *CH*, reintroduced the *MH*, and pasted a green rectangular piece of cardboard (*IMC*; Fig. 1d) on the wall opposite to *MH* to disambiguate the spatial arrangement of tubs during

spatial learning trials. *d)* Experiment 4 consisted of 20 trials (see methods). Immediately before each trial, we shuffled the mulch around to spread chemical cues (except the one underneath MH) and introduced the two new hides (i.e. 'safe' (SH) and 'unsafe' (UH) hides). We started trials by removing the MH. At the end of each trial, we reintroduced the MH and removed the UH. The SH was left inside the tub until lizards switched back to the MH or until the lights were off at the end of the day (at which time it was gently removed).

## CHAPTER 3: MULTIPLE MATING IN A LIZARD INCREASES FECUNDITY, BUT PROVIDES NO EVIDENCE FOR GENETIC BENEFITS

Daniel W.A. Noble, J. Scott Keogh and Martin J. Whiting

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### Abstract

Multiple paternity is taxonomically widespread yet the relative role of direct and indirect (genetic) fitness benefits in explaining the evolution of multiple mating is a topic of intense debate. We test whether female Eastern Water Skinks (*Eulamprus quoyii*) gain direct (fecundity) and/or indirect genetic (increased offspring fitness) benefits through multiple mating. We maintained 216 (108 male, 108 female) *E. quoyii* in six large outdoor enclosures for a single breeding season before bringing gravid females into the lab to give birth. We classified female clutches as being singly (SP) or multiply (MP) sired using six polymorphic microsatellite DNA loci. To test whether females gain genetic benefits, we measured five fitness traits on offspring over their first active season and compared these traits between SP and MP clutches. Contrary to predictions from genetic benefits models, offspring from MP and SP clutches did not differ significantly in snout-vent length, mass, growth rate, sprint speed or endurance. Although MP and SP females did not differ significantly in body size, condition or mass, MP females invested more heavily in reproduction when body size, mass and condition were controlled, producing significantly more offspring compared to SP females. We suggest that polyandry in *E. quoyii* possibly evolved as a mechanism to ensure fertilization of ova, similar to what has been reported in some other taxa.

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## INTRODUCTION

Explaining the widespread occurrence of multiple paternity in nature, given the inherent costs of mating with multiple partners, has been a challenging research endeavor (Jennions and Petrie 2000; Arnqvist and Kirkpatrick 2005; Griffith 2007; Eliassen and Kokko 2008; Uller and Olsson 2008; Slatyer et al. 2012). Female reproductive success is often not predicted to increase as females mate multiply because of the constraints imposed on female reproductive output (Bateman 1948). While female multiple mating appears to be driven by forced copulations in some species, detailed behavioral studies show that females of many species actively engage in multiple mating (Zeh and Zeh 1996; Jennions and Petrie 2000). This observation is puzzling because mating can be a costly process (e.g. Le Galliard et al. 2005; Johnson and Brockmann 2010), yet in order for polyandry to evolve it is expected that the fitness benefits of mating multiply outweigh these costs. In some species polyandry may provide direct benefits to females, ensuring the fertilization of ova and increased female fecundity (Ridley 1988; Slatyer et al. 2012). Such is the case for crickets, where increased investment in nuptial gifts increase female fecundity (Fedorka and Mousseau 2002). However, in many species females gain no obvious direct fitness benefits from polyandry. In these situations polyandrous females are predicted to receive only indirect genetic benefits (Jennions and Petrie 2000). Genetic benefits are generally ascribed to three separate categories: 1) 'good' genes benefits, where females mate multiply to acquire paternal genes that enhance offspring fitness (Jennions and Petrie 2000); 2) genetic compatibility, where females optimize mating with particular males to reduce intragenomic conflict between paternal and maternal alleles (Zeh and Zeh 1996; Neff and Pitcher 2005); or 3) increased genetic diversity, where females ensure that offspring are heterozygous at fitness enhancing loci (Brown 1997). Importantly, all three hypotheses predict that offspring from multiply mated females have increased fitness, on average, compared to singly mated females. However, recent work suggests that this cost-benefit dichotomy maybe an incomplete view of why females may mate multiply (Bleu et al. 2012; Kokko and Mappes 2012). Bleu et al. (2012) show that when mating costs are high, the costs of multiple mating and the risks of remaining un-mated become equally important to male quality and female choosiness is reduced. In contrast, when mating costs are low, mate quality becomes an important factor explaining female mating patterns and choosiness thresholds increase. Indeed, the costs of remaining unmated can be



very high and a more probable null model of female mating patterns may be to simply accept copulations above a female optimum, given stochastic patterns of mate encounter rates (Kokko and Mappes 2012).

Although there is evidence to suggest that females mate multiply to gain genetic benefits (Jennions and Petrie 2000; Garant et al. 2005; Olsson et al. 2005; Byrne and Whiting 2011; Olsson et al. 2011), much of our knowledge comes from studies on birds (Griffith et al. 2002; Westneat and Stewart 2003), frogs (Byrne and Roberts 2011; Byrne and Whiting 2011; Roberts and Byrne 2011), fishes (DiBattista et al. 2008) and invertebrates (Fedorka and Mousseau 2002; Cothran 2008) and we are still only just beginning to understand the complexity with which these benefits manifest themselves in natural populations. Work in lizards and snakes suggest that females may gain genetic benefits that increase offspring survival by enhancing genetic diversity or through the promotion of sperm competition which favors particular genes or sperm (Madsen et al. 1992; Olsson and Madsen 2001; Madsen et al. 2004; Olsson et al. 2005; Lancaster et al. 2009; Olsson et al. 2011), however, these studies have focused on only a few model systems. For example, in an inbred population of European adders (*Vipera berus*), Madsen et al. (1992) show that polyandrous female adders have increased offspring viability compared with singly mated females and they suggest that female polyandry may benefit females by promoting sperm competition that selects for viability genes. Later work where genetically differentiated males were introduced to the same population showed that females that bred with these males produced offspring that had higher survival resulting in an increased recruitment of these offspring in subsequent years (Madsen et al. 2004). In the Swedish sand lizard, *Lacerta agilis*, polyandry has also been shown to increase offspring survival in multiply sired clutches (Olsson et al. 2011). Males have also been shown to vary in MHC haplotypes and such variation is associated with decreased parasitism and a better ability to regulate the development of nuptial coloration by maintaining high levels of corticosterone despite its immuno-suppressive effects (Olsson et al. 2005). These studies provide valuable insight into the genetic benefits of polyandry, however, possible direct benefits gained by females can be difficult to disentangle from indirect benefits and have often been over-shadowed in the context of genetic benefits studies (Griffith 2007).

Here we test whether multiply sired females gain direct and/or indirect genetic benefits in an Australian lizard, the Eastern Water Skink (*Eulamprus quoyii*). Eastern Water Skinks are viviparous, giving birth to 1 – 9 offspring and the genus is known to have a high incidence of

multiple paternity (Morrison et al. 2002; Dubey et al. 2011; Keogh et al. 2012). There is no parental care in *E. quoyii* and females only gain sperm from males during mating, simplifying the possible direct benefits females may receive from males. Using a large genetic dataset to determine paternity, we tested whether single paternity (SP) and multiple paternity (MP) females differed in their fecundity, as would be predicted from direct benefits models. We also tested whether females gain indirect genetic benefits by testing a series of predictions about the difference in fitness traits between offspring from SP and MP clutches. First, offspring from clutches with multiple sires are predicted to be larger (snout-vent length [SVL] and mass) compared to singly sired clutches. Offspring mass and SVL are important fitness components in lizards and many studies have shown that larger offspring at birth have higher survival (Elphick and Shine 1998; Warner and Andrews 2002). Second, offspring from clutches with multiple males are predicted to have enhanced maximal running performance capacity, as measured by sprint speed and endurance. These measures are commonly used as surrogates of fitness and have been shown to enhance survival in other lizard species, particularly in offspring and juveniles (Husak 2006; Irschick et al. 2008; Le Galliard and Ferrière 2008). Third, offspring from singly and multiply sired clutches might also be expected to differ significantly in growth rate. Growth rate is an important fitness measure and is predicted to be correlated with age at sexual maturity, however, the relationship between growth rate and survival can be complex (Sinervo et al. 1992) and we avoided making directional predictions (Olsson and Shine 2002; Warner and Andrews 2002).

## MATERIALS AND METHODS

### *Lizard collection*

We collected 216 sexually mature lizards from five separate locations within the Sydney area in August and September 2010 (Macquarie University, Shrimpton's Creek, Sydney Olympic Park, Fields of Mars and Narrabeen Golf courses). Lizards were captured by noosing and transported back to Macquarie University for further processing. Each lizard was marked with a PIT tag and their snout-vent length (SVL; mm), mass (g), head width (mm) and head length (mm) were recorded. Lizards were sexed by everting the hemipenes and males and females

were kept separate in large outdoor bins prior to their release in large, semi-natural enclosures (details below).

### *Experimental setup*

Lizards were allocated to one of six outdoor enclosures (10 x 16 m) located on Macquarie University campus. Eighteen males and 18 females ( $n = 36$ ) were released in each enclosure. These densities are similar to those found under natural conditions (G. Swann pers. comm.; K. Wechmann unpublished data). Lizards were released into each enclosure so that there were similar ranges in body size and so that each capture location was represented across enclosures. Each enclosure contained two large piles of rocks connected by a series of fallen debris comprised of varying sizes of logs. Four large water bins were placed in each enclosure and three stacked roofing tiles were placed every 2 m to form a grid. The lizards were placed in the enclosures just before their peak breeding period (20 September - 20 October 2010) and were allowed to breed naturally.

Females were collected at the end of the breeding season and brought back into the laboratory and held in individual containers until parturition. Each container (32 x 45 x 27 cm) had UV lighting on one side and was heated using heat cord to create a thermal gradient for thermoregulation. Lizards had constant access to water and were fed vitamin and calcium dusted crickets or canned dog food every other day. At parturition offspring were sexed, weighed and measured and a small piece of tail tissue was excised and stored in ethanol for later genotyping. Offspring were kept in captivity between 8 – 37 days to measure their sprint speed and endurance. Neonates were held individually in containers with a water bowl and paper substrate. They were fed 4 – 5 baby crickets supplemented with vitamin and minerals every other day. At the end of January all the offspring were re-weighed and measured, marked by toe-clipping (Ferner 2007) and then released into one of four large (10 x 16 m) outdoor enclosures. We used surgical scissors sterilized with 70% ethanol for toe-clipping and the removal of tail tips for tissue (DNA) collection. It was necessary to toe-clip offspring because their small size does not permit the use of PIT tags for individual marking. Furthermore, a small amount of tail tissue was necessary because we were not able to acquire adequate DNA concentrations from toe-clips alone. *Eulamprus quoyii* do exhibit tail autonomy and removing a small amount of tail tissue does not have any noticeably negative

effects. Although tail autonomy can be stressful when almost the entire tail is removed (Langkilde and Shine 2006) we only removed ~3 mm of tail tissue from the tip. Furthermore, toe-clipping in lizards does not inflict significant stress beyond what they would encounter in nature (Langkilde and Shine 2006). Offspring were recaptured from enclosures twice a month until May so that lizards could be re-measured and weighed.

### *Offspring sprint speed*

The sprint speed of each offspring was measured once/day over three consecutive days. Offspring were warmed to a temperature of 28°C in an incubator prior to running them on a 1.2 m racetrack. The racetrack was outfitted with photocells positioned every 25 cm for a total running distance of 1.0 m. Lizards were placed at the beginning of the track and were stimulated to run by lightly tapping their tailbase with a paintbrush. As the photocells were broken an internal stopwatch recorded the time it took for each section to be completed. We used the maximum sprint speed over 1 m for analysis because individuals are known to perform sub-optimally in some measurements and using maximal performance has become the standard for lizards (Losos et al. 2002).

### *Offspring endurance*

Offspring endurance was measured immediately after measurements of sprint speed. We constructed a circular racetrack out of cardboard and used rubber matting as substrate. The rubber provided a rough surface enabling the lizards to gain traction while they ran. Lizards were placed in the circular runway and were stimulated to run by gently tapping them on their tailbase. We kept lizards running by gently tapping them each time they stopped for more than 2 seconds. We continued this until each lizard was tapped five times without eliciting movement, at which point the lizard was considered exhausted. The time from the start of the trial to the end of the fifth tap was considered the lizard's endurance. We repeated this over three consecutive days. We used the maximum time to exhaustion for our analysis (Losos et al. 2002).



### *Offspring growth rate*

We calculated offspring growth rates over two time periods. The first period was their growth rate under captive conditions (from when they were born to when they were marked) and the second was calculated after they were marked and released into semi-natural enclosures (from when they were marked to when they were recaptured in the enclosures). This was necessary because of the varying amount of time each offspring spent under captive conditions, but also to increase our sample size because not all offspring could be recaptured in the enclosures. Growth rates were computed using the following formula:  $\text{Growth Rate} = (\text{SVL}_{t_2} - \text{SVL}_{t_1})/(\Delta t)$ . Where  $\text{SVL}_{t_2}$  is the snout-vent length at marking (period 1) or recapture (period 2) and  $\text{SVL}_{t_1}$  is the snout-vent length at either birth (period 1) or marking (period 2).  $\Delta t$  is the number of days between successive captures. We also calculated growth rates based on mass but this gave similar results to SVL and we decided to focus on SVL. Growth rates were averaged for individuals that were recaptured multiple times in our outdoor enclosure and this value was used in analyses.

### *Paternity assignment*

Whole genomic DNA was extracted from tail tissue using a Blood and Tissue Extraction Kit (Qiagen) according to the manufacturers protocol. We assigned paternity to offspring using 6 microsatellite DNA loci (Ek100, Ek107, Ek8, Ek37, Ek39; Scott et al. 2001; Morrison et al. 2002) and (GQ20/21, GQ16/17; Sumner et al. 2001). PCR reactions were carried out in 20  $\mu\text{L}$  reaction volumes containing 1.0  $\mu\text{L}$  of genomic DNA, 10  $\mu\text{L}$  of GoTaq® (Promega), 0.5  $\mu\text{L}$  (10 pmol  $\cdot$   $\mu\text{L}^{-1}$ ) of forward and reverse primers and 8.0  $\mu\text{L}$  of nuclease-free water. PCR conditions for each locus are described in Scott et al. (2001) and Sumner et al. (2001). Forward primers were labeled with different fluorescent dyes (TET, NAD, VIC, FAM) and product from the final PCR reactions were pooled into a single plate, ran on an ABI 3730 DNA analyser (Applied Biosystems) and scored by the Australian Genomic Research Facility (AGRF) using GENEMAPPER software (Applied Biosystems).

Parentage was assigned using the likelihood-based method in the program CERVUS 3.0 (Kalinowski et al. 2007). We simulated 100,000 offspring with 95% loci typed and 1% mistyped loci, using a strict confidence level of 95% and a relaxed confidence level of 80%. The loci used in our study were highly variable, ranging from 3-34 alleles at a single locus with mean polymorphic information content (PIC) of 0.7014. The combined non-exclusion probability for a parent pair was  $4.46 \times 10^{-6}$ . Paternity was assigned conservatively, and we excluded males as being putative sires if they had one or more mismatches with an offspring. In some cases, males could only be compared at four loci with offspring because of differences in the loci missing between the male and offspring. In these situations we assigned paternity to the male only if he had no mismatches and the trio (male, female and offspring combination) LOD scores were significant. We assigned females to multiply sired and singly sired clutches based on the number of males within her clutch. In some clutches we were unable to identify the father. In most cases this was because we were unable to amplify alleles on one of the parents or because females had bred in the wild and we could not identify paternity. To identify whether multiple males or a single male sired the clutch we manually counted the number of unique alleles present across the offspring at each locus. Loci where three or more alleles were present after ruling out female genotype were considered multiply sired clutches. In these cases we could only identify that these were multiply sired clutches but we could not make conclusions about the number of sires.

### *Data analysis*

Female mass, body size and condition (residuals from a regression between SVL and mass) were compared between singly sired (SP) and multiply sired (MP) clutches as well as between females reproducing and those that did not, using ANOVA. Differences in clutch size and relative clutch mass between SP and MP clutches were compared using ANCOVA after controlling for female body condition and SVL. Relative clutch mass was first rank transformed and normalized by calculating quantiles from a normal distribution with a mean = 0 and SD = 1 (hereafter referred to as 'rank transformed') to remove outliers and satisfy the assumptions of normality and homogeneity of variance. We tested whether there was a sex ratio bias between MP and SP females using a GLM with a binomial error distribution (logit

link). Seven females expelled their PIT tags and so these were excluded from analyses because we could not identify them in the dataset.

We analyzed our data on offspring fitness traits and clutch type with generalized linear mixed models (GLMM) using the ‘lme4’ package (Bates and Maechler 2010) in R (R Development Core Team 2010). We used a Gaussian error distribution with an identity link function in all our models. Using an information-theoretic approach, we created a series of candidate models and compared  $AIC_C$  between our candidate set of models and calculated  $\Delta AIC_C$ , Akaike weights ( $\omega_i$ ) and Evidence Ratios (ER). Akaike weights sum to one and are a measure of the probability that the candidate model is the best approximating model out of the set of models evaluated (Symonds and Moussalli 2011). In contrast, evidence ratios provide information on how much more likely the best candidate model is compared with a second candidate model (Symonds and Moussalli 2011). Models with the smallest  $AIC_C$  were considered to be the best approximating model, however,  $\Delta AIC_C$  values  $< 2$  are considered equally plausible. Although, the more parsimonious models (fewest parameters) are preferred when there are equally supported models, we present coefficients from the best approximating model. In all candidate models, clutches were categorized as having multiple fathers (MP) or a single father (SP) and included as a fixed, categorical independent variable we called clutch type. We tested whether clutches with multiple fathers had higher offspring mass or body size at birth, faster growth rates (captive and wild) and whether they had enhanced performance traits (offspring sprint speed and endurance) as predicted if females receive genetic benefits. To account for the possibility of differential effects of clutch type (MP and SP) between the sexes we included candidate models with sex and the interaction between sex and clutch type in our analyses. We also included candidate models that accounted for the possible effects of mother clutch size and body condition in analyses involving offspring mass and body size and offspring SVL in analyses involving mass, sprint speed and endurance. In all models, mother and father identification were included as random effects to account for non-independence in the data. We log transformed sprint speed and endurance to normalize distributions, however, in some variables (indicated in results), the dependent variables had slightly skewed distributions with a few outlying data points (really small or really large values). To test whether our inferences were robust to these outlying points we rank transformed the dependent variable and generated normalized quantiles (mean = 0, SD = 1). We then re-ran analyses to make sure we had comparable results. We ensured that the residuals were normally

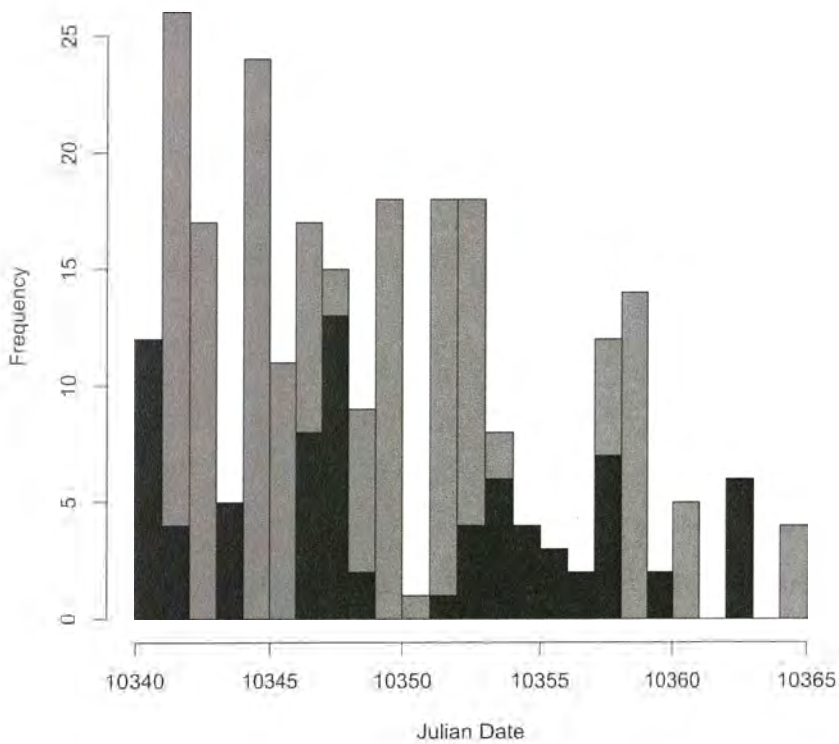
distributed (tested with Shapiro-Wilk normality tests) and that there were no obvious patterns in residual plots. We present effect sizes and t-statistics from our best approximating models. Fitness traits were analyzed with different sample sizes because GLMM's could only be used with complete maternal and paternal information. We do not report p-values from our mixed models because the calculation of denominator degrees of freedom for the derivation of p-values can be difficult to estimate (Pinheiro and Bates 2000). However, in addition to our GLMM analysis we also averaged offspring SVL, mass, sprint speed, endurance and growth rates from a single clutch and subsequently compared MP and SP clutches using ANOVA or ANCOVA and present the F-statistics and p-values for clutch type controlling for important females traits. This analysis did not allow us to incorporate offspring predictors in our models. We used the same set of females for our ANCOVA and GLMM analyses. Significance was assessed at  $\alpha = 0.05$ . Sample sizes for each of the analyses are reported throughout.

## RESULTS

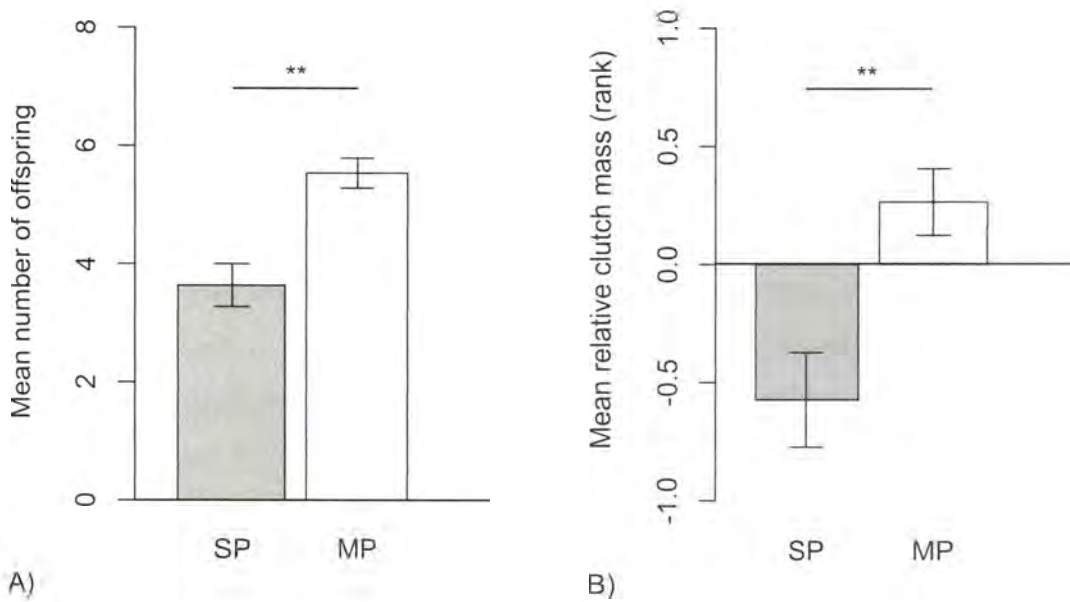
Paternity could be assigned to 226 offspring across 56 females while offspring alleles from seven females were manually counted ( $n = 303$  offspring and  $n = 63$  females total). Forty-five females did not give birth and these females were significantly smaller, on average, than females producing offspring (ANOVA; SVL:  $F_{1, 105} = 19.39$ ,  $P < 0.001$ ; Mass:  $F_{1, 105} = 14.71$ ,  $P < 0.001$ ). Of the 63 females that gave birth, 22 (35%) were sired by one male and 41 (65%) were sired by multiple males. Of the multiply sired clutches, 27 were sired by at least two males, 14 by at least three males and one by four males. The distribution of birthing dates between SP and MP females overlapped extensively (Figure 1). Females produced anywhere from 1-9 offspring in a single clutch. Multiply sired females had a tendency to bias offspring sex towards males, however, this was not significantly different to singly sired females (odds of producing males was 1.62 times that of singly sired clutch:  $Z = 1.80$ ,  $P = 0.07$ ). Multiply and singly sired females did not differ significantly in body size (ANOVA;  $F_{1, 55} = 0.45$ ,  $P = 0.51$ ), mass (ANOVA;  $F_{1, 55} = 0.63$ ,  $P = 0.43$ ) or body condition (ANOVA;  $F_{1, 55} = 0.19$ ,  $P = 0.67$ ). Multiply sired females gave birth to an average of  $5.54 \pm 0.25$  offspring whereas singly sired females gave birth to an average of  $3.64 \pm 0.36$  offspring (Figure 2A). This difference was statistically significant when controlling for body condition and SVL (ANCOVA; Clutch



Type:  $F_{1, 53} = 18.76$ ,  $P < 0.001$ ; Body Condition:  $F_{1, 53} = 19.24$ ,  $P < 0.001$ ; SVL:  $F_{1, 53} = 26.45$ ,  $P < 0.001$ ) even after excluding three females, which gave birth to only a single baby (mean  $\pm$  SE of singly sired clutches =  $4.05 \pm 0.33$ ; ANCOVA; Clutch Type:  $F_{1, 50} = 10.55$ ,  $P < 0.01$ ; Body Condition:  $F_{1, 50} = 17.25$ ,  $P < 0.001$ ; SVL:  $F_{1, 50} = 31.48$ ,  $P < 0.001$ ). Females that had multiply sired clutches also had significantly higher relative clutch mass than females that had singly sired clutches independent of female body size and condition (ANCOVA; Clutch Type:  $F_{1, 53} = 11.18$ ,  $P < 0.01$ ; Body Condition:  $F_{1, 53} = 8.70$ ,  $P < 0.01$ ; SVL:  $F_{1, 53} = 7.19$ ,  $P < 0.01$ ; Figure 2B). Forty-seven offspring died at birth; 12 offspring died from SP clutches (from  $n = 8$  [36%] females) and 35 died from MP clutches (from  $n = 18$  [44%] females).



**Figure 1-** Distribution of offspring birth dates (Julian dates) between singly sired (SP; black bars) and multiply sired (MP; grey bars) clutches of female *E. quoyii*.



**Figure 2-** Mean ( $\pm$  standard error) number of offspring (A) and relative clutch mass (B) for single paternity (SP) and multiple paternity (MP) clutches. (\*\*) indicates that differences are significant at  $P < 0.001$ .

*Offspring Mass and Body Size*

Mean offspring mass at birth was  $1.09 \pm 0.02$  g and  $1.05 \pm 0.02$  g for SP and MP clutches respectively (Figure 3A). Offspring mass was not significantly different between MP and SP clutches (ANCOVA; Clutch type:  $F_{1,45} = 0.24$ ,  $P = 0.62$ ; Clutch size:  $F_{1,45} = 12.62$ ,  $P < 0.001$ ; Female condition:  $F_{1,45} = 12.94$ ,  $P < 0.001$ ; Female SVL:  $F_{1,45} = 11.13$ ,  $P = 0.002$ ). Our GLMM's allowed us to incorporate offspring predictors in analyses and we found four models that were equally supported, none of which included clutch type (Table 1). The best approximating model contained offspring body size ( $\beta = 0.05 \pm 0.005$ ,  $t = 9.99$ ), maternal clutch size ( $\beta = -0.02 \pm 0.009$ ,  $t = -1.97$ ), maternal condition ( $\beta = 0.01 \pm 0.005$ ,  $t = 2.22$ ), and

maternal SVL ( $\beta = 0.006 \pm 0.003$ ,  $t = 2.44$ ) as predictors of offspring mass while there was a weak effect of offspring sex ( $\beta_{\text{Male}} = 0.02 \pm 0.01$ ,  $t = 1.50$ ).

Mean offspring SVL at birth was  $37.78 \pm 0.26$  mm and  $37.39 \pm 0.20$  mm for SP and MP clutches, respectively (Figure 3B). Offspring SVL was not significantly different between MP and SP clutches (ANCOVA; Clutch type;  $F_{1,45} = 0.18$ ,  $P = 0.67$ ; Clutch size;  $F_{1,45} = 20.21$ ,  $P < 0.001$ ; Female condition;  $F_{1,45} = 15.25$ ,  $P < 0.001$ ; female SVL;  $F_{1,45} = 9.69$ ,  $P < 0.01$ ). The best-supported GLMM model contained offspring sex, maternal SVL, maternal condition and maternal clutch size (Akaike  $\omega_i = 0.51$ ; Table 1). However, a second model containing clutch type had similar support. It predicted that offspring from MP clutches were smaller controlling for all other variables ( $\beta = -0.16 \pm 0.38$ ,  $t = -0.42$ ). Model residuals deviated significantly from normality (the result of a few influential points). Transforming SVL to normalized quantiles yielded qualitatively similar results and did not change our inferences. The best-supported model predicted that males were smaller than females at birth ( $\beta_{\text{Male}} = -0.21 \pm 0.16$ ,  $t = -1.30$ ) while females with larger clutches produced smaller sized offspring ( $\beta = -0.68 \pm 0.14$ ,  $t = -5.02$ ) and females that were in better body condition ( $\beta = 0.28 \pm 0.07$ ,  $t = 3.89$ ) and that were larger in size ( $\beta = 0.12 \pm 0.04$ ,  $t = 3.36$ ) gave birth to larger offspring.

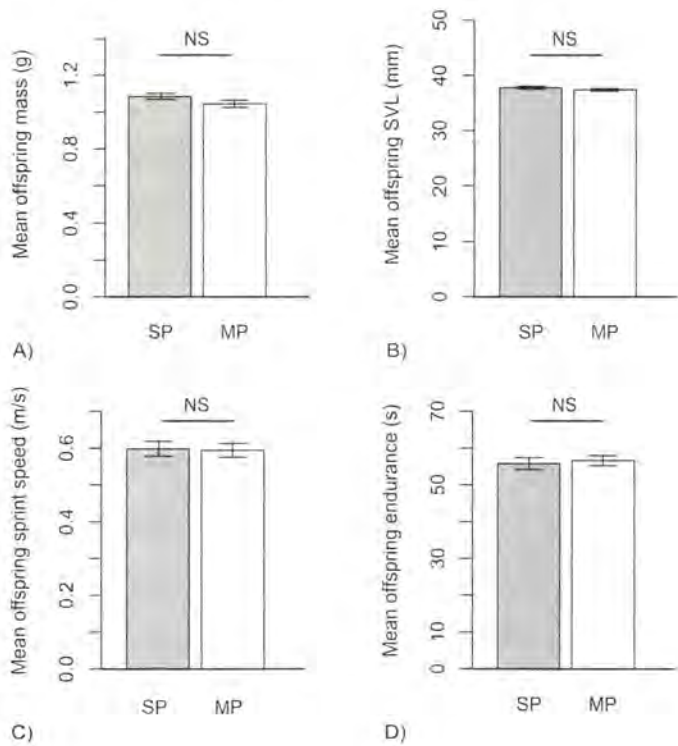
#### *Offspring Sprint Speed and Endurance*

Mean offspring sprint speed was  $0.60 \pm 0.02$  m/s and  $0.59 \pm 0.02$  m/s for SP and MP clutches respectively (Figure 3C). Offspring sprint speed was not significantly different between MP and SP clutches (ANOVA;  $F_{1,48} = 0.23$ ,  $P = 0.63$ ). The best model for predicting log offspring sprint speed contained offspring SVL and sex as predictors (Akaike  $\omega_i = 0.47$ ; Table 1). Males ran faster than females ( $\beta = 0.06 \pm 0.03$ ,  $t = 1.90$ ) and larger offspring ran faster than smaller offspring ( $\beta = 0.03 \pm 0.01$ ,  $t = 2.15$ ).

Mean offspring endurance was  $55.76 \pm 1.63$  s and  $56.61 \pm 1.37$  s for SP and MP clutches respectively (Figure 3D). Offspring endurance was not significantly different between MP and SP clutches (ANOVA;  $F_{1,48} = 0.26$ ,  $P = 0.61$ ). The best model contained only time in captivity before performance measurements as a predictor of endurance (Akaike  $\omega_i = 0.39$ ;  $\beta_{\text{TimeCap}} = -0.008 \pm 0.003$ ,  $t = -2.34$ ; Table 1).

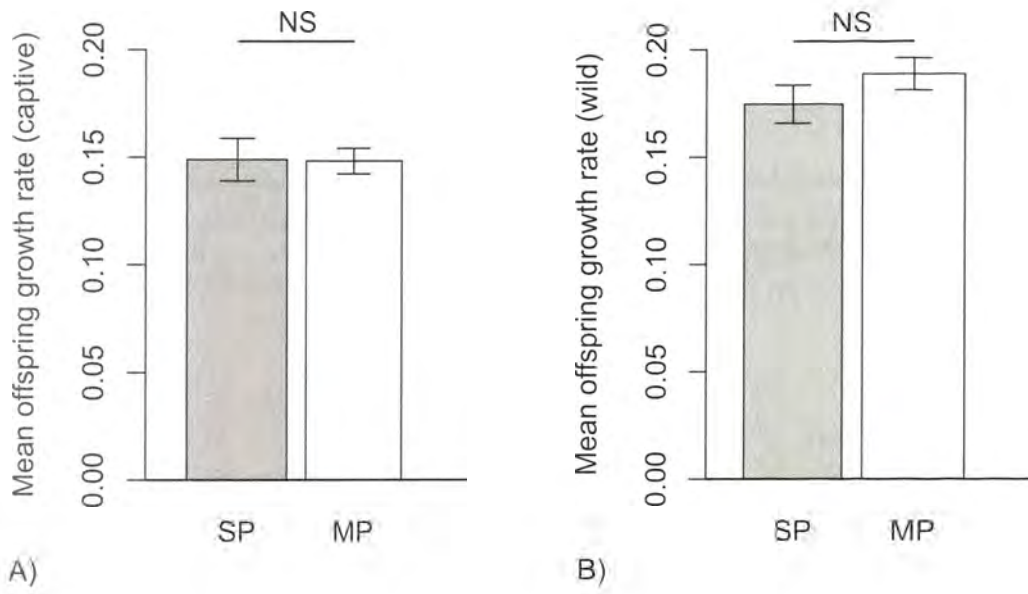
Offspring Growth Rates

We obtained growth rate estimates for 235 (n = 57 SP; n = 178 MP) offspring between birth and marking and 122 (n = 29 SP; n = 93 MP) offspring during our mark-recapture period in our outdoor enclosures. Offspring growth rates were not significantly different between MP and SP clutches during the captive period (Figure 4A: ANOVA;  $F_{1, 51} = 0.98$ ,  $P = 0.33$ ) and the best model contained only sex as a predictor of growth rates in captivity, however, this was a weak effect (Akaike  $\omega_i = 0.50$ ;  $\beta_{\text{Male}} = 0.02 \pm 0.007$ ,  $t = 2.26$ ; Table 1). Offspring growth rates in our semi-natural enclosures were not significantly different between MP and SP clutches (Figure 4B: ANOVA;  $F_{1, 40} = 0.30$ ,  $P = 0.59$ ) and none of our predictors explained a significant amount of variation in wild growth rates (Akaike  $\omega_i = 0.54$ ; Table 1). In both analyses there were outlying points, however, using normalized quantiles gave identical results.



**Figure 3-** Mean ( $\pm$  standard error) offspring mass (A), SVL (B), sprint speed (C) and endurance (D) for single paternity (SP) and multiple paternity (MP) clutches. (NS) indicates that differences are not statistically significant at  $\alpha = 0.05$ .





**Figure 4-** Mean ( $\pm$  standard error) offspring growth rates in captivity (A) and in semi-natural enclosures (B) for single paternity (SP) and multiple paternity (MP) clutches. (NS) indicates that differences are not statistically significant at  $\alpha = 0.05$ .

**Table 1-** Comparison of AIC<sub>C</sub>, ΔAIC<sub>C</sub>, Akaike weights (ω<sub>i</sub>) and Evidence ratios (ER) for our candidate models in analyses of offspring fitness traits (SVL, mass, log(sprint speed), log(endurance) and growth rates). We report the number of females and offspring born to these females in brackets for each analysis because of missing data. Abbreviations are as follows: CT = Clutch type (SP or MP); FCS = Female clutch size; Fcond = Female body condition; FSVL = Female body size; Sex = offspring sex; OffSVL = offspring SVL; TimeCap = Time spent in captivity prior to performance measurement. The abbreviation NL means that the ER is greater than 1200 and thus the model is not likely (NL). Best-supported models are bolded.

Model	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	ω <sub>i</sub>	ER
<i>Offspring SVL (n = 205 offsp., n = 50 fem.)</i>				
SVL~ 1	698.33	15.00	0.00	NL
SVL~ CT + Sex	698.44	15.11	0.00	NL
SVL~ CT	697.97	14.64	0.00	NL
SVL~ Sex	698.75	15.42	0.00	NL
SVL~ CT + Sex + CT*Sex	700.29	16.96	0.00	NL
SVL~ CT + Sex + FCS + Fcond + FSVL + CT*Sex	687.35	4.02	0.07	7.46
SVL~ CT + Sex + FCS + Fcond + FSVL	685.37	2.04	0.18	2.77
SVL~ CT + FCS + Fcond + FSVL	684.85	1.52	0.24	2.14
<b>SVL~ Sex + FCS + Fcond + FSVL</b>	683.33	0.00	0.51	1.00
<i>Offspring mass (n = 204 offsp., n =50 fem.)</i>				
Mass~1	-326.27	85.46	0.00	NL
Mass~ CT + Sex + OffSVL	-408.79	2.94	0.05	2.59
Mass~ CT + Sex + FCS + Fcond + FSVL + OffSVL	-409.57	2.16	0.08	1.75
<b>Mass~ Sex + FCS + Fcond + FSVL + OffSVL</b>	-411.73	0	0.23	0.59
Mass~ OffSVL + Sex	-410.68	1.05	0.13	1.01
Mass~ CT + OffSVL + Sex + FCS + Fcond + FSVL + CT*Sex	-407.40	4.33	0.03	5.18
Mass~ CT + OffSVL	-408.83	2.9	0.05	2.53
Mass~ CT + FCS + Fcond + FSVL + OffSVL	-409.55	2.18	0.08	1.77
Mass~ FCS + Fcond + FSVL + OffSVL	-411.71	0.02	0.22	0.60
<i>Offspring sprint speed (n = 175 offsp., n =50 fem.)</i>				
SS~ intercept	-26.77	2.76	0.09	3.97
SS~ CT + Sex	-25.25	4.28	0.04	8.50
SS~ CT	-24.77	4.76	0.03	10.80
SS~ Sex	-27.27	2.26	0.11	3.10

SS~ CT + Sex + OffSVL + CT*Sex	-25.61	3.92	0.05	7.10
SS~ CT + Sex + CT*Sex	-23.70	5.83	0.02	18.45
SS~ CT + Sex + OffSVL	-27.41	2.12	0.12	2.89
SS~ CT + OffSVL	-26.01	3.52	0.06	5.81
<b>SS~ Sex + OffSVL</b>	-29.53	0	0.35	1.00
SS~Sex + OffSVL +TimeCap	-27.44	2.09	0.12	2.84

*Offspring endurance (n = 175 offsp., n = 50 fem.)*

Endur~ intercept	-90.16	3.28	0.08	1.05
Endur~ CT + Sex	-88.18	5.26	0.03	2.83
Endur~ CT	-88.10	5.34	0.03	2.94
Endur~ Sex	-90.26	3.18	0.08	1.00
Endur~ CT + Sex + CT *Sex	-87.23	6.21	0.02	4.55
Endur~ CT + Sex + OffSVL + CT *Sex	-85.12	8.32	0.01	13.07
Endur~ CT + Sex + OffSVL	-86.13	7.31	0.01	7.89
Endur~ CT + OffSVL	-86.21	7.23	0.01	7.58
Endur~ Sex + OffSVL	-88.24	5.20	0.03	2.75
Endur~ Sex + TimeCap	-93.05	0.39	0.32	0.25
<b>Endur~ TimeCap</b>	-93.44	0	0.39	0.20

*Offspring growth (capt.) (n = 181 offsp., n = 53 fem.)*

Growth_cap~ intercept	-574.82	2.92	0.12	4.31
Growth_cap~ CT + Sex	-576.29	1.45	0.24	2.06
Growth_cap~ CT	-573.41	4.33	0.06	8.71
<b>Growth_cap~ Sex</b>	-577.74	0	0.50	1.00
Growth_cap~ CT + Sex + CT *Sex	-574.14	3.6	0.08	6.05

*Offspring growth (wild) (n = 94 offspr., n = 42 fem.)*

<b>Growth_wild~ intercept</b>	-251.45	0	0.54	1.00
Growth_wild~ CT + Sex	-247.12	4.33	0.06	8.71
Growth_wild~ CT	-249.33	2.12	0.19	2.89
Growth_wild~ Sex	-249.29	2.16	0.18	2.94
Growth_wild~ CT + Sex + CT *Sex	-244.96	6.49	0.02	25.66

## DISCUSSION

We found weak support for the hypothesis that females mate multiply to accrue genetic benefits. Contrary to our predictions there were no significant differences in offspring SVL, mass, sprint speed, endurance, or growth rates between MP and SP clutches and there was little to no support for the inclusion of clutch type in our models. Interestingly, even though MP and SP females did not differ in body mass, SVL or body condition at the beginning of the breeding season, MP females produced significantly larger clutch sizes than SP females and invested a greater proportion of their body mass into their offspring independent of female body size and condition. We discuss the implications of these results to the importance of indirect (genetic) and direct fitness benefits in explaining patterns of multiple paternity in *E. quoyii*.

Although the incidence of multiple paternity was high in *E. quoyii* (65% of clutches), we found little evidence that females mating multiply obtain genetic benefits, at least with respect to the fitness traits we measured. It is possible that our fitness traits do not reflect offspring survival as predicted, given that selection on these traits can be complex in nature (Sinervo et al. 1992); however, we feel that this is unlikely given the large number of traits we measured and their correlation with survival in other lizard species (Elphick and Shine 1998; Warner and Andrews 2002; Husak 2006; Irschick et al. 2008; Le Galliard and Ferrière 2008). Furthermore, similar results to ours have been found in a related species, *E. heatwolei*, where offspring from females with experimentally varied mating rates did not have higher growth rates or survival compared to offspring from singly sired treatments (Keogh et al. 2013).

Importantly, we cannot rule out that females gain no genetic benefits from mating multiply with our experimental design and the level of multiple mating will need to be experimentally varied to conclusively demonstrate a lack of genetic benefits. Moreover, we were unable to assess whether reproductive success of offspring varied between clutch types and genetic benefits might only manifest themselves at later stages in life or may be sex-dependent (e.g. sexy sons hypothesis; Jennions and Petrie 2000; Neff and Pitcher 2005). The lack of differences we found between SP and MP clutches in offspring fitness traits may also be the result of differential sperm competition and/or cryptic female choice between SP and MP females which would optimize fertilizations with compatible males or males with 'good genes' (Uller and Olsson 2008). This may be particularly important given that there does



appear to be some evidence that female *E. quoyii* which mate multiply bias their sex ratio towards males, suggesting that some form of cryptic female choice may be operating. Sex-ratio adjustment has been shown in side-blotched lizards (*Uta stansburiana*) in response to sperm from large and small males (Calsbeek and Sinervo 2004). For example, single paternity females may mate multiply but more strongly bias paternity towards males with good genes, with which they are more genetically compatible, or produce offspring of a particular sex which have higher viability (e.g. Calsbeek and Sinervo 2004). We cannot rule out this hypothesis because we were unable to collect data on mating rates for female *E. quoyii*, however, a recent study investigating offspring survival between MP and SP clutches in *Lacerta agilis* did detect higher survival in offspring from MP clutches using a similar approach to ours (Olsson et al. 2011).

What fitness benefits, if any, do polyandrous females receive if they do not acquire genetic benefits? Although adaptive explanations have dominated the literature, recent work in Zebra finches have shown that polyandrous behavior may evolve not through direct or indirect fitness benefits to females, but simply through indirect selection on male mating behavior (Forstmeier et al. 2011). Assuming promiscuous behavior has a heritable basis, positive selection for multiple mating in males can lead to higher female promiscuity through a correlated evolutionary response (Halliday and Arnold 1987; Forstmeier et al. 2011). Although plausible, given that there is strong sexual selection on male *E. quoyii* to mate multiply, testing this hypothesis requires a substantial dataset on the propensity for males, females and their offspring to mate multiply over a number of generations and this mechanism cannot explain polyandry in many cases because multiple mating often has low heritability (Evans and Simmons 2008; McFarlane et al. 2011).

There are also a number of adaptive explanations that directly affect female fitness in reptiles, which are equally plausible. First, females may mate promiscuously to avoid male harassment (i.e. convenience polyandry; Andersson 1994; Slatyer et al. 2012). Male harassment has been shown to inflict major fecundity and survival costs to female lizards and reduce emigration probability in male biased populations (Le Galliard et al. 2005). Convenience polyandry has been proposed to explain patterns of paternity in marine sea turtles (Lee and Hays 2004) and may be responsible for the patterns of paternity we observed. This assumes that the costs of multiple mating are high; however, female *E. quoyii* are aggressive and able to resist copulations from males (Noble personal observations).

Furthermore, behavioral observations of individuals during the breeding season suggest that male harassment is minimal in this species (D Noble and JS Keogh, personal observations) and copulations are generally inconspicuous (avoiding the potential costs of predation) so it is unlikely that convenience polyandry alone can explain such high levels of multiple mating in *E. quoyii*.

Importantly, our data does show strong fecundity differences between MP and SP females independent of female body size and condition. It is important to consider that this relationship could be driven by the lower probability of detecting multiple paternity in SP females and this has been suggested as an alternative explanation for positive Bateman gradients (e.g. Gerlach et al. 2012). Although we cannot rule out this explanation given that we do not have experimental data, both groups did not differ in SVL, body mass or body condition, which are strongly related to clutch size in *E. quoyii*. The fact that there were no differences in traits between these groups suggests that these patterns could be explained by the fertility insurance hypothesis, where female promiscuity is selected for in females because it guards against producing infertile eggs. Fertility insurance has been suggested to be an important, over-looked hypothesis explaining the evolution of polyandry (Sheldon 1994; Uller and Olsson 2008; Slatyer et al. 2012), yet in many systems it remains un-tested (Sheldon 1994; Griffith 2007). Systems in which females would be most susceptible to inadequate sperm transfer are predicted to have short mating seasons where mate encounter rates maybe low or unpredictable, copulations result in inadequate sperm transfer and/or males vary in sperm quality. Although we do not know the actual mating period in *E. quoyii*, our paternity data suggests that it is short, beginning in late September and ending by the middle of October. In support of this, Vernon (1969) has shown that female *E. quoyii* release ova into the oviduct in October at high altitude populations. In the closely related *E. heatwolei*, Head et al. (2005) has shown that females are receptive for only 7 days in controlled laboratory conditions where detailed behavioral observations on female mating rates could be observed. Such a short mating period among individual females may prevent females from acquiring sufficient sperm supplies prior to ovulation. In addition, one interesting possibility is that female choosiness thresholds may exacerbate this problem with SP females exhibiting higher choosiness thresholds and thus acquiring fewer matings before ovulation (Bleu et al. 2012). Indeed, this could also explain why so many females did not end up reproducing as these females would be expected to have the highest choosiness and therefore run the risk of going unmated altogether

(Bleu et al. 2012; Kokko and Mappes 2012). There is evidence in some species that male lizards vary in their fertility and that multiple mating can guard against infertility (Uller and Olsson 2005) and this is likely a fruitful line of research. The importance of fertility in explaining patterns of multiple mating is further supported by the results of a recent meta-analysis, which used effect sizes from 46 experimental studies across a diversity of taxa (Slatyer et al. 2012). The authors found that the largest effect sizes for direct benefits were for greater fertility and higher clutch production (Slatyer et al. 2012). Experimental tests of the effect of mate limitation on female fecundity in *E. quoyii* as well as an understanding of the variation in female choosiness and male fertility will likely shed important insights into the role of fertility insurance for the evolution of polyandry in this species.

In conclusion, multiple paternity and polyandry are widespread in squamate reptiles (Morrison et al. 2002; Stapley and Keogh 2005; Uller and Olsson 2008), yet the importance of genetic benefits to the evolution of these behaviors has been controversial (Capula and Luiselli 1994; Uller and Olsson 2008) and only studied in a few model reptile systems (Madsen et al. 1992; Olsson and Madsen 2001; Madsen et al. 2004; Olsson et al. 2005; Lancaster et al. 2009; Olsson et al. 2011). Even within species there have been incongruent results with respect to the importance of multiple mating to offspring survival (Madsen et al. 1992; Capula and Luiselli 1994). We show that multiple mating in *E. quoyii* does not appear to be driven by genetic benefits females may receive for the fitness traits we have measured, but is likely the product of fecundity selection: females that mated multiply produced larger clutches with a higher relative clutch mass independently of their body size. This may have the simple benefit of acting as a form of fertility insurance given the short mating period and if males are of variable quality and this is worth investigating further in a diverse range of systems.

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## CHAPTER 4: OFFSPRING FITNESS TRAITS SHOW STRONG MATERNAL EFFECTS AND LOW HERITABILITY IN AN AUSTRALIAN LIZARD

Daniel W.A. Noble, S. Eryn McFarlane, J. Scott Keogh, Martin J. Whiting

Under review

### Abstract

Evolutionary responses to selection require that traits have a heritable basis, yet maternal effects (the effect of a mother's phenotype on her offspring's phenotype) can have profound effects in evolutionary models. It is therefore essential to understand how maternal effects contribute to phenotypic variation in offspring traits and test key assumptions of additive genetic variance in evolutionary models. We measured five traits (offspring endurance, sprint speed, snout-vent length, mass and growth rates) linked to offspring fitness in an Australian lizard (*Eulamprus quoyii*) and estimated the contribution of additive genetic and maternal effects in explaining variation in these traits. We estimated parentage using six microsatellite DNA loci from lizards taking part in a large semi-natural mating experiment and used animal models to partition variance into additive genetic and maternal effects. We found that only offspring endurance was significantly heritable ( $h^2 = 0.38$ , 95% CI 0.17 to 0.49) while all other traits were either strongly influenced by maternal effects (mass, sprint speed and snout-vent length) or were influenced primarily by environmental variance (growth rates). Our study is one of the few to disentangle the relative contributions of additive genetic and maternal effects in contributing to variation in offspring phenotypes in a lizard. While the heritability of fitness-related traits is essential in developing evolutionary models, our results also highlight the important role of maternal effects in explaining variation in lizard offspring phenotypes.

## INTRODUCTION

Evolutionary responses require that traits have a genetic basis and are heritable. An understanding of whether traits are heritable is essential for all facets of evolutionary thinking from understanding the role of ‘good’ genes models in sexual selection (Qvarnstrom & Price, 2001) to determining the causes of phenotypic variation in important fitness traits (Mousseau & Fox, 1998). Importantly, a trait’s response to selection will depend not only on its heritability and the spatial and temporal stability of selection, but also on maternal effects (Lande & Kirkpatrick, 1990; Qvarnstrom & Price, 2001). In addition to the direct genetic contribution from the mother to her offspring (i.e. through additive genetic effects), mothers can also indirectly influence variance in offspring phenotypes through maternal effects (Kirkpatrick & Lande, 1989).

Maternal effects can be caused by the manipulation of the offspring environment and/or differential investment into offspring as a result of the physiological state of the female (Mousseau & Fox, 1998). Such effects have now been recognized across a wide diversity of taxa as being an important contributor to phenotypic variation in offspring, re-enforcing or dampening responses to selection (Qvarnstrom & Price, 2001) and potentially having persistent effects over an individual’s lifetime (Kerr *et al.*, 2007). Maternal effects have also been implicated in playing a major role in our understanding of the evolution of mate preferences and male ornaments and there is increasing interest in the interplay between maternal effects and additive genetic effects on offspring phenotypes (Sheldon, 2000; Qvarnstrom & Price, 2001). For example, the differential allocation by females in offspring from matings with males that are more attractive can increase the response to selection, accelerating the coevolution of male traits and female preferences (Sheldon, 2000; Qvarnstrom & Price, 2001). Determining whether additive genetic or maternal effects drive variation in offspring traits is therefore paramount in predicting evolutionary responses.

Maternally induced changes to offspring phenotypes in lizards come in diverse forms and can be a result of differences in basking behaviour (Shine & Downes, 1999), dietary quality (Warner *et al.*, 2007) and physiological state [i.e. female mass, body size or age] (Noble *et al.*, 2013). These maternal effects have been known to influence a diversity of important offspring fitness traits including body size and mass, performance traits and growth rates (Shine & Harlow, 1993). Although there is ample evidence that maternal effects are important, the

relative contribution of additive genetic and maternal effects to explaining variation in single traits within the same lizard species have seldom been addressed (Tsuju *et al.*, 1989; Sorci *et al.*, 1995; Le Galliard *et al.*, 2004). Furthermore, few studies genetically determine paternity and this does not adequately permit the separation of heritable from maternal effects variation because many estimates are derived from full-sib parent-offspring regressions (Sorci *et al.*, 1995; Le Galliard *et al.*, 2004). Although valuable, such techniques can confound heritability with maternal effects and inflate heritability estimates (Kruuk, 2004).

We measured five traits (endurance, sprint speed, body size and mass and growth rates) in an Australian lizard (*Eulamprus quoyii*) as part of a study testing whether polyandrous females mate multiply to receive ‘good’ genes benefits in the form of increased offspring viability. We showed that females producing single and multiple paternity clutches produce offspring that do not differ in any of the aforementioned traits (Noble *et al.*, 2013). However, in order to explain this pattern fully we need an understanding of whether these traits show evidence for additive genetic variation and the degree to which they are driven by maternal effects. Specifically, we test a key assumption of ‘good’ genes models, namely that traits show evidence of additive genetic variation. We first established maternal and paternal identity of offspring from semi-natural breeding experiments before using animal models to partition phenotypic variance to determine the importance of maternal and heritable effects in key traits known to be under selection in many lizard species (Sinervo *et al.*, 1992; Elphick & Shine, 1998; Warner & Andrews, 2002; Husak, 2006; Irschick *et al.*, 2008; Le Galliard & Ferrière, 2008). This design allowed us to independently assess the role of heritable and maternally driven effects contributing to variation in these offspring traits providing greater insight into the possible importance of viability indicator models in explaining female polyandry in *E. quoyii*.

## MATERIALS AND METHODS

### *Experimental design*

The Eastern Water Skink, *Eulamprus quoyii*, is a large skink species (90 – 118 mm SVL) and is widespread across eastern Australia. They are viviparous, give birth to 1 – 9 offspring in

mid December – January and do not exhibit parental care. We collected 216 (108 males and females) *E. quoyii*, and allowed them to breed in semi-natural enclosures as part of a series of mating system experiments conducted in 2010. Experiments were conducted in six 16 x 10 m outdoor enclosures with 18 males and females in each enclosure. Adults were collected at the end of the breeding season (November) and females were brought back into the laboratory for parturition. At parturition (December – January), offspring snout-vent length (SVL) and mass were recorded and a small tissue sample taken for genetic analysis. Offspring sprint speed and endurance were measured once (approximately a week after birth) over three consecutive days. Lizards were warmed to their optimal body temperature (28°C) prior to all performance measurements using a temperature-controlled incubator. Sprint speed was measured by running lizards down a racetrack outfitted with photocells, and the time between triggering the first and the last photocell over 1 m was used to compute speed. Immediately thereafter, lizards were placed in a circular track and stimulated to run (by gently taping on the tail) until exhaustion. Lizards were considered exhausted if, after five gentle taps, they were no longer stimulated to run at which point we recorded the time. We used these repeated measures of performance in our analyses. Once all measurements were finished we recorded individual mass and SVL again prior to releasing lizards in four semi-natural enclosures where they were re-captured monthly until April prior to winter to estimate individual growth rates. Growth rates were calculated in captivity during their period of stay while being measured on performance traits and also in the wild. We used both measures in analyses. Parentage was assigned using CERVUS 3.0 with six polymorphic microsatellite DNA loci as outlined in Noble *et al.* (2013). Sample sizes varied for each of the analyses (Table 1) depending on the trait because of missing offspring data and/or missing parental identity.

### *Statistical analysis*

We used mixed effect ‘animal models’ that combine phenotypic data with pedigree information and compare the phenotypes of related individuals to estimate the additive genetic variance ( $\sigma^2_a$ ) of each trait (Kruuk, 2004). Full details of our pedigree can be found in Table 1. In addition, we used animal models to estimate the maternal ( $\sigma^2_m$ ) and permanent environmental effects of the traits we were interested in, where  $\sigma^2_m$  is the differences among individuals due to the influence of their mother (Mousseau & Fox, 1998) and  $\sigma^2_i$  is the among



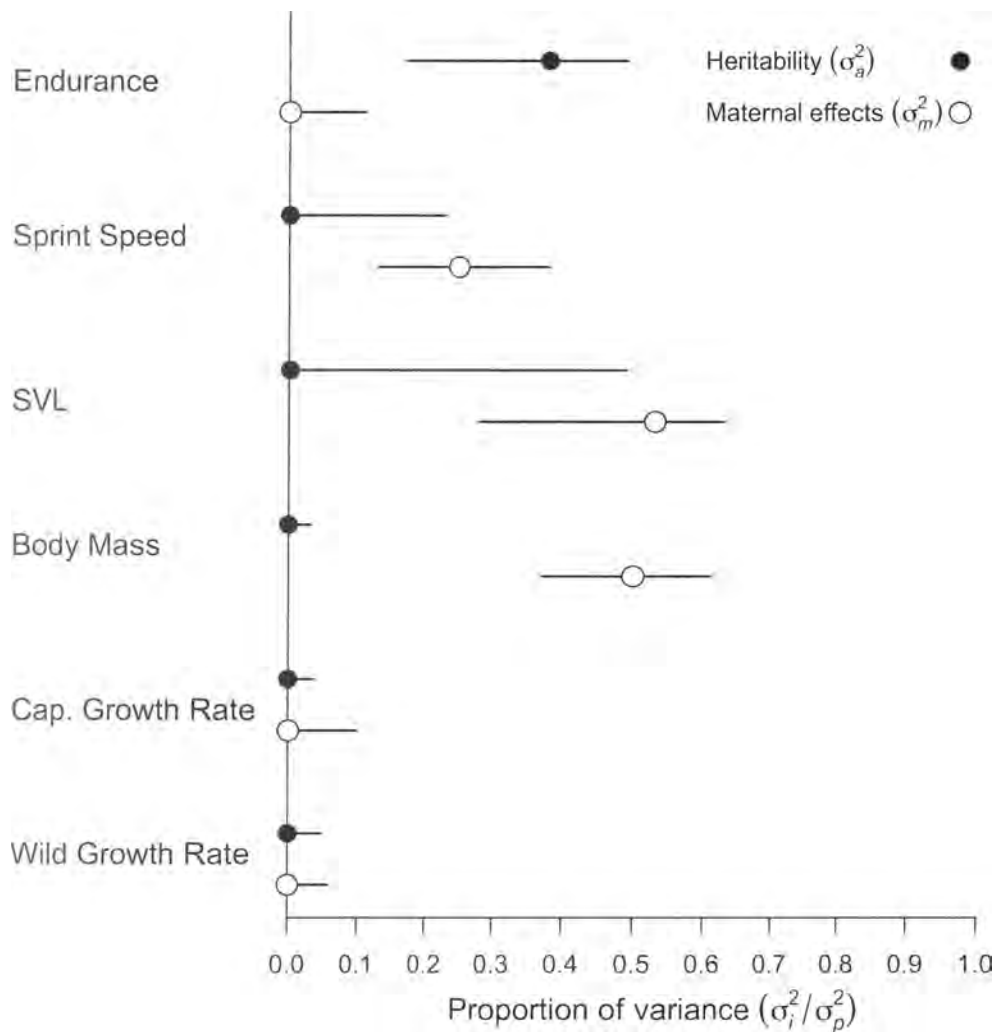
individual differences not accounted for by  $\sigma_a^2$ , and can only be estimated in our performance traits which were repeatedly measured (Kruuk & Hadfield, 2007). We also estimated a residual variance ( $\sigma_r^2$ ). Thus, phenotypic variance ( $\sigma_p^2$ ) was estimated as the sum of all variance components, including  $\sigma_r^2$ . Heritability ( $h^2$ ) was estimated as  $\sigma_a^2/\sigma_p^2$ , maternal effects ( $m^2$ ) were estimated as  $\sigma_m^2/\sigma_p^2$ , and permanent environmental effects was estimated as  $\sigma_i^2/\sigma_p^2$  (Lynch & Walsh, 1998).

All animal models were run in R, using the Markov Chain Monte Carlo for generalized linear mixed models (MCMCglmm) package (Hadfield, 2010; R Development Core Team, 2010). We took a two-step approach to our modeling. First, we ran animal models that incorporated pedigree information and only fitted an intercept. This allowed us to estimate the total additive genetic and maternal effects explaining phenotypic variance in the trait. Second, for traits with strong maternal effects we ran separate GLMMs with specific female traits, which are hypothesized to contribute to the total maternal effect variance. This allowed us to understand what female traits might be contributing to maternal effect variance. In these models we treated maternal identity effects as random in our GLMMs to control for non-independence in the data. We used the “pedantics” package to fix and prune the pedigree, where only informative individuals remained in the pedigree (Morrissey, 2010). We log-transformed endurance and sprint speed, and then assumed a normal distribution for these and the morphological traits. Variance components were estimated as the mode of the posterior distribution and are reported with 95% credible intervals (CI).

We used parameter-expanded priors as we wanted to have non-informative priors (Hadfield, 2010), reflecting our ignorance in making predictions (Ellison, 2004). All models were run with multiple prior specifications to ensure that the estimates were truly derived from the data. Regardless of the prior, the estimates were similar (results not shown). To test for convergence of each model, we used a Heidelberger and Welch convergence diagnostic (Heidelberger & Welch, 1983) and a Geweke convergence diagnostic (Geweke, 1992). We used both diagnostics for every model, as neither is infallible (Cowles & Carlin, 1996). All models had converged as measured by both diagnostics, and additionally, chains were not autocorrelated.

## RESULTS

Offspring endurance showed significant evidence for additive genetic variation ( $h^2 = 0.38$ , 95% CI 0.17 to 0.49; Table 2, 3 & Fig. 1), while heritability estimates for sprint speed, SVL, mass and growth rates were weak and the posterior mode was centered on zero (Table 2, 3; Fig. 1). Our results provide evidence that sprint speed, SVL and mass show evidence for significant maternal effects with large estimates of  $m^2$  (Table 2 & Fig. 1). Female body size, condition and clutch size were strong predictors of offspring mass and SVL and contributed significantly to explaining variation in these traits (Posterior estimates for SVL: Mother SVL  $\beta = 0.13$ , 95% CI = 0.05 to 0.20; Mother condition  $\beta = 0.28$ , 95% CI = 0.13 to 0.42; Mother's clutch size  $\beta = -0.67$ , 95% CI = -0.95 to -0.39 and posterior estimates for offspring mass: Mother SVL  $\beta = 0.012$ , 95% CI = 0.005 to 0.02; Mother condition  $\beta = 0.024$ , 95% CI = 0.01 to 0.04; Mother's clutch size  $\beta = -0.05$ , 95% CI = -0.08 to -0.03) and decreased maternal variance estimates (results not shown) and thus  $m^2$  (Mass:  $m^2 = 0.38$  [95%CI = 0.22 to 0.54]; a 24% decrease from estimates in Table 2; SVL:  $m^2 = 0.43$  [95% CI = 0.21 to 0.61]; a 19% decrease from estimates in Table 2). Residual variance estimates changed little and heritability for offspring traits did not change with the inclusion of maternal traits in the animal model. After controlling for offspring body size and days spent in captivity, offspring birth date had a positive effect on their sprint speed (Offspring birthing date  $\beta = 0.13$ , 95% CI = 0.08 to 0.18; Offspring SVL  $\beta = 0.022$ , 95% CI = -0.002 to 0.05; Days in captivity  $\beta = 0.01$ , 95% CI = 0.0019 to 0.018). Interestingly, residual and additive genetic variance estimates for sprint speed were not affected by the inclusion of fixed effects; however, maternal effects variances decreased although the confidence intervals were large ( $m^2 = 0.002$  [95% CI = 1.03E-08 to 0.25; a 99% decrease based on estimates in Table 2). Growth rate estimates did not show evidence for significant heritability or maternal effects and appeared to be driven by environmental factors (Table 2 & Fig. 1).



**Figure 1** – Estimates of heritability [ $h^2 (\sigma_a^2/\sigma_p^2)$ ], maternal effects [ $m^2 (\sigma_m^2/\sigma_p^2)$ ] for each of the five offspring traits. Growth rates were split into growth in the wild and under captive conditions. Error bars around estimates are 95% Bayesian credible intervals.

**Table 1.** Description of the pedigree structure used for our morphological (SVL, mass, growth rates) and performance (log sprint speed, log endurance) traits. Pedigree structure of performance traits differ from morphological traits because pedigree was pruned for performance traits. Pedigrees were processed in the R package ‘pedantics’; see materials and methods for more details.

Descriptor	Morphological Pedigree	Performance Pedigree
Records	394	308
Maternities	300	222
Paternities	209	160
Full sibs	268	170
Maternal sibs	686	421
Maternal half sibs	418	251
Paternal sibs	884	562
Paternal half sibs	616	392
Maternal grandmothers	0	0
Maternal grandfathers	0	0
Paternal grandmothers	0	0
Paternal grandfathers	0	0
Maximum pedigree depth	1	1
Founders	94	84
Mean maternal sib-ship size	5	4.04
Mean paternal sib-ship size	6.74	5.93
Mean pairwise relatedness	$8.40e^{-03}$	0.0092



**Table 2.** Estimates of heritability [ $h^2$  ( $\sigma_a^2 / \sigma_p^2$ )], maternal effects [ $m^2$  ( $\sigma_m^2 / \sigma_p^2$ )] and permanent environment effects [ $P_e$  ( $\sigma_{pe}^2 / \sigma_p^2$ )] from the  $\mathfrak{Z}$  posterior distribution along with their 95% credible intervals (CI) for five fitness traits: snout-vent length (SVL), mass, growth rate, endurance and sprint speed.  $\sigma_p^2$  denotes the sum of all variance components (i.e.  $\sigma_a^2 + \sigma_m^2 + \sigma_{pe}^2 + \sigma_e^2$ ). Permanent environmental effects ( $P_e$ ) were only estimated for traits with repeated measurements (i.e. sprint speed and endurance)

	offspring	dams	sires						
Trait	(n)	(n)	(n)	$h^2$	95% CI	$m^2$	95% CI	$P_e$	95% CI
SVL	205	50	47	0.001800	$8.9e^{-09} - 0.49$	0.53	0.28 – 0.63	NA	NA
Mass	204	50	47	0.000079	$5.7e^{-10} - 0.03$	0.50	0.37 – 0.61	NA	NA
Growth: Captive	181	53	44	0.000091	$5.0e^{-09} - 0.04$	0.000700	$2.5e^{-10} - 0.10$	NA	NA
Growth: Wild	94	42	33	0.000230	$1.2e^{-09} - 0.05$	0.000330	$2.9e^{-11} - 0.06$	NA	NA
log(Endurance)	175	50	42	0.38	0.17 – 0.49	0.000650	$8.5e^{-12} - 0.11$	0.000540	$6.9e^{-09} - 0.18$
log(Sprint Speed)	175	50	42	0.000760	$2.4e^{-09} - 0.23$	0.25	0.13 – 0.38	0.000760	$7.1e^{-08} - 0.15$

**Table 3.** Posterior mode of variance estimates for each of the random effects in animal models without fixed effects. Brackets below estimates are the upper 95% credible interval (CI). Only the upper 95% CI is shown because variance estimates are constrained to be above zero. Notation for the estimates are as follows:  $\sigma^2_a$  = additive genetic variance;  $\sigma^2_m$  = maternal effects variance;  $\sigma^2_{pe}$  = permanent environmental variance;  $\sigma^2_e$  = residual/environmental variance.

Estimate	SVL	Mass	Captive Growth	Wild Growth	Log sprint speed	Log endurance
$\sigma^2_a$	0.004634761 (1.307)	2.839408e-06 (0.0007292)	3.614199e-11 (0.0002774)	4.291563e-06 (0.0006133043)	0.0001070767 (0.02133)	0.02069261 (0.03098)
$\sigma^2_m$	1.083 (1.956)	0.01065 (0.01667)	1.799165e-06 (0.0007993)	1.627657e-06 (0.0007337)	0.02255519 (0.0374835)	3.063755e-05 (0.006654)
$\sigma^2_{pe}$	NA	NA	NA	NA	6.800645e-05 (0.0132)	5.157077e-05 (0.01041)
$\sigma^2_e$	1.129 (1.358)	0.0112 (0.01333)	0.006927714 (0.00846)	0.01162895 (0.0154005)	0.05114366 (0.05763)	0.033491 (0.03775)

## DISCUSSION

We found evidence for significant heritable variation in offspring endurance, but weak evidence in the other traits we measured. Variation in sprint speed, body size and mass was driven mainly by maternal effects, while environmental factors explained variability in offspring growth rates. Our study is one of the few to disentangle the relative contributions of additive genetic and maternal effects in contributing to variation in offspring phenotypes in a lizard, and represents estimates of such parameters in a phylogenetically disparate group of lizards (Family: *Scincidae*).

Our findings of strong heritability for endurance and weak heritability for sprint speed are remarkably congruent with work in the lizard *Lacerta vivipara*, where full-sib comparisons showed high heritability for endurance [ $h^2 = 0.40$ ; (Le Galliard *et al.*, 2004) and  $h^2 = 0.46$ ; (Sorci *et al.*, 1995)] but weak and non-significant heritability for sprint speed [ $h^2 = 0.12$ , REML and  $h^2 = 0.14$ , ANOVA; (Sorci *et al.*, 1995)]. However, sprint speed results contrast with studies in the lizard *Sceloporus occidentalis* (Tsuju *et al.*, 1989), and the snakes *Thamnophis ordinoides* (Brodie III, 1989) and *Thamnophis sirtalis* (Garland, 1988) in that these species exhibit significant heritability for sprint speed. However, high heritability for speed in these studies may be the result of strong maternal effects, which can inflate heritability estimates in full-sib analyses (Kruuk, 2004). Offspring sprint speed in *E. quoyii* seems to be under strong maternal control and mediated primarily through delayed birthing by females as has been shown in *E. heatwolei* (Shine & Harlow, 1993).

In *E. quoyii*, offspring body size and mass are strongly influenced by maternal effects that are, in part, explained by maternal condition, body size and clutch size. Although it was clear that offspring mass was strongly controlled by maternal effects, offspring body size had large confidence intervals around heritability estimates. Body size is known to have high heritability in many taxa (Hansen *et al.*, 2011) and, in lizards, offspring body size has been shown to be heritable (Calsbeek & Sinervo, 2004). The low heritability estimate we found contradicts these widespread patterns. Our inability to estimate heritability precisely for this trait could be because body size is a complex trait and dominance and/or epistatic effects may be at work. Alternatively, significant gene-by-environment (G  $\times$  E) interactions may also be blurring the patterns we observed (Larsson, 1993). Our pedigree was only a single generation

and does not allow us to estimate dominance effects and experimental work will be necessary to test for G x E interactions with body size.

Our data provide weak evidence for additive genetic variation for most of the offspring traits we measured and this result supports recent work testing ‘good’ genes (i.e. viability indicator models) mechanisms in explaining female polyandry in *Eulamprus*. A lack of additive genetic variance for these traits suggests that females would not be able to choose males with high breeding values resulting in no response to selection. Previous work found no difference in offspring viability between single paternity (SP) and multiple paternity (MP) females in both observational (Noble *et al.*, 2013) and experimental designs (Keogh *et al.*, 2013). If females mating multiply were selecting males to gain ‘good’ genes, which increase offspring viability, then the lack of additive genetic variance would result in a weak/no response to selection. Of course the lack of additive genetic variance does not mean this did not occur in the past, as strong selection can erode heritability in a trait. However, the results from our study suggest that, at least for the traits we measured, there is weak evidence that female preferences for good genes would result in indirect genetic benefits that enhance offspring viability through increased offspring body size, mass, sprint speed or growth rates. Interestingly, endurance has also been linked to fitness in juvenile and offspring lizards in the wild (Le Galliard & Ferrière, 2008) and we did find evidence for significant additive genetic variance for endurance in *E. quoyii*. Nonetheless, it appears that females mating with multiple males do not gain fitness benefits in the form of increased offspring endurance and presumably fitness (Noble *et al.*, 2013).

One explanation for the apparent lack of evidence for ‘good’ genes benefits (Noble *et al.*, 2013) despite the high heritability of endurance, could simply be that endurance is not linked to offspring survival in *E. quoyii* and therefore, females do not gain indirect genetic fitness benefits from mating multiply. This would explain the lack of difference in offspring endurance between singly and multiply sired females (Noble *et al.*, 2013). Indeed, survival for offspring from singly and multiply mated females is similar in *E. heatwolei* (Keogh *et al.*, 2013), supporting this hypothesis. Alternatively, endurance may be under selection, but because this trait is a function of a large number of physiological processes, mutations may maintain additive genetic variation (Sorci *et al.*, 1995). If this is the case then it is still possible that females may mate multiply to acquire ‘good’ genes, which increase offspring endurance. However, the lack of differences in offspring endurance between clutches may be because



there are no reliable signals of male endurance for which females can select. Future experimental work addressing female preferences will shed light on this hypothesis.

In summary, we provide estimates of heritability and maternal effects on five offspring traits predicted to influence fitness in an Australian skink species, *Eulamprus quoyii*. Endurance showed significant additive genetic variance, which is congruent with a number of other studies on lizards and snakes (Sorci *et al.*, 1995; Le Galliard *et al.*, 2004). In contrast, we found that mass and sprint speed were under strong maternal influence and growth rate was largely determined by environmental factors. Offspring body size showed significant maternal effects with low estimates of heritability. However, heritability estimates for body size showed large confidence intervals suggesting that epistatic or dominance effects may be operating on this presumably highly heritable trait and will require multi-generational pedigree information to obtain more precise estimates of narrow-sense heritability in this trait.

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## CHAPTER 5: BEHAVIORAL AND MORPHOLOGICAL TRAITS INTERACT TO PROMOTE THE EVOLUTION OF ALTERNATIVE REPRODUCTIVE TACTICS IN A LIZARD

Daniel W.A. Noble,<sup>1,\*</sup> Kerrie Wechmann,<sup>1</sup> J. Scott Keogh,<sup>2</sup> and Martin J. Whiting,<sup>1</sup>

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### Abstract

Alternative reproductive tactics (ARTs) are predicted to be the result of disruptive correlational selection on suites of morphological, physiological and behavioral traits. ARTs are most obvious when they occur in discrete morphs with concomitant behavioral tactics. However, ARTs driven by behavior, in species lacking obvious phenotypic differences, are rarely documented and poorly understood. We quantified selection acting on phenotypic traits predicted to characterize ARTs by observing marked lizards in six semi-natural populations. We quantified reproductive fitness for each male using 6 microsatellite DNA loci from 226 offspring born to 56 females. Candidate models containing directional and correlational selection gradients were equally supported. As predicted, large males with large home ranges and large males who were observed frequently had the highest reproductive success. We also found evidence that large males that moved little, but were observed frequently and large males which moved frequently, but were observed little, were predicted to have high fitness. Model predictions support our verbal hypothesis regarding the phenotypes characterizing ARTs and suggest that large males may be adopting subtly different tactics to acquire paternity. Our results suggest that disruptive correlational selection between behavioral traits may drive the evolution of ARTs in 'cryptic' systems that lack overt polymorphisms.

## INTRODUCTION

Sexual selection is understood as a powerful evolutionary force that can drive phenotypic differences between the sexes, such as extreme sexual ornamentation and body size dimorphism (Andersson 1994). However, strong sexual selection can also generate phenotypic diversity within the sexes, particularly among males (Andersson 1994; Shuster 2010). Competition for mates generates variance in male mating success and can favor the evolution of alternative phenotypes, which allow males to successfully compete for female mating opportunities (Andersson 1994; Shuster 2010; Taborsky et al. 2008). These alternative reproductive tactics (ARTs) show a remarkable diversity of form across a wide range of taxa and can vary in their degree of genetic determination, with some alternative tactics being the result of allelic variation at a few loci of major effect with lower levels of plasticity (e.g. Sinervo and Lively 1996; Sinervo et al. 2000) and others being determined to a greater degree by environmental influences (i.e. condition-dependent; Byrne and Roberts 2004; Forslund 2003; Lidgard et al. 2005; Müller et al. 2007; Reichard et al. 2004; Schradin and Lindholm 2011; Shine et al. 2005). For example, male side-blotched lizards exhibit one of three discrete morphs (orange, blue and yellow), which are the result of alleles segregating at an autosomal OBY locus (Sinervo and Lively 1996). Orange males are hyper-aggressive and defend large territories, while blue males defend smaller territories and are less aggressive. Yellow males do not defend territories but sneak matings from orange males (Sinervo and Lively 1996). In contrast, male dung beetles, *Onthophagus taurus*, exhibit two alternative phenotypes, horned and hornless males, which develop in response to varying rearing environments and body size thresholds (Emlen 1997). Once males have developed into these alternative morphs the phenotypes are irreversible. Horned males are large and vigorously defend burrows by dung patches, while hornless males adopt 'sneaker-like' tactics, digging intersecting tunnels of their own and sneaking copulations with females within the burrows guarded by large horned males (Emlen 1997; Moczek and Emlen 2000).

Alternative reproductive tactics are predicted to arise through correlational selection for combinations of morphological, physiological and behavioral traits (Miles et al. 2007; Sinervo and Calsbeek 2006; Sinervo and Svensson 2002). The form of selection on these suites of traits is thought to be disruptive, where extreme phenotypes with particular trait combinations experience a fitness advantage over intermediate forms (Gross 1985; Reuffer et

al. 2006; Taborsky et al. 2008). Disruptive selection for ARTs may result from alternative tactics occupying divergent niches (Bergmüller and Taborsky 2010; Taborsky et al. 2008), which permit them to compete successfully for access to resources or mates or through frequency-dependent selection (Sinervo and Lively 1996). Despite the importance of disruptive selection in maintaining mating polymorphisms and potentially driving speciation, most ART examples involve discrete polymorphisms, because of the ability to assign different strategies to each morph. However, few studies have demonstrated evidence for disruptive selection on traits that characterize alternative male mating phenotypes (references within Oliveira et al. 2008), likely due to the lack of intermediate phenotypes in most systems.

Much of our understanding of ARTs comes from systems where the alternative tactics can be clearly defined, however, there is often high variance in reproductive success in systems where males show no clear morphological discontinuities (e.g. Morrison et al. 2002). We would predict that in these systems selection would favor males that adopt alternative behavioral phenotypes, which are generally subtle and less conspicuous. In many systems with ARTs morphological differences between individuals are discrete with few intermediate phenotypes, however, when ARTs are characterized by continuous behavioral differences they provide an excellent opportunity to test for disruptive selection because these traits often contain intermediate phenotypes. Furthermore, the relative contribution of the different behaviors defining ARTs, to male reproductive success, can be quantified (e.g. Baird et al. 2007) and verified using paternity analysis. Trait-based approaches for quantifying the reproductive success of alternative tactics are particularly suitable in these systems when we have an understanding of the traits that are important for fitness.

The lizard genus *Eulamprus* is a widely distributed and well-studied group of skinks in Australia. The genus is known for their high incidence of multiple paternity and high variance in male mating success (Dubey et al. 2011; Morrison et al. 2002; Noble et al. 2013). In *Eulamprus heatwolei*, males exhibit alternative mating tactics where some males act as residents while others are floaters (Keogh et al. 2012; Morrison et al. 2002; Stapley and Keogh 2004). In the wild, resident males have small home ranges and are observed frequently, while floater males have large home ranges and are observed much less often (Morrison et al. 2002; Stapley and Keogh 2004; Stapley and Keogh 2005). Resident males also have a much lower tendency to move in a novel environment compared to floater males and these behaviors form part of a behavioral syndrome (Stapley and Keogh 2004). These activity-related behaviors

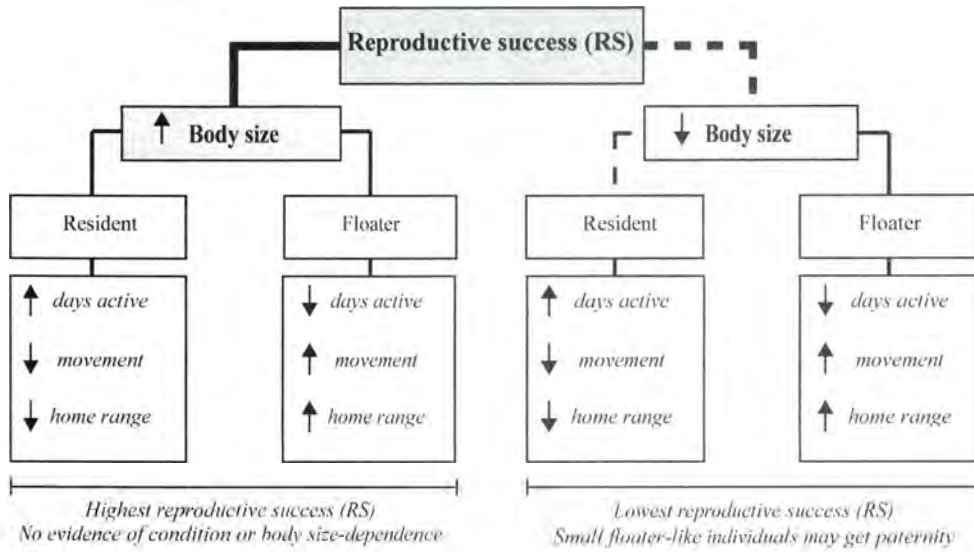
may play an important role in pre-copulatory sexual selection and both resident and floater males have been shown to achieve high reproductive success (Keogh et al. 2012; Morrison et al. 2002; Stapley and Keogh 2005); however, selection on traits that characterize these tactics has not been demonstrated.

We studied a closely related species, *Eulamprus quoyii*, and tested for selection on key behaviors that have previously been used to define ARTs in *E. heatwolei* and which have an important bearing on fitness. Unlike *E. heatwolei*, *E. quoyii* is larger and shows higher levels of aggression yet exhibits very similar behavior and ecology to *E. heatwolei* (D. Noble and J.S. Keogh, personal observations). Given that male *E. quoyii* are aggressive and known to form dominance hierarchies (Done and Heatwole 1977), we predicted that body size would be an important determinant of reproductive success (fig.1). However, the existence of ARTs in the closely related *E. heatwolei* suggests that males adopting particular behavioral traits may elevate their reproductive success further (fig.1). Floater males, which have high rates of movement and larger home ranges (Morrison et al. 2002; Stapley and Keogh 2005), are predicted to acquire paternity by virtue of higher encounter rates with a potentially large number of females, while resident males are predicted to reside with groups of females or focus mating efforts on specific females (i.e. mate guarding). Indeed this is supported by the fact that resident male *E. heatwolei* tend to sire more offspring from resident females. Remaining active for longer might allow these males to mate more regularly with resident females or mate guard (Stapley and Keogh 2005).

Detailed behavioral studies on lizards are difficult to execute in the wild and paternity estimates can be troublesome depending on the proportion of the population sampled. We circumvented these constraints by establishing six breeding populations in large outdoor semi-natural enclosures, where all the individuals were known, and could be followed throughout the breeding season. We specifically addressed two different questions: 1) Is there evidence for disruptive correlational selection acting on behavioral traits which might promote the evolution of alternative male mating tactics? and 2) What behaviors are important contributors to male reproductive success? Since previous work had categorized ‘floater’ and ‘territorial’ males in *E. heatwolei* by the days they are observed active and also demonstrated that they vary in their home range and their movement propensity in the lab (Morrison et al. 2002; Stapley and Keogh 2005), we tested the hypothesis that behaviors (home range, movement rates, total days active) form discrete axes that are under selection in the directions that define



ARTs (fig. 1). To test predictions from our hypotheses, we evaluated the support for a series of candidate models and tested whether predictions from these models (i.e. the directionality and effect sizes of parameter estimates) are congruent with the hypothesis that these traits may promote the evolution of ARTs in *E. quoyii*. We also explore how different behavioral traits contribute to a male's reproductive success by testing how they contribute to the total number of clutches sired by a male.



**Figure 1** - Predictions of how behavioral and morphological traits influence male reproductive success (RS) in Eastern Water Skinks (*E. quoyii*). Body size is predicted to be the most important determinant of male RS, with large males gaining the highest RS because of their higher competitive abilities compared to smaller males. This is represented by the thick black line along the path from RS to body size. The dashed thick line leading to small males indicates that they have dramatically reduced reproductive success by virtue of their small size. For simplicity, males can adopt two different alternative reproductive tactics (ARTs) along a continuum. Males at either extreme of this multivariate trait space are classified as ‘Residents’ (also known as ‘Territorials’; Stapley and Keogh 2004; Stapley and Keogh 2005) and ‘Floaters’. The suites of behavioral traits in the figure are predicted to further elevate the reproductive success of a given male adopting these trait combinations. Solid lines represent higher RS while dashed, lower RS. The solid black line along the small male pathway indicates that small males adopting ‘floater-like’ tactics may elevate their RS in some instances (e.g. Keogh et al. 2012). The thickness of the lines indicates the relative importance of morphology and behavior in determining male RS. For example, large males adopting the

specified trait combinations are predicted to achieve the highest reproductive success. Black arrows indicate larger or smaller (body size & home range) or higher and lower (days active & movement). See Introduction for references to predictions.

## METHODS

### *Field Collection and Experimental Design*

We collected Eastern Water Skinks (*Eulamprus quoyii*) from five sites within 30 km of Macquarie University between 12 August – 17 September 2010. Only sexually mature skinks (SVL > 90 mm) were captured either by hand or by noosing and brought back to the laboratory for further processing.

For each individual, we measured snout-vent length (SVL; to nearest 1 mm) and mass (nearest 0.1 g) and took a small quantity of blood (~50 – 70 uL) or tail tissue for DNA. Lizards were sexed by the presence or absence of hemipenes and individually marked using passive integrated transponder (PIT) tags. Lizards were allocated to one of six outdoor enclosures (16 x 10 m) located on Macquarie University campus (see Noble et al. 2013 for more details on the enclosures). We released 18 males and 18 females ( $n = 36$ ) into each enclosure ensuring that each collection site was represented and that there was natural variation in body size ( $n = 216$  lizards in total). These densities fall within the natural range observed in the wild (unpublished data; G. Swan, personal communication). Each enclosure (fig. S1) contained two piles of large rocks connected by varying sized logs while the remaining areas were more open with no rocks and logs, creating natural heterogeneity in their environment. Four large water containers were placed equidistant in the enclosures and a stack of three ceramic roofing tiles (28 x 45 x 3.2 cm) were positioned on the ground every 2 m to form a grid. These tiles were numbered to facilitate scoring the location of lizards and they also provided shelter for the lizards occupying these areas.

### *Quantifying Behavioral Axes of ARTs*

All lizards were marked with a non-toxic paint marker and cloth tape (gray for females, brown for males) to facilitate individual identification. We released male skinks into the six enclosures two days prior to females. This replicated conditions in the wild where males emerge earlier than females in spring. Lizards were given four days to acclimatize to the enclosures before behavioral observations commenced.

Water skinks were observed for the duration of the breeding season from 22 September to 20 October 2010 (by DWAN and KW) during the active periods of the day (0900-1700 h). Both observers spent a full day sampling together to standardize behavioral recording prior to data collection. Each observer randomly sampled three enclosures per day and we ensured that both observers sampled all six enclosures over a two-day period. We used both scan and focal sampling (Martin and Bateson 1993) to quantify individual behavior. We first scanned each enclosure and recorded the position, sex and identity of all lizards prior to choosing an individual for focal sampling. Scan sampling was done on each enclosure twice a day (once in the morning and afternoon). We recorded positions of lizards on an enclosure map, using the tile markers as reference points. To minimize observer interference, scan samples were conducted by slowly walking the perimeter of the enclosure and using binoculars to identify the ID tags on the backs of lizards. *Eulamprus quoyii* habituates readily to observers and our presence did not have any noticeable effects on individual behavior. Individuals were haphazardly selected for focal sampling and we ensured that a new individual was selected whenever possible to ensure representative sampling within each of the six enclosures. This was done to avoid biasing our behavioral sampling towards particular males that were more active or more easily sampled. Focal samples were 10 min in duration and we recorded whether individuals were moving (locomotion) or remaining stationary. Locomotion was defined as any movement where the lizard moved greater than 10 cm from its initial position. Locomotion bouts were considered independent if lizards stopped for greater than 2 s. If a social interaction took place, the individuals involved, the location, and the outcome were recorded. We observed fighting (stereotypical biting of each others tail) and chasing (rapid approach of one individual followed by the retreat of a second) events between males and copulations between males and females. If individuals were out of view for more than two minutes, the focal sample was abandoned and a new focal individual was located.

We calculated the proportion of time each individual spent moving as the time spent in locomotion divided by the total time in view. Individual locations were transferred from

enclosure maps to electronic maps using GraphClick 3.0. Using these electronic coordinates we calculated minimum convex polygons (MCPs) as estimates of individual home ranges ( $\text{m}^2$ ) using the 'adehabitat' package (Calenge 2006) in the statistical software package R (R Development Core Team 2010). To determine the minimum number of sightings needed to estimate home range area we regressed home range area against the number of re-locations using the same method as Morrison et al. (2002). We found that there was no longer a statistically significant relationship between home range area and the number of sightings when individuals with eight or more sightings were included.

### *Reproductive Success of Behavioral Tactics*

At the end of the breeding season, females were collected from the enclosures and individually housed in plastic boxes (32 x 45 x 27 cm) in a temperature-controlled room until parturition. Heating cable was used to elevate one part of the cage to optimal body temperatures ( $\sim 28 - 32^\circ\text{C}$ ) thereby allowing females to behaviorally thermoregulate. Ultraviolet lighting was provided during daylight hours on a 12:12 hour cycle. Lizards were fed crickets twice per week and dog food once per week, with added calcium and vitamin powder. Once females had given birth, we removed the offspring and weighed and measured each neonate. Lizards were toe-clipped for permanent identification and a small amount of tail tissue was excised and stored in ethanol for DNA extraction.

Genomic DNA was extracted from blood and tissue samples using DNeasy blood and tissue extraction kits (Qiagen) according to the manufacturer's protocol. Six microsatellite DNA loci [Ek100, Ek107, Ek8, Ek37; Scott et al. (2001) and GQ16/17, GQ20/21; Sumner et al. (2001)] were amplified. PCR reactions were carried out in 20  $\mu\text{L}$  reaction volumes containing 1.0  $\mu\text{L}$  of genomic DNA, 10  $\mu\text{L}$  of GoTaq® (from Promega), 0.5  $\mu\text{L}$  ( $10 \text{ pmol} \cdot \mu\text{L}^{-1}$ ) of forward and reverse primers and 8.0  $\mu\text{L}$  of nuclease free water. PCR conditions for each locus are described in Scott et al. (2001) and Sumner et al. (2001). Forward primers were labeled with different fluorescent dyes (TET, NAD, VIC, FAM) and product from the final PCR reactions were pooled into a single plate, run on an ABI 3730 DNA analyzer (Applied Biosystems) and scored by the Australian Genomic Research Facility (AGRF) using AB Genemapper software (Applied Biosystems).



Parentage was assigned using the likelihood-based method in the program CERVUS 3.0 (Kalinowski et al. 2007). We simulated 100,000 offspring with 95% loci typed and 1% mistyped loci, using a strict confidence level of 95% and a relaxed confidence level of 80%. The loci used in our study were highly variable, ranging from 3 – 34 alleles at a single locus with mean polymorphic information content (PIC) of 0.7014. The combined non-exclusion probability for a parent pair was  $4.46 \times 10^{-6}$ . Parentage was assigned conservatively, and we excluded offspring containing one or more mismatches or that had five or less loci compared with putative sires. In some cases, males could only be compared at four loci with offspring because of differences in the loci missing between the male and offspring. In these situations we assigned paternity to the male only if he had no mismatches and the trio (male, female and offspring pair) LOD scores were significant. From these data we calculated: 1) the number of offspring each male sired and 2) the total number of clutches containing offspring sired by each male.

### *Statistical Analyses*

Statistical analyses were carried out using R 2.15.0 (R Development Core Team 2010). In total, 28 (26%) males were not recaptured from the enclosures at the completion of the experiment and we assumed that they had been preyed upon. Unfortunately we could not include survival in our analyses because of the low number of males that died in any given enclosure and the lack of behavioral data on these males. To avoid biasing the results we removed these males from the final analysis of reproductive success because they were part of the experiment for varying amounts of time during the breeding period and therefore we were unable to standardize male mating opportunity. We also excluded males with no focal samples ( $n = 15$ ), that had fewer than 8 re-sightings ( $n = 15$ ) and those that were not genotyped at 5-microsatellite DNA loci ( $n = 1$ ). In total, we obtained complete data for 49 males distributed across the six enclosures with anywhere from 7 – 10 males per enclosure (mean = 8.17). Exclusion of these males may have important consequences for our results so we ran two separate analyses. In the first analysis, as many individuals with complete data (e.g. males that died, but had complete data and sired offspring; too few points for home range estimates) were included. This increased our sample size slightly to 56 males and made our analysis slightly more conservative. Most missing information was because we lacked focal samples for many

individuals. In analysis two, we used a larger sample of males with home range area and days active data along with their morphology. We evaluated a limited number of candidate models (given missing movement data, which seemed to be important) with this larger sample size ( $n = 66$ ) and the results are shown in table S3 - S5. Sample sizes are provided for the different analyses because of the missing information in the data and because while most females could be identified, seven individuals expelled their PIT tags.

We modeled the number of offspring, relative reproductive success ( $w$ ) and number of clutches sired as a function of our predictor variables using a generalized linear model (GLM) with a Tweedie (Poisson-gamma) error distribution and log link function. The Tweedie distribution was ideal for our purposes for a number of reasons. First, absolute reproductive success and clutch number were integer/count variables while relative reproductive success was a positive non-integer variable. The Tweedie distribution allows one to model both types of variables under an over-arching framework by introducing a parameter,  $p$ . When  $p$  is equal to 1 the distribution is equivalent to a Poisson probability distribution where the mean and variance are considered equal and integer/count variables can be modeled accordingly. However, when  $1 < p < 2$  a scale parameter is estimated and both integer and non-integer variables can be modeled. Second, because a scale parameter is estimated when  $1 < p < 2$ , Tweedie models can deal with over-dispersed data (i.e. when the mean and variance do not increase linearly). Indeed Poisson models for our data were slightly over-dispersed and this can lead to smaller standard errors and elevated type-one error rates. To estimate what value of  $p$  was best for our data we fitted our full models and varied  $p$  between 1.1 – 1.6 in intervals of 0.1 and compared  $AIC_C$  between respective models. Models containing the value of  $p$  with the lowest  $AIC_C$  were deemed the best fit and this value of  $p$  was used for all candidate models in the same analysis.

We estimated standardized selection gradients from our multivariate GLMs in a similar fashion to LeBas et al. (2004) and Chaine and Lyon (2008). Linear selection gradients ( $\beta_i$ ) indicate sexual selection that changes the population mean while nonlinear selection gradients ( $\gamma_{ii}$ ; quadratic selection gradients or  $\gamma_{ij}$ ; correlational selection gradients) describe how the phenotypic variance of a trait is changed (Brodie III 1992; Brodie III et al. 1995; Lande and Arnold 1983). We converted the number of offspring sired to relative reproductive success (i.e. the number of offspring sired divided by the population mean within each of the six enclosures) and standardized traits by their mean and standard deviation (Brodie III et al.

1995; Lande and Arnold 1983). Relative reproductive success was calculated using all males in each of the enclosures as we had complete paternity data on these males even though we may not have had complete behavioral data. We present linear selection gradients ( $\beta_i$ ) from our GLM models without the quadratic and cross-product parameters whereas quadratic and correlational selection gradients come from our full models (Brodie III et al. 1995; Lande and Arnold 1983; Mitchell-Olds and Shaw 1987). In addition, quadratic terms and their standard errors were doubled (Stinchcombe et al. 2008).

We generated a set of candidate models based on our verbal hypotheses about how our traits influence reproductive success (fig. 1) and compared the fit of our models using  $AIC_C$ , which is a more robust information criteria for model selection when the sample size to parameter ratio is small (Burnham and Anderson 2002). We estimated directional selection on snout-vent length (SVL; body size), home range area, total days active and the proportion of time spent moving. In all models the proportion of time spent moving was arcsine transformed because its distribution was highly right skewed and we attempted to reduce its influence on the selection analysis (see *supplemental materials* for additional analyses we performed to ascertain the effects of predictor distributions on model selection). Since previous studies suggest that males may be adopting alternative reproductive tactics, it is possible that traits may evolve as modules (Morrison et al. 2002; Stapley and Keogh 2004; Stapley and Keogh 2005). Given our smaller sample size, we restricted our models to contain important two-way interactions between traits that we predicted would be under correlational selection (home range area, total days active and proportion of time spent moving). We also included quadratic estimates for each of the behavioral traits (home range area, total days active and proportion of time spent moving), which might be under disruptive selection. Since our behavioral traits (particularly total days active and movement rates) may be strongly influenced by a male's body condition we included condition (residuals from a linear regression between mass and SVL) in some candidate models. This is a commonly used measure of body condition for lizards and removes the strong collinearity between SVL and mass in the models. We included enclosure as a covariate ("block") in all models to control for the differences in the number of females giving birth and the number of offspring produced in each enclosure. Although it is possible to model the enclosure variable as a random effect, we chose not to because of the small number of random effect levels, which can lead to imprecise variance estimates. Generally it is recommended that only variables with greater than 5 – 6

levels be modeled as random effects (Bolker et al. 2008). Although we are on the cusp of this recommendation, we erred of the side of caution and treated enclosure as a fixed effect. We repeated the above analyses using absolute fitness (i.e. number of offspring) and using standardized traits. We retained our standardized traits for modeling absolute fitness because it improves the interpretation of regression coefficients, particularly in the presence of interactions (Schielzeth 2010).

In addition to testing hypotheses about how male traits influence absolute and relative reproductive success and whether there was evidence for correlational disruptive selection we were also interested in testing hypotheses concerning the mechanisms by which these traits may influence the number of females mated. Since it was difficult to get accurate estimates of the number of females that overlapped a male's home range and because this may not necessarily be a good indicator of the number of females actually sired, we used the number of clutches in which a male sired offspring as our dependent variable and modeled it as a function of our predictor variables. We tested three alternative hypotheses. First, males that spend more time active will sire offspring from more females because remaining active would allow males to mate with females that become sexually receptive at different times or possibly exclude other males from accessing these females. If this were supported then models with total days active would be best supported over models with home range area after controlling for body size. Second, males who move more over a larger area sire more offspring across more females because they are able to encounter more spatially dispersed females for which they may mate. If this were supported then models with home range area and/or moving would be best supported over models with total days active. Lastly, both home range area and total days active may both be important predictors of male mating success, particularly if males are adopting alternative tactics to acquire paternity. Support for this hypothesis would mean that models containing home range area and total days active would have the best support and possibly models with an interaction between home range area and total days active. Furthermore, we would predict a positive relationship between the number of clutches sired and the number of offspring produced.

In all analyses, we calculated  $\Delta AIC_C$  between our best approximating model and set of competing candidate models along with each models Akaike weight,  $w_i$ , which provides an estimate of model support. Each models Akaike weight can be interpreted as the probability of model  $i$  being the true model out of the models in the candidate set (Symonds and Moussalli



2011). We avoided discounting models with  $\Delta AIC_C$  of less than 6 from the best approximating model (Richards 2005; Symonds and Moussalli 2011). This was particularly true with our data because of our smaller sample sizes. Simpler models are often ranked as the best approximating model when the quantity of data is low, however, it has been shown that the addition of more data can improve the rank of lower ranked models (Richards 2005). In addition to evaluating model uncertainty, we were also interested in testing specific predictions from our models. Therefore, models containing specific interactions that were within 6 IC units of each other were of particular interest to test whether their predictions were congruent with verbal models. To account for differences in parameter estimates between models we took a model averaging approach (Grueber et al. 2011; Symonds and Moussalli 2011). We used ‘conditional’ or ‘natural’ model averaging where parameters are only averaged over the models where that particular parameter is estimated (Grueber et al. 2011). We did this because interactions appeared to be weaker, but potentially important. Model averaged coefficients were estimated from our candidate model set for models that were within 6 IC units of the top model and weighted by the Akaike weight of the each model. For all model averaging we used our standardized traits (mean = 0 and sd = 1).

To visualize how our dependent variables change with respect to multiple predictor variables we used the *vis.gam* function in the ‘mgcv’ package in R (Wood 2006). This allows one to use a fitted parametric GLM model to predict data while controlling for the influential effects of all other variables in the model. We visualized the predicted surfaces of our top models to determine whether behavioral and morphological traits influence reproductive success in the predicted direction based on our hypotheses (fig. 1). We also explored how these traits affect the number of clutches a male sired to understand the mechanisms by which males obtain copulations. Only bivariate plots can be visualized and we compared these plots to determine whether they support our specific predictions about what phenotype combinations would be expected to have high fitness. We avoided extrapolating predicted surfaces too far beyond the bounds of our data and computed predicted estimates only in areas that were within 0.15 – 0.18 units of a nearest data point. Since our models explained a large amount of variation in fitness traits we also predicted the theoretical fitness landscape if we were to have particular combinations of phenotypes. In these instances we were particularly interested in model predictions from equally supported models with interactions. Although the use of non-parametric cubic splines can be used to visualize fitness surfaces (Brodie III et al.

1995; Schluter 1988), we avoided these because of our limited dataset and enclosure effects. Datasets for these analyses can be found in Dryad (*doi: upon acceptance*).

## RESULTS

### *Paternity and Reproductive Success*

A total of 303 offspring were born from 63 females and the number of offspring a female produced was positively related to both her body condition and SVL (multiple linear regression  $n = 56$ ; condition,  $\beta = 0.26 \pm 0.06$ ,  $t = 4.0$ ,  $P < 0.0001$ ; SVL,  $\beta = 0.16 \pm 0.03$ ,  $t = 4.8$ ,  $P < 0.0001$ ). Two hundred twenty-six offspring from 56 females could be assigned to a single sire (zero mismatches at all loci compared and significant trio LOD scores). A total of 21 males (42%) had no reproductive success while 28 (57%) males sired at least one offspring. Twenty-nine (52%) clutches had more than one male sire offspring based on paternity assignment and allelic diversity within the clutch. The number of offspring sired ranged from 1 – 17 and these males sired offspring across 1 – 6 unique clutches. Mean reproductive success varied across enclosures, ranging from an average of 1.11 (enclosure 5) offspring to 3.56 (enclosure 6) offspring with an overall average of 2.10 ( $se = 0.33$ ) across enclosures.

### *Behavioral Observations*

A total of 17.3 hours of focal observations were conducted on 56 individual males across the six enclosures. Aggressive interactions between males were uncommon and only three fights and nine chases were recorded. Male home range size ranged from 3.07 – 72.7 m<sup>2</sup> (mean =  $30.70 \pm 2.50$  m<sup>2</sup>,  $n = 66$ ) while they remained active anywhere from 5 – 20 days (mean =  $11 \pm 0.40$  days,  $n = 66$ ). SVL and home range size were not significantly correlated (Spearman rank correlation:  $r_s = -0.05$ ,  $p = 0.67$ ,  $n = 66$ ). Home range area was also not significantly correlated with the number of days a male was active ( $r_s = 0.12$   $p = 0.33$ ,  $n = 66$ ).

### *Correlational Disruptive Selection for ARTs and Predictors of Reproductive Success*

Our models explained anywhere from 59 – 80% of the variation in relative and absolute reproductive success (table 1, 2). There was a strong positive relationship between relative reproductive success and male body size and total days active (fig. 2a & b). However, there was substantial variation and no relationship between relative reproductive success and male home range area and the proportion of time spent moving (fig. 2c & d). The lack of relationship in fig. 2c & d was partly due to the fact that individuals with high fitness but small home ranges were observed active a lot and had lower than average movement rates. The best-supported models were those containing directional selection gradients for male body size (SVL), total days active, home range area and the proportion of time spent moving (table 1, 2, 3). Two models were equally supported in most of our analyses, one containing only main effects and a second containing an interaction between the proportion of time spent moving and total days active with the  $\Delta AIC_c$  between the two models ranging from 0.12 – 0.38 IC units from each other (table 1;  $n = 49$ ; full model coefficients table S6). This difference increased in the analyses with a larger sample size to 1.26 – 2.33 (table 1;  $n = 56$ ) and condition was included in these models. We found similar support for these two models when using predictor variables that were converted to normalized quantiles prior to analyses (table S2), suggesting that this pattern could not simply be explained by the skewed distribution of the proportion of time spent moving. Model averaged estimates for parameters in the tied models indicated that body size ( $\beta_{\text{mean}} = 1.30 \pm \text{adj se } 0.26$ ), home range area ( $\beta_{\text{mean}} = 0.44 \pm \text{adj se } 0.16$ ), total days active ( $\beta_{\text{mean}} = 0.46 \pm \text{adj se } 0.14$ ) and proportion time spent moving ( $\beta_{\text{mean}} = 0.13 \pm \text{adj se } 0.46$ ) had positive effects on relative reproductive success, while there was support for an interaction between total days active and the proportion of time spent moving ( $\beta_{\text{mean}} = -0.26 \pm \text{adj se } 0.16$ ). We used our top models to predict reproductive success and test whether these were in line with predictions from our verbal hypothesis (fig. 1), while controlling for other variables in the model. Both the main effects and the interaction model (model 1 and 2; table 1, 3) predict that large males that are observed frequently and large males that have large home ranges obtain high reproductive success when holding other variables in the model constant (fig. 3 a, b, e, f). Model 1 and 2 also predict that large males with large home ranges that move more are expected to have high reproductive success (fig. 3c & g). The interaction between total days active and proportion of time spent moving predicted disruptive selection in that large males which moved little, but which were observed frequently and males that moved a lot but were observed less were predicted to have high

reproductive success (fig. 3d & h). This was also evident when we predicted the entire fitness landscape (fig. 4). Correlational selection was still evident when male 151 was removed, however, it was no longer disruptive (see *supplementary materials* for details on analysis).

There was a strong positive relationship between the number of offspring sired and the number of clutches in which a male sired offspring ( $r_s = 0.98$ ,  $n = 49$ ,  $p < 0.001$ ; fig. S2). There was much model uncertainty in our candidate set and we found equal support for two candidate models (table 2, 4; model 3 & 4), however, a number of other models were within 6  $AIC_C$  units of these models (models 2, 5, 6, 7, 9; table 4). Model averaged coefficients from models within 6 IC units of our top model showed that body size ( $\beta_{\text{mean}} = 0.90 \pm \text{adj se } 0.26$ ), total days active ( $\beta_{\text{mean}} = 0.28 \pm \text{adj se } 0.15$ ) and home range area ( $\beta_{\text{mean}} = 0.40 \pm \text{adj se } 0.15$ ) all had a positive effect on the number of clutches sired. We predicted the expected number of clutches a male of a particular phenotype would be expected to sire from models 3 and 4, but also present model 2 because both home range area and total days active are present in the same model and estimates may change slightly (table 5). Our models made qualitatively similar predictions; individuals with large body size and with large home range areas were predicted to sire offspring across more clutches (fig. 5a & c), while larger individuals who were active longer were also predicted to sire offspring across more clutches (fig. 5b & d). In the best-supported models, the coefficient for home range area was larger than the total days a male was active (table 5). Thus the influence of home range on number of clutches sired exceeded that of total days active.



**Table 1** – Candidate models evaluated based on standardised predictor variables (mean = 0; sd = 1) and relative reproductive success and the number of offspring (absolute fitness) as response variables. Results are presented from two separate analyses with different sample sizes. The number of model parameters, sample size corrected Akaike information criterion ( $AIC_C$ ), the difference in  $AIC_C$  between the top model and each candidate model ( $\Delta AIC_C$ ), Akaike weights ( $w_i$ ) and adjusted  $R^2$  (Adj  $R^2$ ) for each model are also presented. The set of predictors in each of the candidate models are listed in Table 3 under “Relative and Absolute Reproductive Success”. The Tweedie parameter is listed for each analysis and refers to the best-supported parameter for each of the analyses. Bolded models indicate models that are within 2  $\Delta AIC_C$  units of each other and have equal support

Relative Reproductive Success									
Candidate model	k	Adj $R^2$	n = 49 – Tweedie $p = 1.1$			Adj $R^2$	n = 56 – Tweedie $p = 1.2$		
			$AIC_C$	$\Delta AIC_C$	$w_i$		$AIC_C$	$\Delta AIC_C$	$w_i$
Model 1	11	<b>0.76</b>	<b>147.41</b>	<b>0</b>	<b>0.3780</b>	0.63	169.25	2.44	0.1200
Model 2	10	<b>0.71</b>	<b>147.53</b>	<b>0.12</b>	<b>0.3560</b>	<b>0.61</b>	<b>167.47</b>	<b>0.66</b>	<b>0.2923</b>
Model 3	12	0.76	150.60	3.19	0.0767	0.71	169.14	2.33	0.1268
Model 4	11	0.73	149.77	2.36	0.1162	<b>0.70</b>	<b>166.81</b>	<b>0</b>	<b>0.4065</b>
Model 5	12	0.74	150.97	3.56	0.0637	0.62	171.22	4.41	0.0448
Model 6	13	0.73	154.84	7.43	0.0092	0.60	174.36	7.55	0.0093
Model 7	15	0.73	163.05	15.64	0.0002	0.59	181.90	15.09	0.0002
Model 8	16	0.75	166.96	19.55	0.0000	0.59	185.87	19.06	0.0000
Model 9	17	0.77	170.93	23.52	0.0000	0.70	186.72	19.91	0.0000
Model 10 (Null)	1	NA	283.97	136.56	0.0000	NA	233.87	67.06	0.0000

Number of Offspring (Absolute Fitness)									
Candidate model	k	Adj $R^2$	n = 49 – Tweedie $p = 1.1$			Adj $R^2$	n = 56 – Tweedie $p = 1.1$		
			$AIC_C$	$\Delta AIC_C$	$w_i$		$AIC_C$	$\Delta AIC_C$	$w_i$
Model 1	11	<b>0.76</b>	<b>181.54</b>	<b>0</b>	<b>0.3831</b>	0.63	208.14	5.52	0.0365
Model 2	10	<b>0.72</b>	<b>181.92</b>	<b>0.38</b>	<b>0.3168</b>	0.62	207.61	4.99	0.0476
Model 3	12	0.77	184.39	2.85	0.0921	<b>0.73</b>	<b>203.88</b>	<b>1.26</b>	<b>0.3070</b>
Model 4	11	0.75	183.67	2.13	0.1321	<b>0.72</b>	<b>202.62</b>	<b>0</b>	<b>0.5765</b>
Model 5	12	0.75	185.05	3.51	0.0662	0.64	208.83	6.21	0.0258
Model 6	13	0.74	188.92	7.38	0.0096	0.63	211.68	9.06	0.0062
Model 7	15	0.73	197.19	15.65	0.0002	0.62	218.9	16.28	0.0002
Model 8	16	0.77	200.65	19.11	0.0000	0.64	221.99	19.37	0.0000
Model 9	17	0.80	204.19	22.65	0.0000	0.77	219.04	16.42	0.0002
Model 10 (Null)	1	NA	336.96	155.42	0.0000	NA	377.48	174.86	0.0000

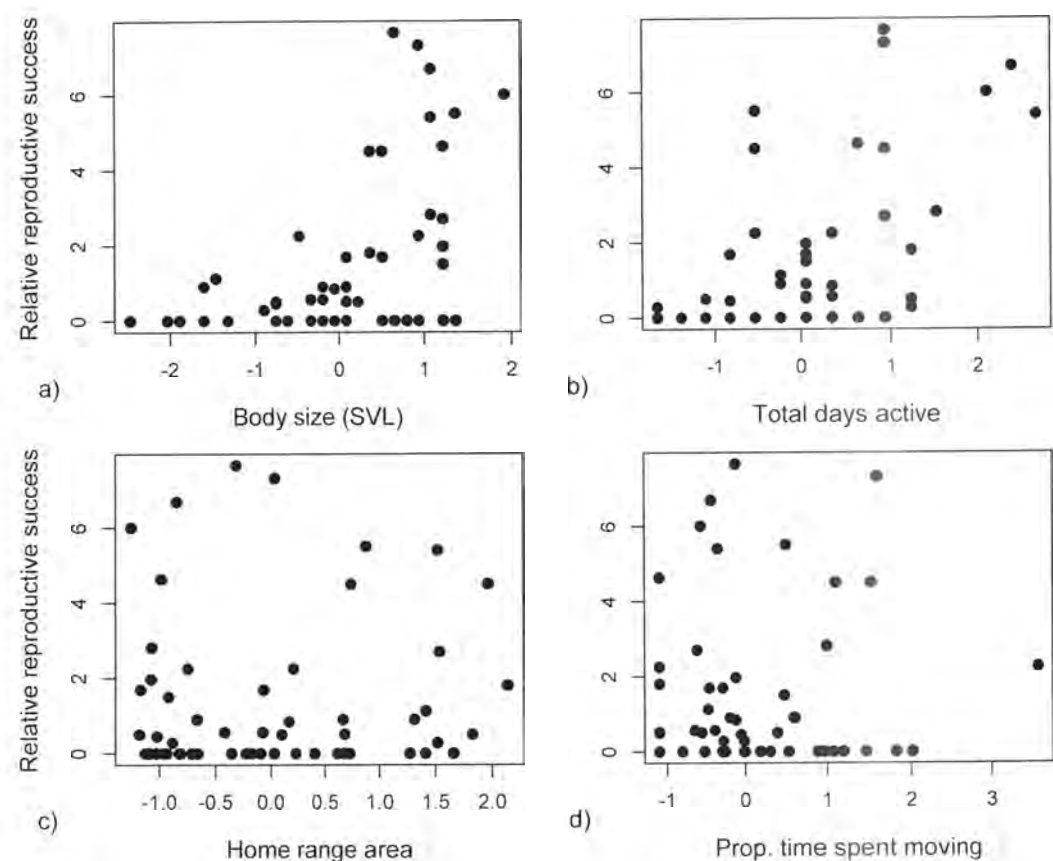
**Table 2** – Predictors in each candidate model evaluated. Abbreviations are as follows: ENCL = enclosure, SVL = snout-vent length, HRA = Home range area, MOVE = Proportion of time spent moving, TOTDAY = Total days active, COND = condition.

Relative and Absolute Reproductive Success	
Candidate Model	Predictors
Model 1	ENCL + SVL + HRA + MOVE + TOTDAY + MOVE*TOTDAY
Model 2	ENCL + SVL + HRA + MOVE + TOTDAY
Model 3	ENCL + SVL + COND + HRA + MOVE + TOTDAY + MOVE*TOTDAY
Model 4	ENCL + SVL + COND + HRA + MOVE + TOTDAY
Model 5	ENCL + SVL + HRA + MOVE + TOTDAY + MOVE*TOTDAY + MOVE*HRA
Model 6	ENCL + SVL + HRA + MOVE + TOTDAY + MOVE*TOTDAY + MOVE*HRA + HRA *TOTDAY
Model 7	ENCL + SVL + HRA + MOVE + MOVE <sup>2</sup> + TOTDAY + TOTDAY <sup>2</sup> + MOVE*TOTDAY + MOVE* HRA + HRA *TOTDAY
Model 8	ENCL + SVL + HRA + HRA <sup>2</sup> + MOVE + MOVE <sup>2</sup> + TOTDAY + TOTDAY <sup>2</sup> + MOVE*TOTDAY + MOVE* HRA + HRA *TOTDAY
Model 9	ENCL + SVL + COND + HRA + HRA <sup>2</sup> + MOVE + MOVE <sup>2</sup> + TOTDAY + TOTDAY <sup>2</sup> + MOVE*TOTDAY + MOVE* HRA + HRA *TOTDAY
Model 10	INT (NULL)

Number of clutches sired	
Candidate Model	Predictors
Model 1	ENCL + SVL + TOTDAY + TOTDAY <sup>2</sup> + MOVE + HRA + HRA <sup>2</sup> + TOTDAY*HRA + MOVE*TOTDAY + MOVE*HRA
Model 2	ENCL + SVL + TOTDAY + HRA
Model 3	ENCL + SVL + TOTDAY
Model 4	ENCL + SVL + HRA
Model 5	ENCL + SVL + TOTDAY + HRA + TOTDAY*HRA
Model 6	ENCL + SVL + HRA + MOVE
Model 7	ENCL + SVL + TOTDAY + MOVE
Model 8	ENCL + SVL + TOTDAY + TOTDAY <sup>2</sup> + HRA + HRA <sup>2</sup>
Model 9	ENCL + SVL + TOTDAY + HRA + HRA <sup>2</sup>

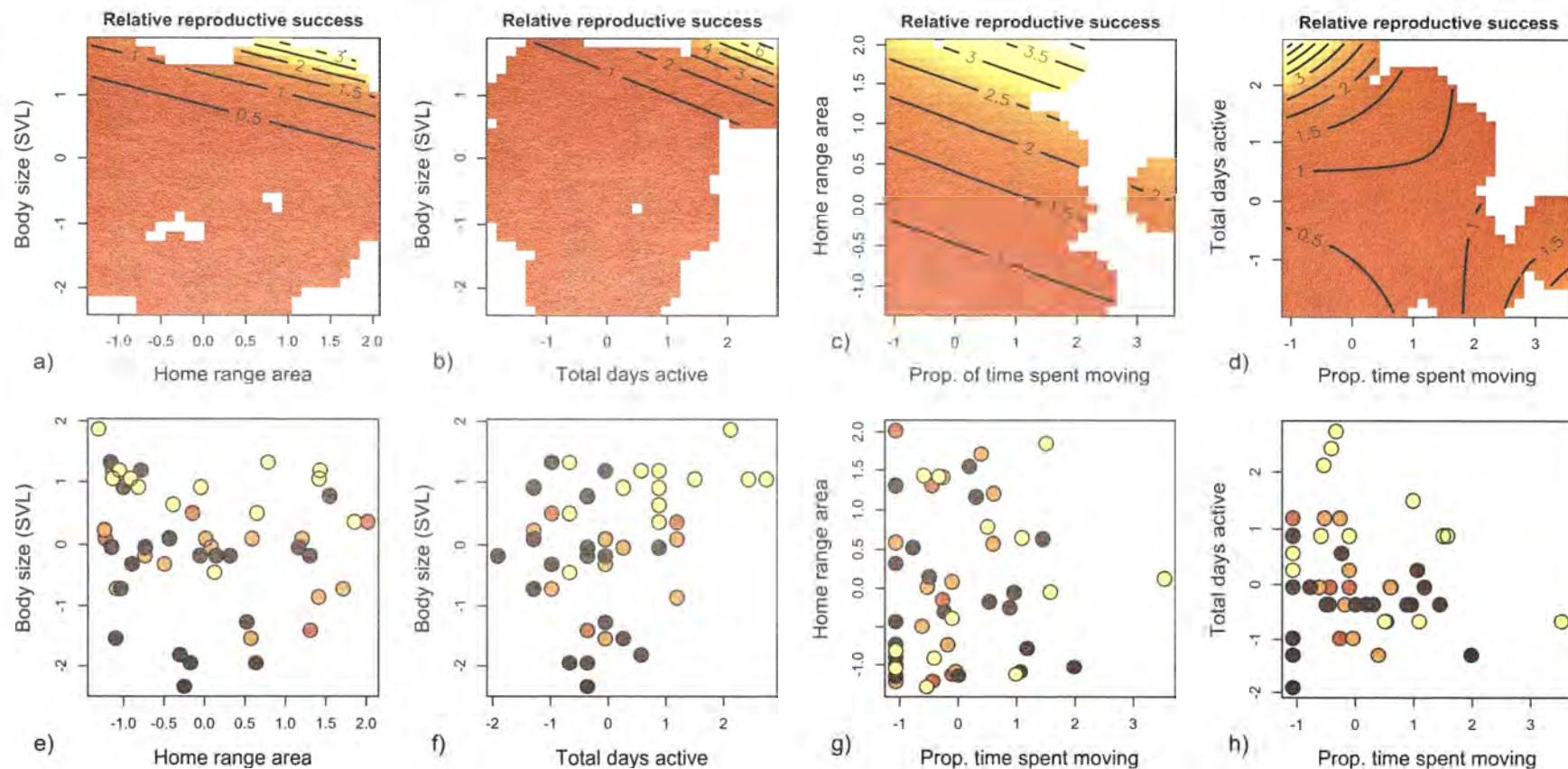
**Table 3** – Standardized selection gradients from GLMs (Tweedie probability distribution; log link) from our two top supported models of relative reproductive success as a function of the standardized traits. Models can be found in Table 1,  $n = 49$  analysis.

Variable (standardized trait)	Model 1				Model 2			
	$\beta/\gamma_{ij}$	SE	t – value	p – value	$\beta$	SE	t – value	p – value
Body size	1.35	0.25	5.42	<0.001	1.23	0.24	5.19	<0.001
Home range area (HRA)	0.46	0.16	2.92	<0.001	0.41	0.15	2.68	0.01
Total days active (TOTDAY)	0.45	0.13	3.50	<0.001	0.49	0.13	3.74	<0.001
Prop. time spent moving (MOVE)	0.11	0.16	0.71	0.48	0.13	0.17	0.77	0.44
TOTDAY*MOVE	– 0.26	0.15	– 1.69	0.09	NA	NA	NA	NA

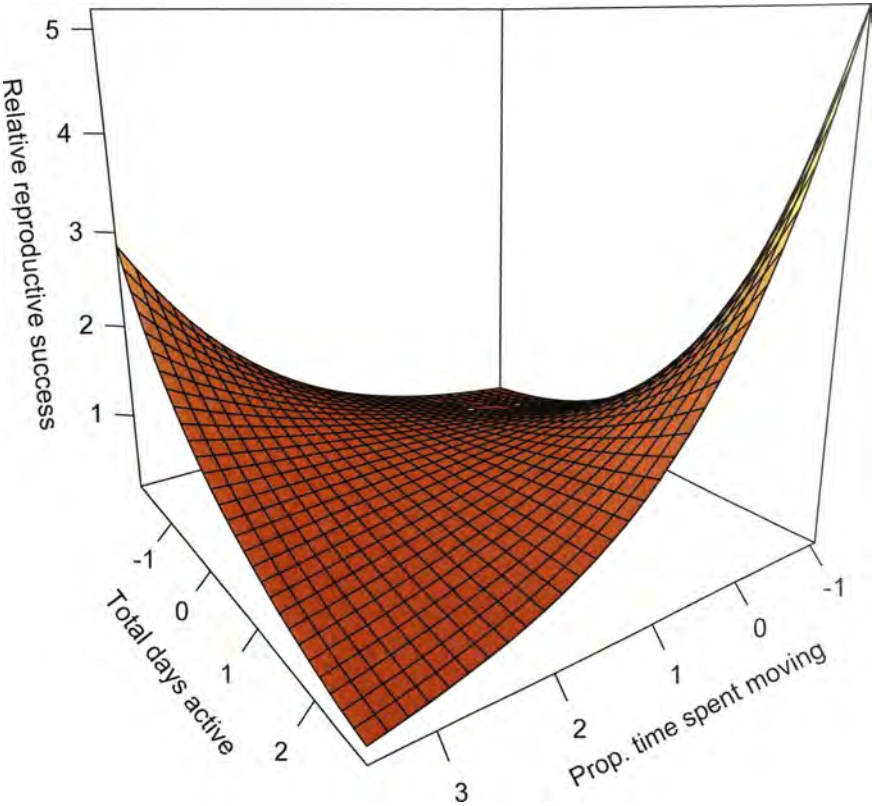


**Figure 2** – Relationships between relative reproductive success and each of our standardized traits. a) male body size (snout-vent length; SVL); b) total days a male was observed active; c) home range area (minimum convex polygon); and d) the proportion of time a male spent moving in a 10 minute focal sample.

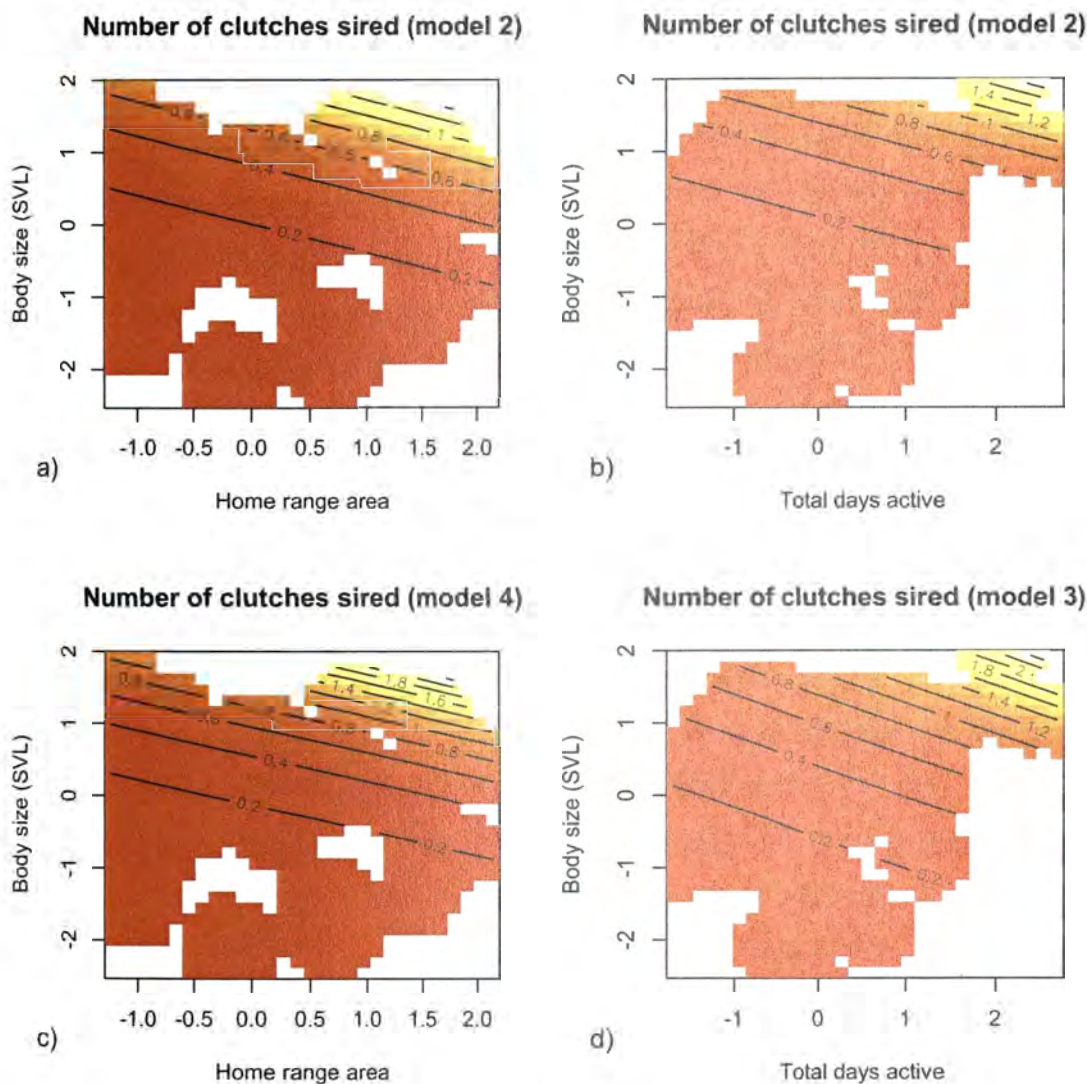




**Figure 3** – (a – d) Predicted parametric contour plots of relative reproductive success as a function of our standardized traits from GLMs with a Tweedie error distribution and log link function. Contours are predicted where data are available and controls for other influential parameters in the model. (e – h) Raw data for multiple predictor variables plotted together. Colors indicate the relative reproductive success of individual lizards: ‘dark red’ – no offspring; ‘red’ – relative reproductive success of 1 and below; ‘orange’ – relative reproductive success of greater than 1 and less than 2; ‘yellow’ – relative reproductive success that is greater than 2.



**Figure 4** – Predicted surface plot from parametric GLM model of relative reproductive success and standardized traits (total days active and proportion of time spent moving) while controlling for other variables in the model. Surface plots are extrapolated over the entire trait space and offers a theoretical expectation of an individual’s reproductive success if all possible trait combinations were observed.



**Figure 5** - Predicted parametric contour plots of the number of clutches sired as a function of our standardized traits from our top two supported GLMs (Tweedie distribution; log link) (model 4 & 3; c & d) and a model with both home range and days active (model 2; a & b). Plots explore the predicted number of clutches sired as a function of body size (SVL) and either total days active or home range area. We only predicted areas of the fitness surface that were close to existing data.



**Table 4** – Candidate models evaluated based on standardised predictor variables (mean = 0; sd = 1) and the number of clutches sired as the dependent variable. Results are presented from two separate analyses with different sample sizes. The number of model parameters, sample size corrected Akaike information criterion ( $AIC_C$ ), the difference in  $AIC_C$  between the top model and each candidate model ( $\Delta AIC_C$ ), Akaike weights ( $w_i$ ) and adjusted  $R^2$  ( $Adj R^2$ ) for each model are also presented. The set of predictors in each of the candidate models are listed in Table 3 under the “Number of clutches sired”. The Tweedie parameter is listed for each analysis and refers to the best-supported parameter for each of the analyses. Bolded models indicate models that are within 2  $\Delta AIC_C$  units of each other and have equal support.

Candidate Model	k	Adj R <sup>2</sup>	Number of clutches sired							
			n = 49 – Tweedie <i>p</i> = 1.1				n = 56 – Tweedie <i>p</i> = 1.1			
			AIC <sub>C</sub>	ΔAIC <sub>C</sub>	<i>w<sub>i</sub></i>	Adj R <sup>2</sup>	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	<i>w<sub>i</sub></i>	
Model 1	15	0.64	164.08	22.3	0.0000	0.52	177.97	25.2	0.0000	
Model 2	9	0.61	144.47	2.69	0.1091	0.51	157.43	4.66	0.0446	
Model 3	<b>8</b>	<b>0.39</b>	<b>141.78</b>	<b>0</b>	<b>0.4186</b>	0.36	<b>152.77</b>	<b>0</b>	<b>0.4580</b>	
Model 4	<b>8</b>	<b>0.59</b>	<b>142.52</b>	<b>0.74</b>	<b>0.2892</b>	0.50	<b>153.46</b>	<b>0.69</b>	<b>0.3244</b>	
Model 5	10	0.59	147.77	5.99	0.0209	0.50	160.58	7.81	0.0092	
Model 6	9	0.60	145.70	3.92	0.0590	0.52	156.59	3.82	0.0678	
Model 7	9	0.43	145.43	3.65	0.0675	0.39	156.14	3.37	0.0849	
Model 8	11	0.63	150.53	8.75	0.0053	0.49	163.86	11.09	0.0018	
Model 9	10	0.63	147.02	5.24	0.0305	0.50	160.58	7.81	0.0092	

**Table 5** – Parameters important in predicting the number of clutches a male sired. Parameters are presented for the top supported models (3 & 4) and model 2 with all parameters in the same model.

Parameter	Model 3			Model 4			Model 2		
	Estimate	SE	p – value	Estimate	SE	p – value	Estimate	SE	p – value
Body size	0.75	0.20	<0.001	1.10	0.21	<0.001	0.94	0.21	<0.001
Total days active	0.28	0.15	0.07	NA	NA	NA	0.28	0.14	0.04
Home range area	NA	NA	NA	0.40	0.14	<0.01	0.40	0.14	<0.01



## DISCUSSION

By quantifying male behavior in semi-natural enclosures, in combination with paternity testing of a known population, we provide evidence for directional selection on both behavioral and morphological traits related to reproductive success. Our analysis also suggests that disruptive correlational selection may also play an important role in shaping the suites of behavioral traits males adopt in this system. Models with correlational selection gradients were almost equally supported in our candidate set and predictions from these models supported combinations of traits that are predicted to define alternative reproductive tactics. We showed that males with higher reproductive success also sired offspring across more females and there was equal support for models containing a positive effect for total days active and home range area on the number of clutches sired. Model predictions regarding how these traits influence the number of clutches sired were also in accordance with ART predictions. Interestingly, home range area had a larger estimate in models with clutch number while total days active had a larger estimate in models predicting offspring number controlling for body size effects.

The contributions of different behavioral and morphological attributes to male reproductive success are seldom addressed in a single statistical framework. This is particularly important with respect to understanding the independent contributions of a male's phenotype to his reproductive success and elucidates the pre-copulatory mechanisms contributing to paternity outcome. We found that male body size, total days active and home range area were strong independent predictors of male reproductive success. In *E. quoyii*, large body size positively affected male reproductive success and these results are congruent with a wide range of organisms, which show body size effects on reproductive success (Andersson 1994 and references therein). Body size is an important determinant of contest outcome and dominance status (Andersson 1994; Rowland 1989), with larger males often gaining access to higher quality territories or larger groups of females (Andersson 1994; Baird et al. 2007; Wells 1977). Larger *E. quoyii* are known be more dominant then smaller ones (Done and Heatwole 1977). This may explain why larger males achieve higher reproductive success; however, we do not yet understand the reasons for dominance or territoriality in *E. quoyii* and future work

will be necessary to understand how large males, using potentially different reproductive tactics, monopolize female mating opportunities.

Interestingly, labile behavioral traits may be more important to male reproductive success in systems where overt aggression is low (Baird et al. 2007). In their study on the mating success of male collard lizards (*Crotaphytus collaris*), Baird et al. (2007) found that patrol rate (total distance travelled divided by the observation time), territory area and distant displays (total number of displays divided by observation time) were all more important predictors than morphological variables to male mating success. They suggest that this may be due to the lower occurrence of aggression between males in their population (Baird et al. 2007). Even though *E. quoyii* are aggressive, we found evidence for important roles for movement rates, home range size and total days active to male reproductive success. Home range size and presumably movement rates are likely key determinants of male reproductive success when females are spatially and temporally dispersed (Emlen and Oring 1977) because males capable of covering larger areas are expected to interact with a larger number of sexually receptive females increasing their chances of mating. Indeed, our data support home range area as a behavioral trait that is important for mate acquisition since male *E. quoyii* with larger home range areas sired offspring across more clutches, suggesting that these lizards interact and copulate with more females. However, males that spend more time active during the breeding season are also expected to mate with more females, particularly if females are temporally variable in their receptivity or spatially clumped (Emlen and Oring 1977). Staying active longer, particularly with aggregations of females may also allow males to copulate more frequently with females, giving them an advantage in post-copulatory sexual selection. We found evidence that total days active also positively influenced the number of clutches a male sired because models with total days active were equally supported to models with home range area. Interestingly, comparing the strength of estimated coefficients between total days active and home range area between models of offspring number and clutch number suggests that total days active has a larger effect on the number of offspring sired, while home range area has a larger effect on the number of clutches sired. Although these differences are small this finding suggests that males that are observed frequently and those with large home range areas may elevate their reproductive success in two subtly different ways. Males remaining active longer may mate more frequently and/or prevent females from mating with other males. Indeed, resident *E. heatwolei* are more likely to sire offspring with resident females (Stapley

and Keogh 2005) and the proportion of females choosing to remain sedentary may have important consequences for their reproductive success. In contrast, males with larger home ranges probably copulate with spatially dispersed females and it does appear they are mating with more females than 'resident-like' males, however, they are not siring as many offspring within these clutches. Although a plausible hypothesis, this will require further testing.

*Is there evidence for correlational disruptive selection on behavioral traits?*

Disruptive selection has been championed as a major force generating and maintaining phenotypic diversity in populations and may play a major role in speciation (Reufferer et al. 2006). Recent advances in multivariate statistics have allowed for greater insight into the modes of selection in natural populations and studies identifying both correlational and disruptive selection have increased over the years (Bolnick and Lau 2008; Brodie III 1992; Calsbeek and Smith 2007; Hendry et al. 2009; Kingsolver et al. 2001; McGlothlin et al. 2005; Sinervo and Svensson 2002). However, measurements of selection on phenotypic traits have been biased, with greater than 90% of selection estimates based on morphological and life-history traits (Kingsolver et al. 2001). We provide evidence for correlational disruptive selection acting on behavioral traits that are in accordance with phenotypic axes of ARTs in water skinks. To our knowledge, we provide the first evidence for such patterns. Large male lizards with high and low movement rates that are observed rarely or often, respectively, are predicted to achieve higher reproductive success when controlling for all other variables. This supports previous work in the related *E. heatwolei*, which defined and categorized ARTs using residency and the total days active (Morrison et al. 2002; Stapley and Keogh 2005) and which has shown that resident and floater males differ in their propensity to move in the lab (Stapley and Keogh, 2004). We did not find strong evidence for interactions between other behavioral traits we hypothesized to be part of the male phenotypes achieving high reproductive success; however, their coefficients were in the predicted direction and it is possible that our sample size was insufficient to detect these effects as they may be much weaker (Brodie III 1992; Brodie III et al. 1995).

In general, our selection estimates were large compared to many reported estimates in the literature (Kingsolver et al. 2012; Kingsolver et al. 2001). This likely reflects the smaller sample sizes used in our study, which can bias selection estimates upwards. However, such

large selection estimates are also the result of strong sexual selection in this system as there was high variance in male reproductive success (Kingsolver et al. 2012). Although larger sample sizes will be necessary to be completely confident about the strength of correlational selection gradients and to clarify model uncertainty, our data does suggest interactions between behavioral traits are important in this system, particularly given that we detected evidence for it with relatively small sample sizes. Importantly, we also found that predictions from our statistical models that included biologically plausible interactions were in agreement with what we predicted from our verbal models based on previous studies (Keogh et al. 2012; Morrison et al. 2002; Stapley and Keogh 2004; Stapley and Keogh 2005). Nonetheless, this needs to be interpreted with caution because strong directional selection can also drive significant correlational selection gradients (Brodie III et al. 1995; Lande and Arnold 1983; Phillips and Arnold 1989) and may be responsible for variation among males in the tactics they adopt to acquire paternity. This may be the case given that directional selection gradients predict phenotypes that are also in line with the traits predicted to characterize alternative tactics [i.e. strong directional selection for large home range (floater) but many days active (resident)]. This may be an alternative explanation for the patterns we observed and it is possible it might lead to somewhat ‘discrete’ variation in populations, particularly if constraints exist on what phenotypes males can adopt. For example, energetic constraints may limit the behavioral repertoire of individual males and a trade-off may exist between remaining active for long periods and moving a lot over a large area. Selection may favor males adopting these different behavioral phenotypes, as they appear to be alternative mechanisms for acquiring paternity.

#### *Implications for our Understanding of ARTs*

Understanding the evolutionary dynamics between alternative reproductive tactics requires data on each tactic’s relative fitness (Austad 1984; Gross 1996; Shuster 2010; Taborsky et al. 2008). Our results provide interesting possibilities with respect to understanding the relative fitness of ARTs in *E. quoyii* because behavioral traits in ectotherms are strongly influenced by environmental conditions during mating (Olsson et al. 2011). Long-term studies in *Lacerta agilis* have shown the incidence of multiple paternity to be higher in warmer years and the authors attribute these changes to an increase in mate encounter rates



and increased male activity (Olsson et al. 2011). Since alternative reproductive tactics in *E. quoyii* are linked to activity related behavioral traits it is reasonable to predict that environmental variation may effect the relative fitness of ARTs and highlights the importance of environmental variation to the relative fitness of alternative tactics, which has been highlighted by previous authors (Shuster 2010; Taborsky 1998).

In summary, we provide evidence for strong directional selection on behavioral traits predicted to be important for male reproductive success in *E. quoyii* and our models suggest that correlational disruptive selection may also be acting on large males to potentially promote the evolution of alternative male mating tactics. Our study highlights how morphological and labile behavioral traits may interact in complex ways to create a fitness landscape, which might promote the evolution of alternative male mating tactics in systems where there are no obvious morphological differences between tactics. Testing the generality of this finding and understanding the behavioral mechanisms generating ARTs will be a fruitful avenue for future research.

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## SUPPLEMENTARY MATERIALS

### Materials and Methods

#### *Model selection validation and influential points*

We plotted the residuals from our final models against each of the predictor variables to look for patterns in the residuals. We also checked that residuals were centered on the mean and that there were no patterns in the residuals when plotted against fitted values. We also checked for outliers or influential points in the final models. There was evidence that some points were influential in our analysis and we explored how removal of these data points affected our model selection procedures and inferences. Visual plots (fig. 4h) suggest that male 151 had the highest movement rates and appeared to have higher reproductive success (relative reproductive success for this male was 2.25). Exclusion of this male removes the fitness peak for model predictions of high reproductive success for males that are observed little but which move a lot. However, models containing an interaction between days active and movement were still equally supported compared with only main effect models ( $AIC_C$  of models: Main effects,  $AIC_C = 143.74$ ; Interaction model,  $AIC_C = 145.19$ ;  $\Delta_{IC} = 1.45$ ). Model residuals also indicated that male 48 was a highly influential point in most analyses. However, exclusion of this male did not affect our results (table S1).

Although GLMs make no assumption regarding the distribution of predictor variables, strongly skewed distributions can lead to the presence of highly influential points. In addition to exploring how our inferences were affected by removing influential points we transformed our predictor variables to normalized quantiles to reduce the influential affects these distributions may have on model fitting. Traits were first rank transformed and then we used these ranks to extract quantiles from a normal probability distribution with a mean = 0 and standard deviation = 1. Our normalized quantiles were strongly correlated with our original traits (Pearson's  $r = 0.79 - 0.99$ ) however extreme distributions were no longer a concern. Furthermore, transforming in this way ensured that all variables were standardized around a distribution with a mean of 0 and standard deviation of 1. Unfortunately, interpreting the quantiles based on trait values is no longer possible, however, we compared our analyses using

normalized quantiles with those from our selection analysis to ensure that there was similar support for models. In all cases there was similar support for the same top models (table S2).

**Table S1** – Candidate model selection after removing influential points from analysis. In all analyses male 48 was consistently influential. This point was removed and models were re-run to check that it did not change the final results. With its removal evidence for correlational disruptive selection was stronger in all cases.

Relative Reproductive Success									
Candidate model	k	Adj R <sup>2</sup>	n = 48 – Tweedie p = 1.1			n = 55 – Tweedie p = 1.1			
			AIC <sub>C</sub>	ΔAIC <sub>C</sub>	w <sub>i</sub>	Adj R <sup>2</sup>	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	w <sub>i</sub>
Model 1	11	<b>0.75</b>	<b>136.51</b>	<b>0</b>	<b>0.5328</b>	0.62	160.79	2.5	0.1353
Model 2	10	<b>0.69</b>	<b>138.66</b>	<b>2.15</b>	<b>0.1818</b>	0.60	161.86	3.57	0.0792
Model 3	12	0.75	139.56	3.05	0.1159	<b>0.71</b>	<b>159.39</b>	<b>1.1</b>	<b>0.2724</b>
Model 4	11	0.72	140.47	3.96	0.0736	<b>0.70</b>	<b>158.29</b>	<b>0</b>	<b>0.4722</b>
Model 5	12	0.74	140.23	3.72	0.0829	0.62	163.50	5.21	0.0349
Model 6	13	0.75	143.98	7.47	0.0127	0.61	167.10	8.81	0.0058
Model 7	15	0.74	152.77	16.26	0.0002	0.59	174.90	16.61	0.0001
Model 8	16	0.74	157.48	20.97	0.0000	0.58	179.07	20.78	0.0000
Model 9	17	0.77	161.54	25.03	0.0000	0.69	179.26	20.97	0.0000
Model 10	1	NA	277.97	141.46	0.0000	NA	319.75	161.46	0.0000

Number of Offspring (Absolute Fitness)									
Candidate model	k	Adj R <sup>2</sup>	n = 48 – Tweedie p = 1.1			n = 55 – Tweedie p = 1.1			
			AIC <sub>C</sub>	ΔAIC <sub>C</sub>	w <sub>i</sub>	Adj R <sup>2</sup>	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	w <sub>i</sub>
Model 1	11	<b>0.75</b>	<b>174.81</b>	<b>0</b>	<b>0.4356</b>	0.62	200.97	5.01	0.0423
Model 2	10	<b>0.71</b>	<b>175.70</b>	<b>0.89</b>	<b>0.2792</b>	0.60	200.96	5	0.0425
Model 3	12	0.76	177.85	3.04	0.0953	<b>0.73</b>	<b>196.59</b>	<b>0.63</b>	<b>0.3778</b>
Model 4	11	0.74	177.47	2.66	0.1152	<b>0.72</b>	<b>195.96</b>	<b>0</b>	<b>0.5177</b>
Model 5	12	0.75	178.60	3.79	0.0655	0.63	202.84	6.88	0.0166
Model 6	13	0.74	182.55	7.74	0.0091	0.62	206.26	10.3	0.0030
Model 7	15	0.73	191.33	16.52	0.0001	0.61	213.81	17.85	0.0001
Model 8	16	0.75	195.71	20.9	0.0000	0.62	217.47	21.51	0.0000
Model 9	17	0.79	199.79	24.98	0.0000	0.76	214.52	18.56	0.0000
Model 10	1	NA	321.15	146.34	0.0000	NA	362.51	166.55	0.0000



**Table S2-** Candidate models evaluated based on normalised quantiles of predictor variables (mean = 0; sd = 1) and relative reproductive success and the number of offspring (absolute fitness) as dependent variables. Results are presented from two separate analyses with different sample sizes. The number of model parameters, sample size corrected Akaike information criterion ( $AIC_C$ ), the difference in  $AIC_C$  between the top model and each candidate model ( $\Delta AIC_C$ ), Akaike weights ( $w_i$ ) and adjusted  $R^2$  ( $Adj R^2$ ) for each model are also presented. The set of predictors in each of the candidate models are listed in Table 3 of the main manuscript under “Relative and Absolute Reproductive Success”. The Tweedie parameter is listed for each analysis and refers to the best-supported parameter for each of the analyses. Bolded models indicate models that are within 2  $\Delta AIC_C$  units of each other and have equal support.

Relative Reproductive Success									
Candidate model	k	Adj $R^2$	n = 49 – Tweedie p = 1.1			n = 56 – Tweedie p = 1.1			
			$AIC_C$	$\Delta AIC_C$	$w_i$	Adj $R^2$	$AIC_C$	$\Delta AIC_C$	$w_i$
Model 1	11	<b>0.81</b>	<b>143.84</b>	<b>0.00</b>	<b>0.42</b>	0.69	165.91	4.42	0.0594
Model 2	10	<b>0.73</b>	<b>145.09</b>	<b>1.25</b>	<b>0.23</b>	0.64	167.47	5.98	0.0272
Model 3	12	0.82	145.92	2.08	0.15	<b>0.79</b>	<b>161.49</b>	<b>0.00</b>	<b>0.5413</b>
Model 4	11	0.77	146.32	2.48	0.12	<b>0.74</b>	<b>162.39</b>	<b>0.90</b>	<b>0.3451</b>
Model 5	12	0.80	147.40	3.56	0.07	0.68	167.83	6.34	0.0227
Model 6	13	0.79	151.32	7.48	0.01	0.67	171.35	9.86	0.0039
Model 7	15	0.79	157.89	14.05	0.00	0.66	177.73	16.24	0.0002
Model 8	16	0.79	162.40	18.56	0.00	0.68	181.20	19.71	0.0000
Model 9	17	0.81	166.28	22.44	0.00	0.77	178.39	16.90	0.0001
Model 10	1	NA	283.97	140.13	0.00	NA	325.13	163.64	0.0000

Number of Offspring (Absolute Fitness)									
Candidate model	k	Adj $R^2$	n = 49 – Tweedie p = 1.1			n = 56 – Tweedie p = 1.1			
			$AIC_C$	$\Delta AIC_C$	$w_i$	Adj $R^2$	$AIC_C$	$\Delta AIC_C$	$w_i$
Model 1	11	<b>0.81</b>	<b>178.83</b>	<b>0.00</b>	<b>0.37</b>	0.68	203.63	5.11	0.0395
Model 2	10	<b>0.76</b>	<b>179.46</b>	<b>0.63</b>	<b>0.27</b>	0.65	204.73	6.21	0.0228
Model 3	12	0.82	180.95	2.12	0.13	<b>0.79</b>	<b>198.52</b>	<b>0</b>	<b>0.5081</b>
Model 4	11	0.79	180.40	1.57	0.17	<b>0.76</b>	<b>198.95</b>	<b>0.43</b>	<b>0.4098</b>
Model 5	12	0.80	182.47	3.64	0.06	0.68	205.39	6.87	0.0164
Model 6	13	0.79	186.42	7.59	0.01	0.67	208.69	10.17	0.0031
Model 7	15	0.80	193.06	14.23	0.00	0.66	214.49	15.97	0.0002
Model 8	16	0.80	197.42	18.59	0.00	0.69	217.04	18.52	0.0000
Model 9	17	0.83	201.53	22.70	0.00	0.79	214.33	15.81	0.0002
Model 10	1	NA	336.96	158.13	0.00	NA	377.48	178.96	0.0000

**Table S3** – Candidate model selection based on a larger sample of complete data for home range area and total days active. Note that this analysis *does not* contain the proportion of time spent moving and thus does not control for the effects of moving. The models are based on a sample of 66 individuals, which had complete home range and days active information. Only individuals with at least 8 or more sightings had home range estimates. The number of model parameters, sample size corrected Akaike information criterion ( $AIC_C$ ), the difference in  $AIC_C$  between the top model and each candidate model ( $\Delta AIC_C$ ), Akaike weights ( $w_i$ ) and adjusted  $R^2$  ( $Adj R^2$ ) for each model are also presented. The set of predictors in each of the candidate models are listed in Table S4 of the main manuscript under “Relative and Absolute Reproductive Success”. The Tweedie parameter is listed for each analysis and refers to the best-supported parameter for each of the analyses. Bolded models indicate models that are within 2  $\Delta AIC_C$  units of each other and have equal support.

Candidate models	Relative reproductive success				
	n = 66 – Tweedie = 1.3				
	k	Adj R <sup>2</sup>	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	w <sub>i</sub>
Model 1	9	0.19	235.75	4.68	0.0469
Model 2	10	0.17	235.90	4.84	0.0433
<b>Model 3</b>	<b>10</b>	<b>0.26</b>	<b>231.06</b>	<b>0.00</b>	<b>0.4866</b>
<b>Model 4</b>	<b>11</b>	<b>0.24</b>	<b>231.79</b>	<b>0.73</b>	<b>0.3378</b>
Model 5	13	0.32	234.54	3.48	0.0854

**Table S5** – Model coefficients for analysis with n = 66 for home range area and total days active for both the main effects model (model 3) and the interaction model (model 4) (Table S3 & S4).

Parameter	Model 3				Model 4			
	Estimate	SE	t-value	p-value	Estimate	SE	t-value	p-value
Body size	0.67	0.19	3.50	<0.001	0.67	0.19	3.54	<0.001
Condition	0.36	0.15	2.40	0.02	0.35	0.15	2.34	0.02
Home range area	0.10	0.17	0.58	0.56	0.20	0.19	1.10	0.28
Total days active	0.33	0.15	2.18	0.03	0.31	0.15	2.04	0.05
HRA*TOTDAY	NA	NA	NA	NA	−0.19	0.15	−	0.21
							1.30	

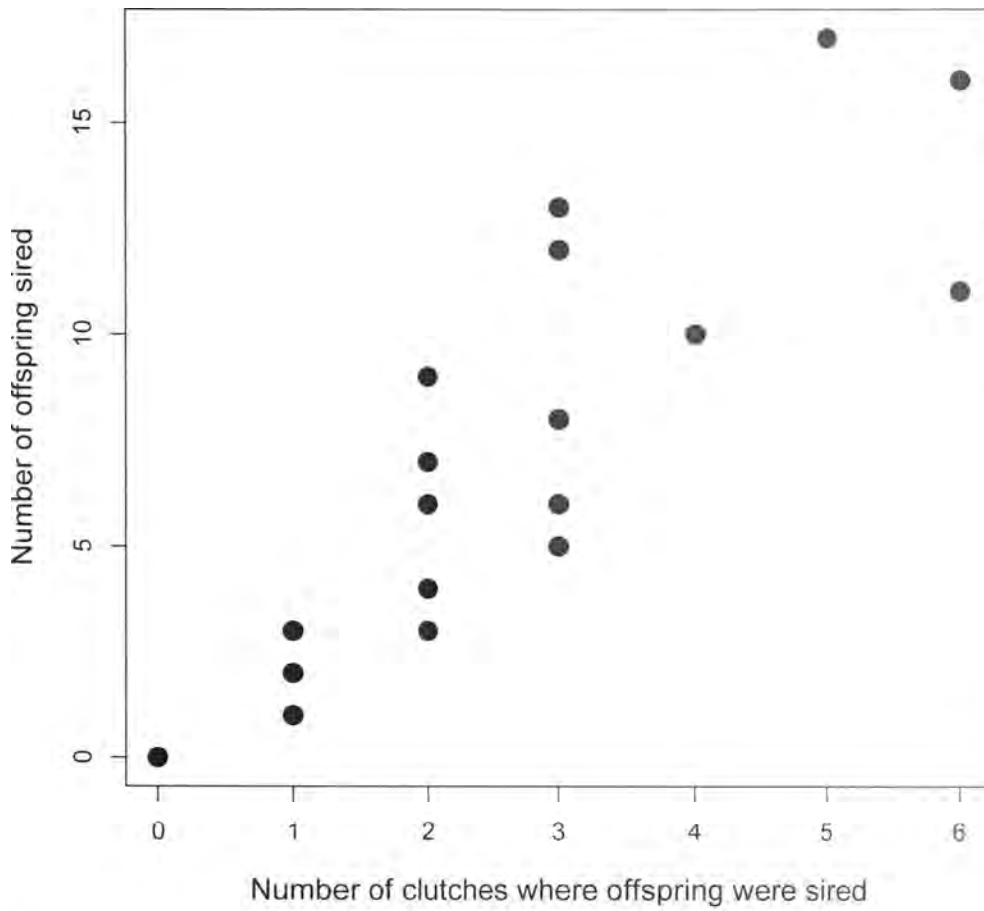
**Table S6** – Full model of standardized selection gradients from GLMs (Tweedie error distribution; log link) of relative reproductive success as a function of standardized (mean = 0, sd = 1) traits using the predictors variables found in the best-supported model in Table 1 of manuscript. Linear selection gradients are taken from a model without quadratic and correlational selection gradients whereas quadratic and correlation selection gradients are taken from the full model. Quadratic gradients and their standard errors are doubled. Note that enclosure is included in the model, but not shown for simplicity.

Variable (standardized trait)	$\beta/\gamma_{ii}/\gamma_{ij}$	Standard error	t - value	p - value
Body size	1.23	0.24	5.19	<0.001
Home range area (HRA)	0.41	0.15	2.68	0.01
Total days active (TOTDAY)	0.49	0.13	3.74	<0.001
Prop. time spent moving (MOVE)	0.13	0.17	0.77	0.44
HRA <sup>2</sup>	- 0.32	0.42	- 0.74	0.47
TOTDAY <sup>2</sup>	- 0.10	0.26	- 0.41	0.69
MOVE <sup>2</sup>	- 0.08	0.24	- 0.08	0.71
TOTDAY*MOVE	- 0.32	0.21	- 1.52	0.14
TOTDAY*HRA	- 0.01	0.16	- 0.04	0.97
MOVE*HRA	0.13	0.20	0.66	0.52



**Figure S1** - An example of one of the six experimental enclosures used in this study. For more photographs of the lizard enclosures see [http://whitinglab.com/?page\\_id=75](http://whitinglab.com/?page_id=75).





**Figure S2** - Relationship between the number of offspring produced and the number of clutches in which a male sired offspring.

## **CHAPTER 6: SEX, TESTOSTERONE AND PERFORMANCE IN AN AUSTRALIAN LIZARD WITH SEXUALLY DIMORPHIC BODY SHAPE BUT NOT SIZE**

Daniel W.A. Noble, Kerry V. Fanson and Martin J. Whiting

Under Review

### **Abstract**

Understanding underlying physiological differences between the sexes in circulating testosterone (T) and how hormonal variation affects morphology-performance relationships may help inform on the evolution of sexual dimorphism in diverse taxa. Using a widely distributed Australian lizard (*Eulamprus quoyii*) with weak sexual dimorphism and no dichromatism we tested whether circulating plasma T differed between the sexes and whether variation in plasma T covaried with morphological and performance traits (bite force, sprint speed, endurance). Males had larger head dimensions, bite force, sprint speed and endurance compared to females. We found that the sexes did not differ in circulating T and that it was weakly associated with both morphological and performance traits, except for sprint speed. Plasma T was associated with decreased sprint speed in both males and females. Interestingly, high circulating T showed a non-linear relationship with bite force in males and not females and this relationship may be related to alternative male reproductive tactics in this species. Our results suggest that T does not directly affect performance or morphology, although it may play an important organizational role during the development of morphological traits, which could explain the differences in morphology and thus performance between the sexes. Differences in performance between the sexes suggest differential selection on these functional traits between males and females. We argue that greater focus on sexually monomorphic species will provide a unique opportunity to explore androgen-morphology-performance relationships and their role in mediating sex differences.

## INTRODUCTION

Males and females of many species are sexually dimorphic, varying in size, shape and/or colour (Hedrick & Temeles, 1989; Andersson, 1994). Sexual dimorphism (SD) may be sufficiently pronounced that it exceeds differences in morphology among related species and therefore, may have important effects on an organism's behaviour, ecology and life history (Butler *et al.*, 2007). Sexual selection is typically invoked as the ultimate explanation for SD where intra-sexual competition leads to increased body size or elaborate ornamentation in males (Hedrick & Temeles, 1989; Andersson, 1994). In contrast, fecundity selection, where increased reproductive investment leads to selection for larger body size in females, may lead to males and females being similar in size (Andersson, 1994). SD may also arise through natural selection when the sexes occupy different ecological niches and thereby reduce competition over resources (Shine, 1989).

Although SD in morphological traits has been studied in numerous taxa (Hedrick & Temeles, 1989; Andersson, 1994), few studies have attempted to quantify and understand sex differences in performance traits (Lailvaux *et al.*, 2003; Lailvaux, 2007; Van Damme *et al.*, 2008) such as running speed, stamina, jumping performance and biting force. This is important given that performance traits are predicted to be more direct targets of selection (Arnold, 1983; Miles *et al.*, 2007b) and understanding inter-relationships between morphology and performance can therefore inform on the evolution SD. Although theoretically we would predict sex differences in performance given genetic and organizational differences between the sexes, this may not always be the case (Zajitschek *et al.*, 2012).

Individual variation in morphology is predicted to be related to performance through a complex path of cause and effect (Arnold, 1983; Miles *et al.*, 2007b). Androgens, such as testosterone (T), play a key role in mediating this interaction via their organizational and activational effects on morphology and performance (Figure 1). Androgens can indirectly affect performance by modifying growth rates and organizing the development of morphological traits, such as body size and shape (Crews *et al.*, 1998; Sinervo *et al.*, 2000; Cox & John-Alder, 2005; Sinervo & Calsbeek, 2006; Whiting *et al.*, 2006; John-Alder *et al.*, 2007; Miles *et al.*, 2007b; Husak & Irschick, 2009). Alternatively, androgens may mediate plastic changes to internal physiology and muscle mass and fiber type, which would lead to a more active and direct role in shaping variation in morphology and performance (Sinervo *et*

*al.*, 2000; Irschick *et al.*, 2006; Gowan *et al.*, 2010; Huyghe *et al.*, 2010). Testosterone is generally predicted to be higher in males compared with females (Sinervo *et al.*, 2000; Miles *et al.*, 2007b; Van Damme *et al.*, 2008; Huyghe *et al.*, 2010), and this widespread pattern may provide a proximate explanation for sex differences in performance by coordinating developmental and physiological changes that would give rise to performance differences. However, testosterone can have complex effects on both male and female morphology, growth and behaviour (Owens & Short, 1995; Crews *et al.*, 1998; Cox & John-Alder, 2005; John-Alder *et al.*, 2007), and recent work has challenged the general assumptions about sex differences in T. In several species, both circulating levels of T and the biological effects of T are similar for both sexes and this may lead to the sexes being similar in behaviour, morphology and performance (Adkins-Regan, 2005; Ketterson *et al.*, 2005; Hews *et al.*, 2012).

Much of our understanding about androgen-morphology-performance relationships come from systems where the sexes are markedly different in colour, size and shape (Whiting *et al.*, 2006; Huyghe *et al.*, 2010). We know much less about species with low levels of SD and whether they exhibit similar patterns. Studying sexually size dimorphic species can make understanding sex differences in performance difficult because body size often leads to isometric changes in suites of traits such as limb size, body mass, and lung volume (Van Damme *et al.*, 2008), which can be strongly related to performance capacity (Van Damme *et al.*, 2008). If males and females differ in their sensitivity to T and if some systems, notably those that show no clear sexual dimorphism have similar levels of circulating T, do we see similar differences in performance between the sexes?

Lizards are model systems for addressing questions on functional ecology because of the clearly developed methods for quantifying performance and our understanding of their relationships with fitness (Sinervo *et al.*, 2000; Sinervo & Zamudio, 2001; Lailvaux *et al.*, 2003; Vanhooydonck *et al.*, 2005; Husak, 2006; Sinervo & Calsbeek, 2006; Miles *et al.*, 2007b; Irschick *et al.*, 2008; Cox *et al.*, 2009). Even in light of this, few studies explicitly test for sex differences in performance (Lailvaux *et al.*, 2003; Lappin *et al.*, 2006; McBrayer & Anderson, 2007; see references in Miles *et al.*, 2007b; Zajitschek *et al.*, 2012). Of the studies that have, males generally perform better than females in performance traits such as bite force (Lappin *et al.*, 2006; McBrayer & Anderson, 2007), endurance (Van Damme *et al.*, 2008) and sprinting speed (Lailvaux *et al.*, 2003; Van Damme *et al.*, 2008), but this is not always the case (Lailvaux, 2007; Van Damme *et al.*, 2008; Zajitschek *et al.*, 2012) and may be related to



the differences in the organizational and activational roles of androgens as a result of differential selective pressure between the sexes.

Eastern Water Skinks (*Eulamprus quoyii*) are a model system to explore sex-dependent differences in androgen-morphology-performance relationships. This species is not sexually dichromatic and males and females do not differ in body size, but only in head and limb shape and body mass (Schwarzkopf, 2005). The similarity in size between the sexes and the lack of sexual dichromatism provides a unique opportunity to examine testosterone-morphology-performance paradigm because body dimensions are independent of body size and any organizational (and possibly activational) effects of T among the sexes may be similar providing a unique natural manipulation of functional relationships. We were particularly interested in the following questions: 1) Do males and females differ in circulating testosterone levels and performance traits and does circulating testosterone have activational effects on head size and performance? 2) If so, does the relationship between testosterone and performance vary between the sexes? We tested hypothesized relationships between testosterone and its effects on morphology and performance (Fig. 1) along with whether sexual dimorphism in shape and mass mediate differences in three different performance traits (sprint speed, bite force and running endurance).

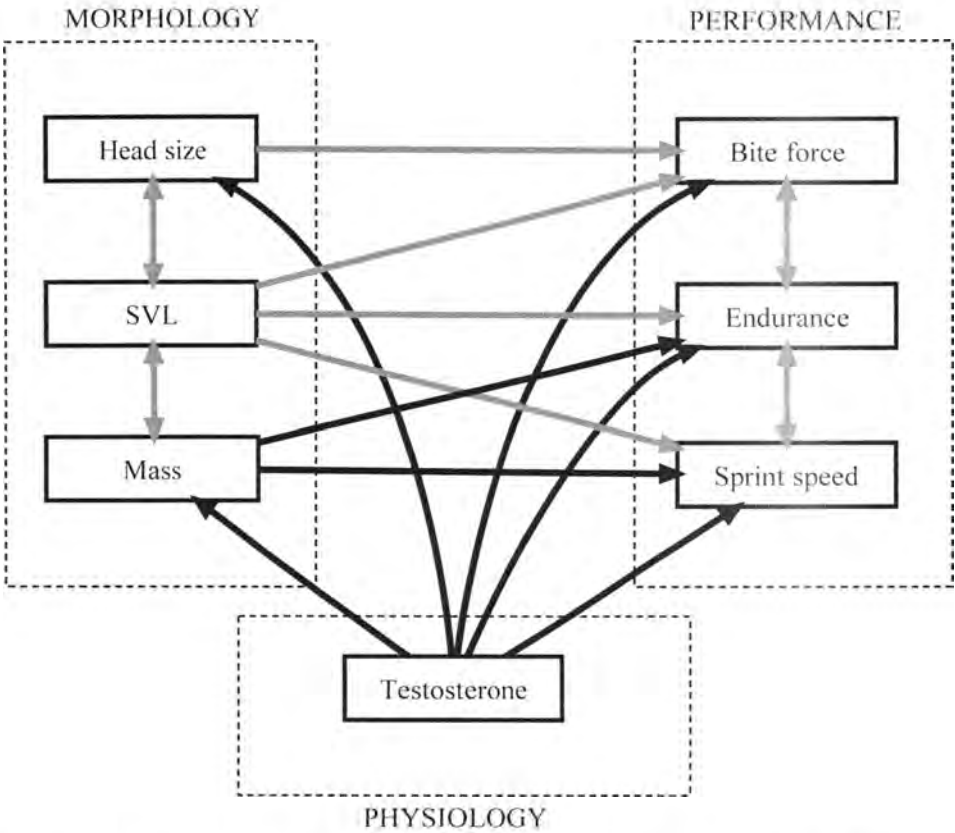


Figure 1 – Hypothesized relationships between testosterone, morphology (head size, snout-vent length [SVL], mass) and performance (bite force, sprint speed and endurance). Unidirectional arrows indicate that increases in one variable lead to corresponding increases in the connected variable. Gray arrows indicate that the relationships are predicted to be the same for both males and females, while black arrows indicates that relationships will either be stronger or only exist among males

## MATERIALS AND METHODS

### *Lizard collection*

We collected 216 adult *E. quoyii* from five sites in the Sydney region (see Noble *et al.*, 2013a for details). Within minutes (mean =  $5.26 \pm 0.45$  min; median = 4 min,  $n = 201$ ) of capture we took an ~30–50  $\mu$ L blood sample for hormone analysis. Blood was placed on ice until it could be processed in the lab. Lizards were then brought back to the laboratory at Macquarie University where they were sexed by checking for the presence of hemipenes. We measured body dimensions (snout-vent length [SVL], inter-limb length) body mass, tail length and head dimensions (head width, head length, head depth). Lizards were then transferred to plastic bins [Keiji; 487 mm (L) x 350 mm (W) x 260 mm (H)] in a temperature-controlled room for three days to measure whole-organism performance traits. Each bin had a hide box and a water bowl with newspaper as a substrate. Lizards were maintained at ambient temperatures of ~22–26°C with an elevated basking side of ~28–30°C. Ultraviolet lighting and water were provided at all times and lizards were fed once during the three days with crickets or mealworms.

### *Performance measurements*

Lizard bite force, sprint speed, and endurance were measured daily over three consecutive days. The order of each performance measurements was as follows: 1) bite force, 2) sprint speed and 3) endurance. Prior to all measurements lizards were heated to their preferred body temperature (~28°C; Law & Bradley, 1990) by placing lizards in plastic zip-lock bags and floating them in warm water that had been heated to approximately 34°C. For each lizard we recorded the time lizards began performance trials and the body temperature of lizards before the start (prior to bite force) and at the end (after endurance measurements) of performance measurements using a Miller-Weber® cloacal thermometer. Given the large number of lizards tested it was not possible for the same person to record all measurements and we compared whether there were inter-recorder differences in performance measurements across researchers

in our analysis. We used the maximal measurement for each performance variable in analyses (Losos *et al.*, 2002).

### *I. Bite force*

Bite force (N) was measured twice a day over three consecutive days for a total of six measurements per lizard. We took a second measurement of bite force after sprint speed because lizards appeared to be more motivated to bite after they had run. Bite force was measured using a Kistler force transducer (Kistler Inc., Winterthur Switzerland) that was connected to a Kistler charge amplifier (Model 5995, Kistler Inc.) We induced lizards to bite the two plates by gently pinching the sides of their mouth.

### *II. Sprint speed*

Sprint speed was measured directly after the first bite force measurement using a 2 m racetrack. The running surface of the racetrack was lined with rubber matting and at each 25 cm interval a white line indicated distance. A bucket was placed at the end of the racetrack, which the lizard fell into once the run was complete. Lizards were placed at the starting line and stimulated to run by tapping them gently on their tail-base. In most cases lizards ran continuously to the end of the finish line, with only occasional incidences of stops and reversals. We recorded sprinting lizards using a Panasonic HD video camera (30 fps) and quantified the speed of each of the three runs using MotionPro: Motion Analysis Software (<http://www.motionprosoftware.com/>).

### *III. Endurance*

Endurance was measured immediately following sprinting. Lizards were run on a modified human treadmill to measure their maximal endurance. A transparent plexi-glass box with adjustable compartments was placed on top of the treadmill. The middle compartment was adjusted so that the lizards could run unobstructed, while also ensuring that they could not easily turn around. All trails were run at a speed of  $1.0 \text{ km}\cdot\text{hr}^{-1}$ . Lizards were placed on the treadmill and were stimulated to run by gently tapping the base of their tail. After each tap of



the tail base we gave the lizard a few seconds to continue running. If the lizard was not stimulated to run we allowed the lizard to move closer towards the end of the treadmill and tapped the lizard again. We continued this until the lizard could no longer run at which point we allowed the lizard to fall into a container at the base of the treadmill. We then placed the lizard back on the treadmill and continued the same procedure until the lizard had fallen into the container three times, at which point we considered the lizard exhausted. Endurance was defined as the total time from when the lizard was placed on the treadmill to when it fell off the third time.

### *Testosterone assays*

We measured plasma testosterone (T) using a double-antibody enzyme-immunoassay (EIA). The antibody, biotinylated enzyme label, and testosterone standard were obtained from R. Palme (University of Veterinary Medicine, Vienna). The antibody (rabbit anti-testosterone, described by (Palme & Möstl, 1994) cross-reacted with the following steroids: testosterone 100%, 5  $\alpha$ -dihydrotestosterone (DHT) 24%, 5-DHT 12%, 4-androstene-3  $\beta$ ,17  $\beta$ -diol 8%, and 5  $\alpha$ -androstane-3  $\alpha$ ,17  $\beta$ -diol 6% (for further details see Hirschenhauser *et al.*, 1999). Assay sensitivity was 0.006 ng/mL. The assay was biochemically validated in our lab by demonstrating parallelism between a serially diluted plasma pool and the standard curve.

Assay procedures were similar to those previously described (Palme & Möstl, 1994; Touma *et al.*, 2003). Briefly, we coated microtiter plates (Nunc maxisorp) with 250  $\mu$ L of protein A solution (Sigma P7837, 2  $\mu$ g/mL) and incubated overnight at room temperature. Wells were emptied and blocked with 300  $\mu$ L of a second coating buffer for at least 3 h. Plates were washed and loaded in duplicate with 25  $\mu$ L of standard, control or diluted plasma sample (1:10 in phosphate buffer) before immediately adding 100  $\mu$ L of biotinylated label (working dilution = 1:5,000,000) and antibody (working dilution = 1:75,000) were added to the plate. Plates were incubated overnight at 4 °C while shaking. After washing, we added 250  $\mu$ L streptavidin-peroxidase conjugate (Sigma S2438, 0.2  $\mu$ g/ml) were added to each well. Plates were incubated for 45 min at 4 °C while shaking and then washed again. Substrate solution (250  $\mu$ L/well, tetramethylbenzadine (TMB), Sigma 87748, 66.7  $\mu$ g/ml) was added to the plate and incubated for 45 min as above. The enzymatic reaction was stopped by adding 50  $\mu$ L sulphuric acid (2 mol/L) to each well and absorbance was measured at a wavelength of 450

nm (reference filter = 630 nm) using a BioTek ELx808 microplate reader (BioTek Instruments, Winooski, VT). To monitor precision and reproducibility, low (~70% binding) and high (~30% binding) quality control samples were run on each plate. The intra-assay coefficient of variation (CV) was <15%, and the inter-assay CVs were 17.8% and 11.5% ( $n = 7$ ) for low and high controls, respectively. Samples that were too concentrated were re-run at a 1:100 dilution. For samples with low T concentrations, we extrapolated the concentration if the sample was just off the curve and assigned a concentration of 0 if the percent binding was > 105%.

### *Statistical analysis*

All data were analyzed using R 2.15.0 (R Development Core Team, 2010). Prior to analysis, we excluded lizards with no morphological data ( $n = 1$ ) and two lizards (one male and one female) that were extreme outliers in plots of body mass most likely due to measurement error. Due to insufficient plasma it was not possible to obtain T data for all lizards therefore for each of the analyses, we indicate the final sample size used throughout the paper.

First, we compared body size, inter-limb length, head dimensions (width, depth, length) and tail length between the sexes using generalized linear models (GLMs) with a Gaussian error distribution (identity link function). All morphological traits were log transformed prior to analysis. We included sex, SVL and an interaction between SVL and sex in all models to account for heterogeneity of slopes between the sexes. We compared models using information criteria approaches, using a sample size corrected Akaike information criteria (AICc) (Burnham & Anderson, 2002). We also calculated Akaike weights ( $w$ ) which are a measure of the probability of a model out of the candidate set (Symonds & Moussalli, 2011). For models within 2  $\Delta$ IC units of one another we chose the most parsimonious model even though models within 2  $\Delta$ IC units are considered equally plausible (Burnham & Anderson, 2002).

To test for sex differences in T, we used Quantile regression (Cade & Noon, 2003) and controlled for residual body mass, SVL, and collection date. Quantile regression is a semi-parametric regression technique that is suitable for highly skewed, non-normal response distributions given that it does not make assumptions about the error distribution and is robust to highly influential data points (Cade & Noon, 2003). To make coefficients more comparable, we mean centered and standardized each trait by dividing by 2 standard deviations prior to

analysis, making continuous and binary categorical predictors more comparable (Gelman, 2008).

Changes in muscle mass may be subject to activational effects of T, and may thereby cause plasticity in some morphological measurements, particularly head width and depth, and body mass. These traits have been shown to change through the season, presumably as a result of plasma T (Irschick *et al.*, 2006). To test whether circulating T directly affected head dimensions and body mass we added T to our top supported models for these morphological traits (above), controlling for collection date.

To test for sex differences in performance and examine whether morphological traits and circulating T explain variance in performance, we used GLMs with a Gaussian error distribution. Sprint speed, bite force and endurance were dependent variables and we log transformed maximal endurance to ensure normality of residuals prior to analysis. There was substantial collinearity between morphological traits, but because we were interested in understanding each trait's contribution to performance, we included residual mass and residual head width in our models. These were the residuals from a regression between log transformed mass/head width and SVL. We chose only to use head width for bite force models because it was strongly correlated with head length and depth and it appeared to explain the most variation. In our sprint speed and endurance models, we included main effects of SVL, residual mass, sex, testosterone (log transformed + 1), collection date, researcher and cloacal temperature at the time of performance. Since we were also interested in whether there might be differences in the relationship between testosterone, SVL, residual mass, speed and endurance between the sexes, we included interactions between these variables and sex. Males and females differed substantially in their head dimensions and attempts to model residual head width and sex together lead to strong collinearity between these parameters [variance inflation factors (VIFs) approaching 4]. Therefore, we decided to model bite force separately for males and females. In both male and female models we included SVL, residual mass, residual head width, testosterone, collection date, researcher and temperature as main effects.

In all models we ensured that residuals were normally distributed (visual inspection and using Shapiro-Wilks normality tests) and that there were no strongly influential data points. We also plotted residuals against each predictor to look for non-linearity. In cases where non-linearity was identified in continuous predictors we re-fitted models and included a quadratic term to test whether this improved the overall model fit. *Eulamprus* exhibit

alternative reproductive tactics and it is possible that some predictors are non-linearly related to performance measurements in males (Morrison *et al.*, 2002; Stapley & Keogh, 2004; 2005; Keogh *et al.*, 2012; Noble *et al.*, 2013b). If quadratics were included in models we compared the  $AIC_C$  between our main effects model and our new model and predicted performance measurements from our new model to try and understand the nature of this non-linearity. In bite force models there were three strongly influential points with extremely low bite force residuals. We assumed that this might be due to a lack of motivation to bite the measurement plates, given that there were already a number of lizards that did not attempt to bite. We therefore excluded these three lizards from the final analysis. Coefficients were again standardized for these analyses (Gelman, 2008). We present our full models and standard errors along with  $\Delta AIC_C$  between our main effects model and models excluding interactions of interest.

To understand the relationships between our performance traits in each of the sexes we regressed residuals for each performance trait conditional on the most important variables in models from Tables 3 & 4 where confidence intervals did not overlap with zero. We did not include testosterone in sprint speed models given that performance traits needed to have a balanced sample size and including testosterone led to a major drop in the overall sample size for each correlation. We tested whether performance residuals were correlated with each other between the sexes using Pearson correlation tests.

## RESULTS

### *Sexual size and shape dimorphism*

Snout-vent length (SVL) was not different between males and females (GLM comparing null model with model containing sex:  $\Delta AIC_C = 1.50$ ,  $n = 213$ ; Fig. 2); however, males were heavier than females when controlling for SVL and there was weak evidence for heterogeneity of slopes between sexes (Table 1 & Fig. 3e). Males and females differed in head dimensions and there was significant heterogeneity of slopes between body size and head width (Table 1 & Fig. 3a), head length (Table 1 & Fig. 3b) and head depth (Table 1 & Fig. 3c). In all cases males had larger head dimensions compared with females (Fig. 3a – c). Many lizards had broken or re-growing tails (low points; Fig. 3g) and this made the relationship between tail



length and body size complicated. We therefore present just the scatterplot of this relationship. Although there was a trend for larger tails as body size increased, there was substantial spread in the data and the relationship appeared to be weak (Fig. 3g). Females had significantly larger inter-limb length compared to males after controlling for body size (Table 1 & Fig. 3f). We found weak evidence for significant heterogeneity of slopes (Table 1) and the relationship appeared mostly linear, with the most parsimonious additive model explaining the data adequately.

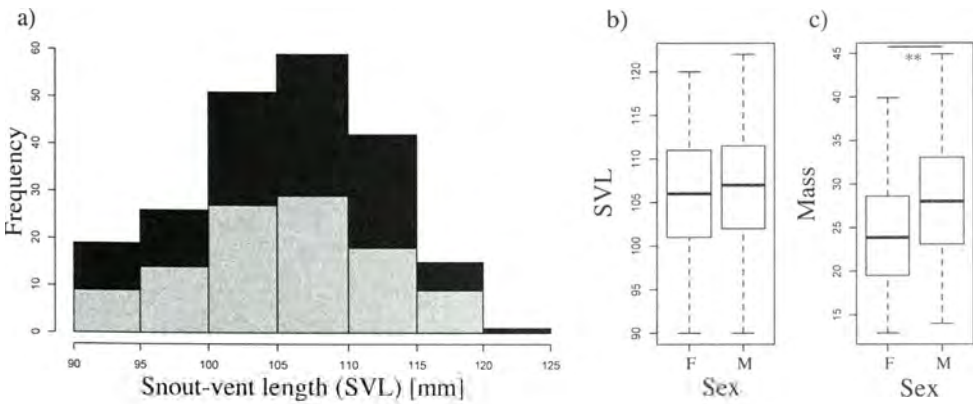


Figure 2 – (a) Body size distributions of male (‘black’) and female (‘gray’) *E. quoyii* along with boxplots (b). (c) Boxplots of the distributions of mass between male and female *E. quoyii*. \*\* indicates that mean differences are significant  $P < 0.01$ .

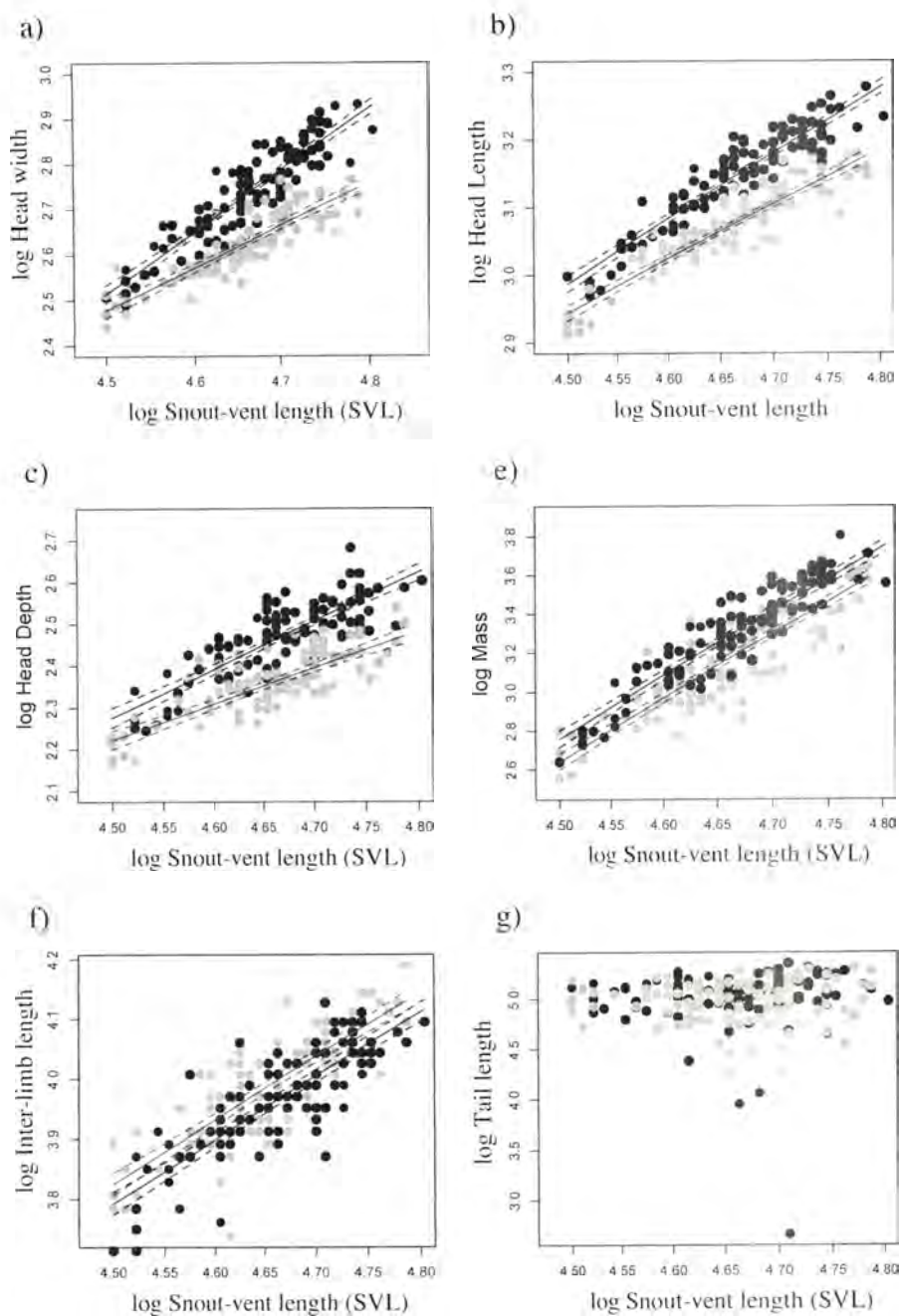


Figure 3 – Relationships between log snout-vent length (SVL) and morphological measurements for males ('black') and females ('gray'). (a) log head width; (b) log head length; (c) log head depth; (d) log inter-limb length and (e) log tail length. Solid lines are best-fit lines from models in Table 1. Dashed lines are the upper and lower 95% confidence intervals.

Table 1 – Candidate model set testing for body size (log transformed) and sex effects on natural log transformed head length, width, depth and inter-limb length (ILL). Sample-size corrected Akaike information criteria ( $AIC_C$ ), the difference in  $AIC_C$  between the best-supported model and model  $i$  ( $\Delta AIC_C$ ) and the Akaike weight ( $w$ ) of each model are provided. Sample sizes for each analysis = 213.

Model	Log (Head width)			Log (Head length)			Log (Head Depth)			Log (ILL)			Log (Mass)		
	$AIC_C$	$\Delta AIC_C$	$w$	$AIC_C$	$\Delta AIC_C$	$w$	$AIC_C$	$\Delta AIC_C$	$w$	$AIC_C$	$\Delta AIC_C$	$w$	$AIC_C$	$\Delta AIC_C$	$w$
Intercept only (NULL)	-352.48	446.63	0.00	-495.12	477.9	0.00	-775.23	336.88	0.00	-423.02	226.3	0.00	-32.58	356.35	0.00
log(SVL)	-541.93	257.18	0.00	-755.65	237.37	0.00	-526.86	165.25	0.00	-639.08	19.24	0.00	-273.45	50.32	0.00
log(SVL) + Sex	-750.99	48.12	0.00	-964.64	8.38	0.00	-684.82	729	0.00	-658.32	0	0.72	-323.77	0	0.50
log(SVL) + Sex + Sex*log(SVL)	-799.11	0	1.00	-973.02	0	0.99	-692.11	0	0.97	-656.32	-1.87	0.28	-323.74	0.03	0.50
Parameter	Est.	UCL	LCL	Est.	UCL	LCL	Est.	UCL	LCL	Est.	UCL	LCL	Est.	UCL	LCL
Intercept	-1.73	-1.24	-2.21	-0.68	-0.37	-0.99	-1.08	-1.08	-2.28	-0.98	-0.48	-1.42	-12.12	-11.09	-13.35
Sex (M)	-1.93	-1.22	-2.63	-0.67	-0.22	-1.12	-1.25	-0.39	-1.17	-0.03	-0.02	-0.05	0.13	0.43	-0.09
log (SVL)	0.93	1.04	0.83	0.81	0.87	0.74	0.87	0	0.73	1.08	0.76	-0.33	-3.28	-3.50	-5.06
log (SVL) *Sex	-0.44	0.59	-0.28	0.10	0.76	-0.06	0.28	0.48	-0.10						

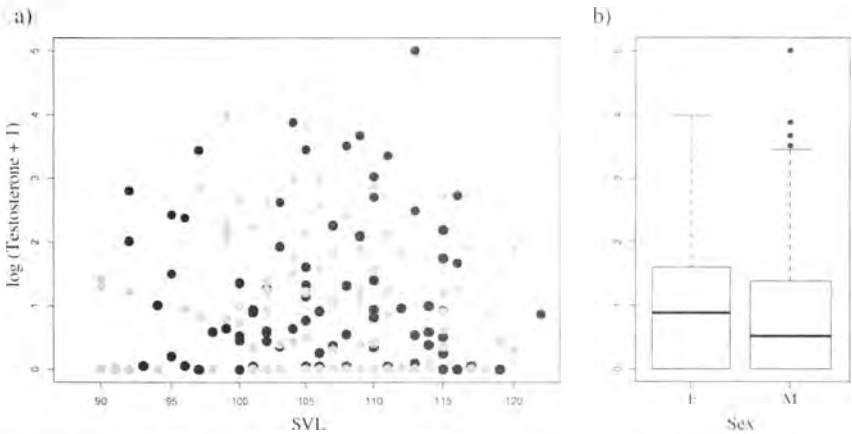


Figure 4 – (a) Relationship between body size and circulating plasma testosterone for male (‘black’) and female (‘gray’) *E. quoyii* along with boxplots (b).

Sex differences in testosterone

Median plasma testosterone increased with collection date ( $\beta_{0.5} = 0.02$ , CI = 0.01 – 0.04) and males had a tendency to have lower median plasma T than females but these distributions largely overlapped (Fig 4; Intercept:  $\beta_{0.5} = 0.87$ , CI = 0.50 – 0.98; Sex (M):  $\beta_{0.5} = -0.34$ , CI = -0.61 – 0.05). Median plasma T was not related strongly to either SVL ( $\beta_{0.5} = -0.12$ , CI = -0.36 – 0.07) or residual mass ( $\beta_{0.5} = 0.19$ , CI = -0.19 – 0.35).

Effect of T on morphology across the sexes

We found only weak evidence that testosterone positively influenced head width, depth or mass directly (Table 2). Collection date was positively associated with mass and head depth and negatively associated with head depth (Table 2).

Table 2 – The effects of plasma testosterone (T) on head width, head depth and body mass in *Eulamprus quoyii*. “Top models” are the best-supported models from Table 1. The change in AIC between the top model and the models containing log transformed T and Julian collection date are provided. Samples sizes for each analysis are provided in brackets. We present the full main effects models along with their lower and upper 95% confidence intervals (‘L CI’ and ‘U CI’). All estimates are standardized (mean = 0, sd = 2).

Model	Log (Head Width) (n = 193)			Log (Head Depth) (n = 193)			Log (Mass) (n = 193)		
	AIC <sub>c</sub>	Δ AIC <sub>c</sub>	w	AIC <sub>c</sub>	Δ AIC <sub>c</sub>	w	AIC <sub>c</sub>	Δ AIC <sub>c</sub>	w
Top Model from Table 1 or null (SVL)	-709.63	15.74	0.00	-638.25	9.53	0.01	-303.67	16.34	0.00
Top Model + log T	-709.03	16.34	0.00	-636.85	10.93	0.00	-303.67	16.34	0.00
Top Model + CD	<b>-725.37</b>	<b>0</b>	<b>0.63</b>	<b>-647.78</b>	<b>0.00</b>	<b>0.68</b>	<b>-320.01</b>	<b>0.00</b>	<b>0.65</b>
Top Model + log T + CD	-723.30	2.07	0.22	-645.62	2.16	0.23	-318.15	1.86	0.26
Top Model + log T + CD + Sex*log T	-722.42	2.95	0.14	-643.44	4.34	0.08	-316.05	3.96	0.09
Parameter	Estimate	L CI	U CI	Estimate	L CI	U CI	Estimate	L CI	U CI
Intercept	2.68	2.678	2.688	2.42	2.41	2.42	3.23	3.22	3.25
Log (Testosterone + 1)	0.002	-0.01	0.012	-0.0005	-0.01	0.01	0.01	-0.02	0.04
Log (SVL)	<b>0.16</b>	<b>0.15</b>	<b>0.17</b>	<b>0.14</b>	<b>0.13</b>	<b>0.15</b>	<b>0.45</b>	<b>0.42</b>	<b>0.47</b>
Sex (M)	<b>0.12</b>	<b>0.11</b>	<b>0.13</b>	<b>0.09</b>	<b>0.08</b>	<b>0.11</b>	<b>0.15</b>	<b>0.12</b>	<b>0.18</b>
Sex*log (SVL)	<b>0.05</b>	<b>0.03</b>	<b>0.08</b>	<b>0.04</b>	<b>0.01</b>	<b>0.06</b>	-	-	-
Collection Date	<b>0.02</b>	<b>0.01</b>	<b>0.04</b>	<b>-0.02</b>	<b>-0.04</b>	<b>-0.01</b>	<b>0.07</b>	<b>0.04</b>	<b>0.10</b>

Effect of morphology and testosterone on performance across the sexes



We also found weak evidence that T was linearly related to sprint speed, endurance and bite force in both males and females, however, it did appear to show a non-linear relationship with bite force in males (Fig. 5e). Males with non-detectable levels of T and males with high levels of T appeared to both have high bite force (Fig. 5e), while increased plasma T concentrations had a tendency to be associated with lower levels of endurance (Fig. 5c), but seemed to be driven by a single male. The top-supported model for sprint speed and endurance included only main effect parameters and there was no evidence for heterogeneity of slopes in morphology and performance traits between the sexes (Table 3 & 4). Tail length decreased endurance and body size increased endurance controlling for all other variables. Males had higher endurance than females (Table 3: See estimates). Testosterone did not strongly influence endurance and there was no evidence for non-linearity in testosterone after controlling for all other variables. Residual mass was positively related to sprint speed while SVL was positively related to speed, but only weakly (Table 3). Males had higher sprint speed compared to females and increased plasma testosterone decreased maximal sprint speed independently of sex and there was no evidence for a significant interaction between testosterone and sex (Table 3). Body size and residual head width both strongly affected bite force in males and females, while T did not appear to be linearly related to bite force after controlling for all other variables in the model. Residual plots identified non-linearity between T and residual head width and bite force for males, but not in females. We re-fitted male bite force models by including quadratics for the latter parameters. This improved model residuals, but did not strongly improve model fit, although both models were almost equally supported ( $\Delta AIC_c$  between main effects model in Table 4 and model with quadratics for T and residual head width = 1.76). Plotting the predicted bite force between residual head width and log testosterone while controlling for all other variables in the model revealed that males with high residual head width with low and high testosterone had the highest bite force (Fig. 6).

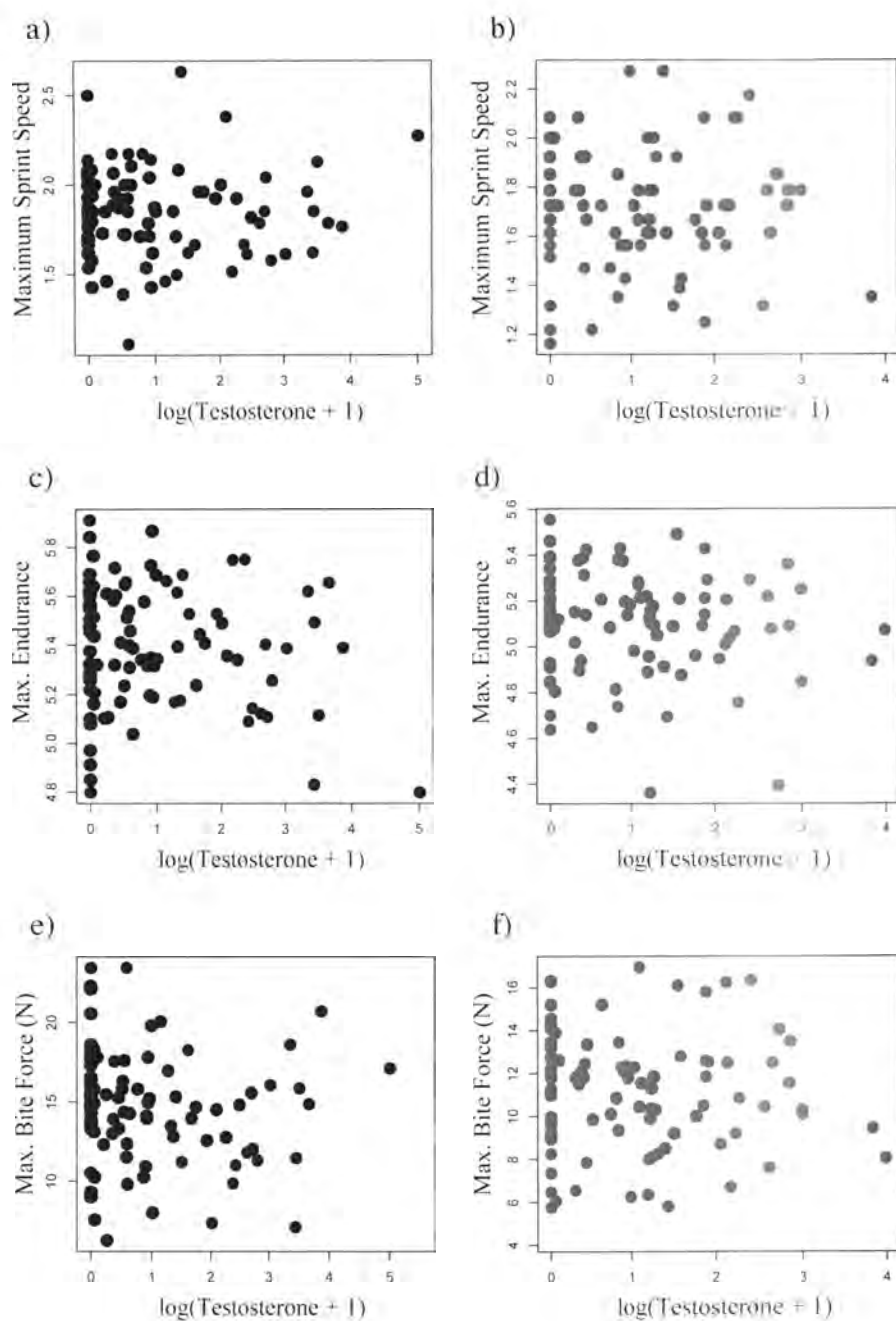


Figure 5 – Relationship between circulating plasma testosterone and performance measurements (sprint speed, endurance and bite force) for males (a, c, e) and females (b, d, f).

Table 3 – Full models testing the relationship between performance traits (Maximal endurance, sprint speed and bite force), sex and morphology (body size PC1, tail length, head size PC1). Interactions were removed from the full model to evaluate the overall effect of their removal

and are provided under the lower table. Estimates for the main effects models are provided. “Std. Est.” are standardized regression coefficients (z-transformed continuous predictors, mean = 0, sd = 2; Gelman, 2008). Confidence intervals of bolded estimates do not overlap zero.

Parameter	Log Maximal Endurance (n = 193)				Maximal Speed (n = 190)			
	Estimate	SE	Std. Est.	SE	Estimate	SE	Std. Est.	SE
Intercept	<b>37.973</b>	<b>23.063</b>	<b>5.256</b>	<b>0.015</b>	-49.7	43.9	<b>1.79</b>	<b>0.04</b>
Sex (M)	<b>0.297</b>	<b>0.039</b>	<b>0.297</b>	<b>0.039</b>	<b>0.11</b>	<b>0.04</b>	<b>0.11</b>	<b>0.04</b>
SVL	<b>0.015</b>	<b>0.002</b>	<b>0.215</b>	<b>0.031</b>	<b>0.006</b>	<b>0.002</b>	<b>0.08</b>	<b>0.03</b>
Res. Mass	-0.064	0.156	-0.016	0.039	<b>0.45</b>	<b>0.159</b>	<b>0.11</b>	<b>0.04</b>
Tail Length	<b>-0.002</b>	<b>0.001</b>	<b>-0.106</b>	<b>0.033</b>	0.0008	0.0006	0.05	0.03
Log (Testosterone + 1)	-0.027	0.014	-0.057	0.031	<b>-0.03</b>	<b>0.02</b>	<b>-0.07</b>	<b>0.03</b>
Collection Date	-0.002	0.002	-0.051	0.035	0.003	0.003	0.08	0.07
Temperature	-0.018	0.013	-0.044	0.032	<b>0.05</b>	<b>0.01</b>	<b>0.11</b>	<b>0.03</b>
Researcher 1	<b>0.089</b>	<b>0.042</b>	<b>0.089</b>	<b>0.042</b>	-0.09	0.07	-0.09	0.07
Researcher 2	-	-	-	-	<b>0.11</b>	<b>0.043</b>	<b>0.11</b>	<b>0.04</b>
<hr/>								
Interaction Parameter	AIC <sub>c</sub>	Δ			AIC <sub>c</sub>	Δ		
Main effects model	-53.31	0			-56.79	0		
Sex*Testosterone	-48.86	4.45			-52.60	4.19		
Sex*Res. Mass	-48.79	4.52			-52.43	4.36		
Sex*SVL	-48.86	4.45			-52.64	4.15		

Table 4 – Full model estimates (unstandardized and standardized) for the effects of morphology and testosterone on bite force in males and females. Sexes were separated due to strong collinearity between head dimensions and sex. Bolded estimates are significant at  $P < 0.05$ . Three lizards were excluded from models because they were extreme outliers in the analysis.

Parameter	Bite Force - Males (n = 88)				Bite Force - Females (n = 90)			
	Estimate	SE	Std. Est.	SE	Estimate	SE	Std. Est.	SE
Intercept	1731.94	453.07	14.00	0.71	-420.26	567.83	9.75	0.57
SVL	<b>0.33</b>	<b>0.03</b>	<b>4.49</b>	<b>0.46</b>	<b>0.28</b>	<b>0.03</b>	<b>4.23</b>	<b>0.46</b>
Res. Mass	0.84	2.77	0.16	0.51	2.24	2.09	0.56	0.52
Res. Head Width	<b>16.36</b>	<b>6.27</b>	<b>1.43</b>	<b>0.55</b>	<b>24.96</b>	<b>6.40</b>	<b>1.95</b>	<b>0.50</b>
Log (Testosterone + 1)	0.11	0.19	0.25	0.45	-0.02	0.19	-0.05	0.39
Collection Date	<b>-0.12</b>	<b>0.03</b>	<b>-2.82</b>	<b>0.73</b>	0.03	0.04	0.47	0.67
Temperature	-0.04	0.22	-0.09	0.43	0.05	0.17	0.14	0.43
Researcher 1	1.55	0.95	1.55	0.95	<b>2.80</b>	<b>0.84</b>	<b>2.80</b>	<b>0.84</b>
Researcher 2	0.80	0.73	0.80	0.73	0.77	0.61	0.77	0.61

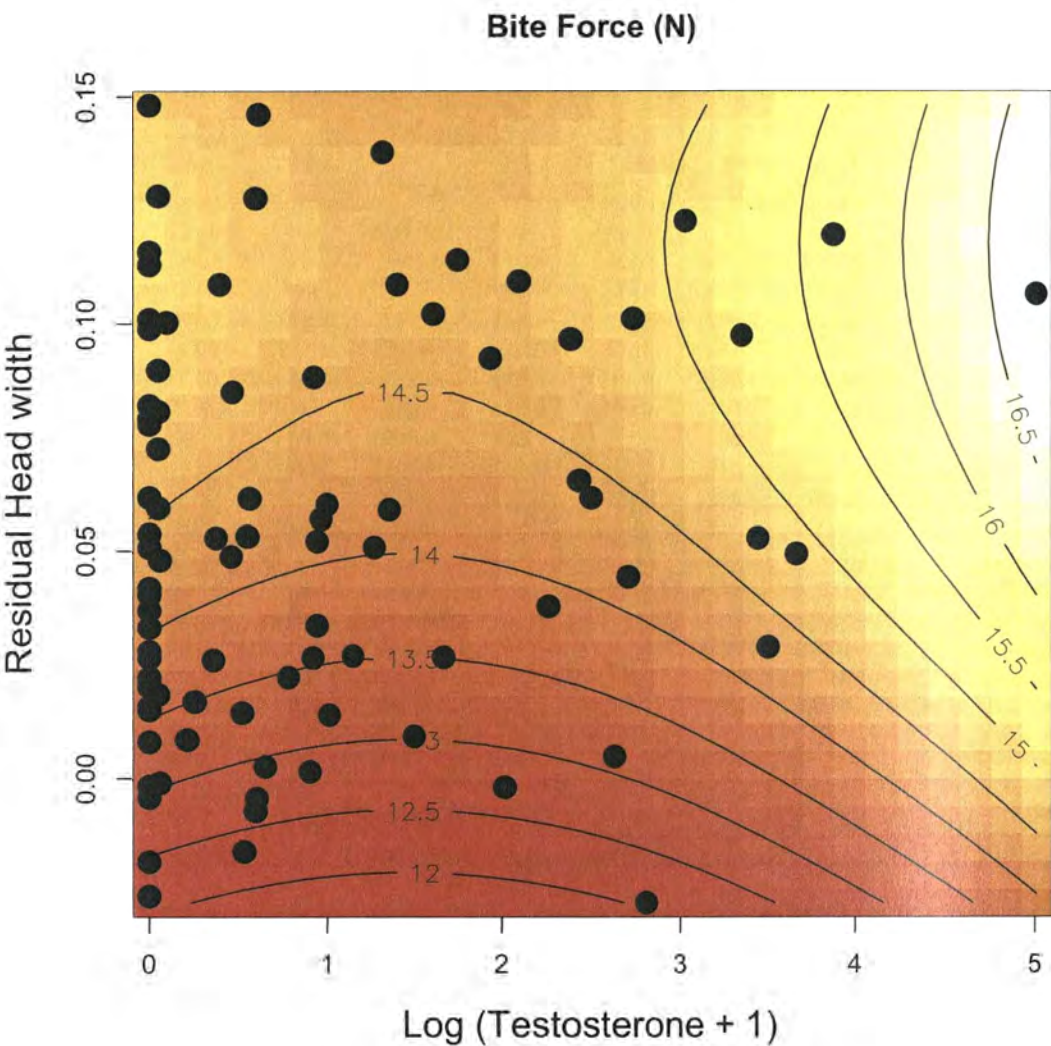


Figure 6 – Contour plot of predicted bite force varying with head width and T conditional on all other variables in the model (Table 4; all other variables at median values). Note that this model contains quadratics for residual head width and log T given that these showed evidence for non-linearity. All other parameters are the same as Table 4. Points are individual males (n = 88).

*Relationships between residual bite force, endurance and sprint speed*

Residual bite force, sprint speed and endurance were weakly correlated in males (Table 5; Fig. 7 a – c) and females (Table 5; Fig. 7 d – f).



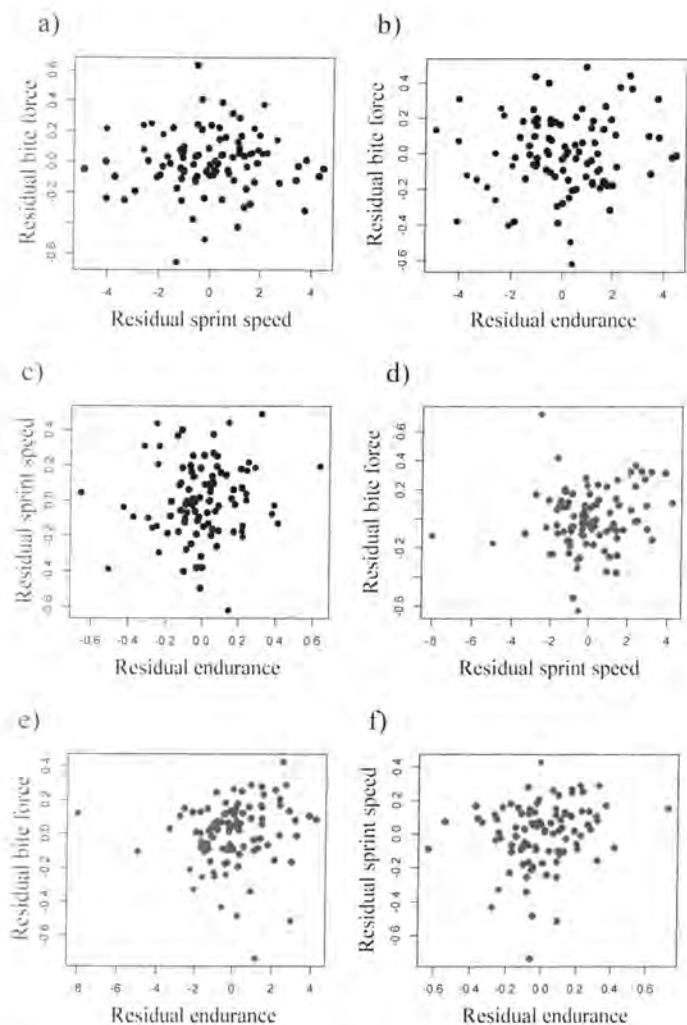


Figure 7 – Plots of residual bite force, sprint speed and endurance in males ('black': a-c) and females ('gray': d-f). Note that residuals are taken from regressing each performance trait against variables explaining most variance in each of the traits. See methods for details.

Table 5 – Pearson’s correlation coefficients between residual sprint speed, bite force and endurance for males (below diagonal) and females (above diagonal). 95% confidence intervals are provided in brackets after each estimate.

	Residual Sprint Speed	Residual Endurance	Residual Bite Force
Residual Sprint Speed	–	0.19 (-0.02 – 0.37)	0.16 (-0.05 – 0.35)
Residual Endurance	0.10 (-0.10 – 0.30)	–	0.11 (-0.10 – 0.30)
Residual Bite Force	0.03 (-0.18 – 0.23)	0.09 (-0.12 – 0.28)	–

DISCUSSION

Species lacking sexual body size dimorphism provide a unique opportunity to explore androgen-morphology-performance relationships because functional relationships between traits and body size can be disentangled. In *E. quoyii*, where males and females do not differ in body size or colouration, we found that males ran longer and faster than females and had stronger bite force which was a result of differences in mass and shape between the sexes. Plasma T levels did not differ between the sexes and there was weak evidence that T was related to any of the morphological or performance traits except sprint speed. Interestingly, high circulating T showed a non-linear relationship with bite force in males and not females and this relationship may be related to alternative male reproductive tactics in this species (Noble *et al.*, 2013b). Our results suggest that T does not have a strong activational effect on performance or morphology, although it may play an important organizational role during the development of morphological traits. Such strong differences in the sexes in performance suggest that there may be differential selection on these functional traits between males and females (Lailvaux, 2007; Van Damme *et al.*, 2008), which is plausible given the important role of these performance traits to fitness (Sinervo *et al.*, 2000; Husak *et al.*, 2006; Husak, 2006)

Enhanced sprint speed, endurance and bite force of male *E. quoyii* is consistent with patterns in a wide range of taxa, which have shown that males generally perform better than females (Lailvaux, 2007; Van Damme *et al.*, 2008). Our results are interesting in that these differences map more closely to shape and energy store differences between the sexes rather than corresponding changes in suites of traits that scale with body size (Van Damme *et al.*, 2008). Male *E. quoyii* have wider heads than females and head size is known to positively

affect bite force in many lizard species because increased cranial size can accommodate more musculature (Lappin *et al.*, 2006; Husak *et al.*, 2007; McBrayer & Anderson, 2007). Male *E. quoyii* are also heavier and have longer limbs (Schwarzkopf, 2005) than females. Both limb size (Losos, 1990; Bonine & Garland, 1999) and energy stores are thought to be associated with greater running performance given their predicted link with stride length and hind-limb size (Losos, 1990) and energy assimilation during exercise (Van Damme *et al.*, 2008). Such strong differences in the sexes in performance suggest that there may be differential selection on these functional traits between males and females (Lailvaux, 2007; Van Damme *et al.*, 2008), which is plausible given the important role of these performance traits to fitness (Sinervo *et al.*, 2000; Husak *et al.*, 2006; Husak, 2006).

Interestingly, plasma T did not differ between male and female *E. quoyii*, although there was substantial variation in T within each sex. The lack of T differences between the sexes has been observed in other taxa (Ketterson *et al.*, 2005), including lizards (Hews *et al.*, 2012) and may be related to the degree of sexual dimorphism in a given species and/or behavioural similarities between the sexes (Ketterson *et al.*, 2005). Testosterone is known to be important in sexual signal development and in affecting behaviours such as home range, movement rates and aggression in lizards (Sinervo *et al.*, 2000; Cox *et al.*, 2005; Whiting *et al.*, 2006; Miles *et al.*, 2007b; Cox *et al.*, 2009). Both male and female *E. quoyii* are aggressive and males do not develop obvious sexual signals commonly observed in many lizard taxa (D. Noble, personal observation). In addition, male *E. quoyii* exhibit alternative reproductive tactics (ARTs) that are linked to activity related behaviours and home range size differences (Noble *et al.*, 2013b). This variation in home range and activity related behaviour appears to also manifest itself in females in *E. heatwolei*, a closely related species (Stapley & Keogh, 2005). Indeed, extreme differences in circulating T within sexes may provide a proximate explanation to ARTs in this system and may drive similarities in behaviour and morphology in males and females, particularly if the same traits are sensitive to changes in plasma T across the sexes. Future experimental work will be necessary to understand the role T plays in mediating changes in these behaviours across the sexes and its organizational and activational role in both males and females.

Plasma testosterone explained variation in sprint speed, but not other morphological or performance traits in *E. quoyii*. Testosterone has been implicated as a proximate explanation for differences in performance both between (Lailvaux, 2007; Van Damme *et al.*, 2008) and

within the sexes (Irschick *et al.*, 2006; Miles *et al.*, 2007b). The fact that there were differences in performance between the sexes and yet there are no body size or T differences between them suggests that, if T does affect morphology-performance relationships, it does so during ontogeny through organizational effects on body shape (Crews *et al.*, 1998). Concentrations of androgen receptor proteins and aromatizing enzymes may also play a role in how tissues, such as muscle, respond to circulating androgens and thus shape sex and individual variation in morphology and performance and these variables should be explored in the future. Alternatively, variation in morphology and performance could also be explained by other important hormones, such as corticosterone (Miles *et al.*, 2007a) or individual variation in mitochondrial enzyme activity (Seebacher *et al.*, 2003). Few studies have directly tested whether testosterone covaries with performance and morphological traits. In an experimental study on *Gallotia galloti*, Huyghe *et al.* (2010) found that pre-implant circulating T was positively correlated with bite force but not with sprint speed, body size or any other morphological trait in *G. galloti*. Treatment with exogenous T affected muscle mass but also did not result in changes in head dimensions or performance (sprint speed or bite force) (Huyghe *et al.*, 2010). Husak *et al.* (2007) found that T was positively related to SVL, dewlap size and bite force in 'lightweight' *Anolis carolinensis*. However, when removing the confounding effects of SVL, the relationship between T, bite force and dewlap size no longer existed (Husak *et al.*, 2007). In contrast, exogenous T increased endurance in yellow and blue-throated morphs of *Uta stansburiana* (Sinervo *et al.*, 2000). Given our results, and those from the literature on other lizards, it is clear that the role of T in enhancing performance is still poorly understood and considering the interacting effects of other hormones and levels of androgen receptors and enzymes will be essential to understand the proximate role T plays, if any, in shaping individual performance and morphology.

Interestingly, we did find evidence for a non-linear relationship between plasma T and bite force in males but not females, which is congruent with theoretical expectations of alternative reproductive tactics (ARTs) in *E. quoyii* (Noble *et al.*, 2013b). Males with large residual head width and high and low testosterone were predicted to have the highest bite forces, with individuals with high plasma T being predicted to bite the hardest. Although still a tentative result which requires experimental work, this does provide some tantalizing clues into the proximate underpinnings of ARTs in *E. quoyii*. Large males exhibit different behavioural tactics, with territorial/resident males having small home range areas and lower



movement rates, but spending more days active, while floater males have large home range areas, move a lot, but are not observed as frequently (Stapley & Keogh, 2004; 2005; Noble *et al.*, 2013b). Selection has also been shown to favor these alternative behavioural tactics (Noble *et al.*, 2013b). Territorial/resident and floater males are of similar body size and therefore will have similar head widths, given the strong pattern of scaling between these traits, supporting this hypothesis. It is not clear from this pattern whether differences in bite force are caused by plasma testosterone levels or the result of behavioural differences in aggression or motivation to bite, which might be causing the slight differences between these groups of males and explain other behavioural differences with respect to ARTs in this system.

In conclusion, few studies explicitly test for sex differences in performance (Lailvaux, 2007) and fewer explore their proximate underpinnings. Most work has focused on sexually dimorphic species, where the sexes vary in a number of physiological and morphological traits thereby making an understanding of the causal mechanisms behind performance differences more difficult to grasp. We argue that a greater focus on sexually monomorphic species and species with sex differences in shape will shed greater insight into physiology-morphology-performance relationships and help ascertain whether important androgens, such as testosterone play a role in driving variation in morphology, performance and fitness.

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## CHAPTER 7: NATURAL AND SEXUAL SELECTION ON WHOLE-ORGANISM PERFORMANCE TRAITS IN AN AUSTRALIAN LIZARD WITH ALTERNATIVE REPRODUCTIVE TACTICS

Daniel W.A. Noble, J. Scott Keogh and Martin J. Whiting

### Abstract

Natural and sexual selection shape the adaptive landscape in complex ways that may lead to the integration of suites of traits. Much of our understanding of selection comes from studies of morphological traits with few studies attempting to quantify the form and direction of selection on whole-organism performance and behaviour even though they are predicted to be more direct targets of selection in nature. We quantified natural and sexual selection on whole-organism performance traits (bite force, sprint speed and endurance) in an Australian lizard with alternative male reproductive tactics, the Eastern water skink (*Eulamprus quoyii*). We established six breeding populations in semi-natural enclosures and estimated survival and reproductive success (molecular paternity assignment) during the breeding season for 216 lizards. We found natural selection to be weak on performance traits during the breeding season for both males and females. There was evidence for sexual selection on sprint speed in males but not females and selection was non-linear with fast and slow large males having the highest reproductive success. Body size, bite force and endurance were strongly correlated in males and females and there was weak evidence for direct selection on bite force and endurance when selection on body size was controlled. Our results provide an explanation for sex-differences in running speed and suggest that alternative reproductive tactics in males maybe integrated with whole-organisms performance traits, highlighting the importance of considering behaviour in understanding patterns of selection on whole-organism performance.

## INTRODUCTION

Natural and sexual selection act on phenotypic variability in powerful and complex ways that shape the adaptive landscape. Sexual selection in particular has played a prominent role in the evolution of sexual size dimorphism (Andersson 1994), elaborate male ornamentation (Andersson 1994) and alternative male reproductive tactics (Miles et al. 2007b; Sinervo et al. 2000). Directional selection is now recognized as a pervasive evolutionary force (Hoekstra et al. 2001; Kingsolver et al. 2001), which can lead to rapid evolutionary responses over ecological timescales (e.g. Grant and Grant 1995; Reznick et al. 1997). However, traits are not often selected upon in isolation and correlational selection can favor the integration of traits (Brodie III 1992; McGlothlin et al. 2005; Miles et al. 2007b). Although there are thousands of selection estimates from natural populations (Kingsolver et al. 2012; Kingsolver et al. 2001), much of our understanding about the strength and form of selection comes from studies of morphological traits (Kingsolver et al. 2001). Only a few studies have estimated selection gradients on more direct targets of selection, such as behaviour and performance both of which are the outcome of morphological, physiological, developmental and environmental variability (Arnold 1983; Irschick et al. 2008; Kaplan and Phillips 2006). Kingsolver et al. (2012) recently emphasized the need to quantify non-linear/correlational selection gradients, particularly on behavioural and physiological traits in natural populations. Such studies are difficult to implement given the inherent complexity of many biological systems and the difficulty in generating clear hypotheses about the form and direction of selection on these traits in nature.

Alternative reproductive tactics (ARTs) are prevalent across the animal kingdom (Oliviera et al. 2008) where strong sexual selection leads to the evolution of individuals that express different behavioural, physiological and/or morphological traits which allow them to gain access to female mating opportunities (Andersson 1994; Miles et al. 2007b; Sinervo and Lively 1996). In these systems, intra-sexual competition often leads to dominant and subordinate males adopting different behavioural strategies. These ARTs or behaviors may can be closely linked with whole-organism functional performance (Miles et al. 2007b), or the ability of individuals to perform an ecologically relevant task (Lailvaux and Irschick 2006), such as sustained running endurance for winning male contests or defending territories. For example, male side-blotched lizards (*Uta stansburiana*) exhibit one of three throat color



morphs (orange, blue and yellow) (Sinervo and Lively 1996). Hyper-aggressive orange males guard large territories and have high endurance capacities, presumably to aid in their defense (Sinervo and Lively 1996; Sinervo et al. 2000). In contrast, blue morphs have small territories and are less aggressive while yellow morphs sneak matings. Importantly, both of these male morphs having lower endurance capacities compared to orange males (Sinervo et al. 2000). In dung beetles (*Onthophagus taurus*), horned males are large and vigorously defend dung patches, whereas small hornless males adopt ‘sneaker-like’ behavioural tactics, digging intersecting tunnels which allow them to sneak copulations with females (Emlen 1997). Horn size is a strong predictor of endurance capacity and pulling force suggesting that the connection between large horns and behavioural tactic is mediated by whole-organism performance traits (Lailvaux et al. 2005). The integration of behavior with functional performance capacities in systems with ARTs provides an excellent opportunity to quantify selection gradients in a conceptual framework, which permits the testing of mechanistic hypotheses (Miles et al. 2007b). Although most ARTs are characterized by discrete variation, a powerful approach to understanding their evolution is to quantify continuous traits predicted to co-vary with behavioural reproductive tactics to test whether they match the associated predictions (Noble et al. 2013c).

Lizards are ideal model systems to explore hypotheses about selection on performance traits in the context of ARTs because we have a good understanding about the links between functional performance and key behaviours important to reproductive success, such as dominance and territoriality (Lailvaux et al. 2004; Losos et al. 2002; Miles et al. 2007b). Moreover, performance traits are also subject to natural selection in predictable ways (Irschick et al. 2008) allowing one to compare the role of natural and sexual selection in shaping the adaptive landscape. A number of performance traits have been identified as targets of natural and sexual selection in lizards. For example, dominance and resource holding potential (RHP) can often be predicted by large body size and biting force in lizards because larger, stronger biting lizards are capable of winning contests over sub-ordinate males allowing them to gain access to more resources and mates (Husak et al. 2006b; Lailvaux et al. 2004). Dominance has also been linked with locomotor performance in lizards such that more dominant individuals have higher endurance than subordinate males because winners of contests are capable of sustained displaying and fighting (Perry et al. 2004; Sinervo et al. 2000). Sprint speed has also been implicated as an important locomotor trait associated with male dominance because it

allows males to guard larger territories more effectively. However, there are alternative hypotheses for why sprint speed can be important to male reproductive success that may be unrelated to male dominance. Sprint speed and stamina (endurance) were both correlated with winning dyadic contests in *Urosaurus ornatus* (Robson and Miles 2000). Similarly, in collard lizards (*Crotaphytus collaris*), faster lizards sire more offspring because they are able to more effectively defend their territories and prevent other males from usurping them (Husak et al. 2006a; Husak et al. 2008). In contrast, sprint speed is not important for male dominance in the territorial lizard *Anolis cristatellus* (Perry et al. 2004). Indeed, a rarely tested alternative hypothesis is that sprint speed is important for sneaking copulations in systems with ARTs (Husak et al. 2008). The role of sprint speed in dominance and reproductive success likely depends on the behavioral traits adopted by males and the mating system of the given species. Furthermore, locomotor performance has been shown to be strongly related to survival in many lizard taxa (Irschick et al. 2008) and may feedback in complex ways that affect sexual selection.

Using a widely distributed Australian lizard, the Eastern Water Skink (*Eulamprus quoyii*), we conducted a large scale breeding experiment under semi-natural conditions by placing lizards in outdoor enclosures and quantifying selection on whole-organism performance traits (sprint speed, endurance and bite force) that are predicted to be important for survival and reproductive success (Husak and Fox 2008; Irschick et al. 2008; Lailvaux and Irschick 2006). Our study is the first to quantify both natural and sexual selection on a multitude of performance traits across sexes in lizards. *Eulamprus* are an ideal model system for such studies because they are abundant, habituate quickly and breed readily under semi-natural conditions and have been the topic of considerable study (Keogh et al. 2012; Keogh et al. 2013; Noble et al. 2012; Noble et al. 2013b; Scott et al. 2001; Stapley and Keogh 2004; Stapley and Keogh 2005). Male *Eulamprus* adopt behaviorally driven ARTs with, as of yet, no known morphological correlates. Noble et al. (2013c) proposed a theoretical framework for which to understand ARTs in *E. quoyii* that relate to subtle, but important, behavioural differences among the tactics. Territorial/resident males are predicted to be observed often, move little and have small home ranges, while floater males are predicted to have large home ranges, to be observed little and move frequently while active (Keogh et al. 2012; Noble et al. 2013c; Stapley and Keogh 2004; Stapley and Keogh 2005). Territorial and floater males differ in their reproductive success, yet there is evidence for correlational disruptive selection on

behavioural traits among large males (Noble et al. 2013c; Stapley and Keogh 2005). Behavioural differences among ARTs may also be linked with whole-organism performance traits (Miles et al. 2007a; Miles et al. 2007b; Sinervo et al. 2000) in *E. quoyii* given their association with ARTs in other systems.

We tested three alternative hypotheses about how locomotor performance and bite force influence survival and reproductive success (Fig. 1a-c; Husak and Fox 2008) and test the hypothesis that the relationship between performance traits and reproductive success is sex-dependent as would be predicted if such traits are under strong sexual selection (Fig. 1a-c). Given the existence of behaviourally driven ARTs in *E. quoyii* and our understanding of the relationship between performance, dominance and reproductive success in other lizards (Husak et al. 2006a; Miles et al. 2007b; Robson and Miles 2000), we also made *a priori* predictions about how correlational/non-linear selection might result in different fitness optima that may relate to the different mechanisms by which ARTs obtain paternity (Fig. 1d & e; Predictions 1–2). Territorial and floater male lizards may both be selected to have high sprint speed and/or endurance but for different reasons. Territorial males may require fast speeds to prevent usurping and higher endurance for pro-longed displaying and/or winning contests, while floater males may require speed for sneaking copulations with females and endurance and speed for transversing over large areas in search of female copulations (Fig. 1d & e; Prediction 1) (Husak et al. 2008). Alternatively, sprint speed and endurance may only be selected in floaters to allow individuals to sneak copulations from the territories of more dominant males and move over large areas given that territory holders are predicted to have small home ranges (Morrison et al. 2002; Stapley and Keogh 2005) and contests in *E. quoyii* are commonly resolved before escalating to fights (D. Noble personal observation; Fig. 1d & e; Prediction 2). Importantly, body size is one of the most important predictors of reproductive success in *E. quoyii* (Noble et al. 2013c) and as a result we would expect these fitness peaks to be among large males as these individuals can adopt either floater or territorial tactics and have the highest reproductive success (Noble et al. 2013c; Stapley and Keogh 2005). Under this hypothesis we would further predict that floaters would sire fewer offspring across more clutches, while territorial males would sire more offspring over few clutches and sprint speed would be a strong predictor of the number of clutches sired.



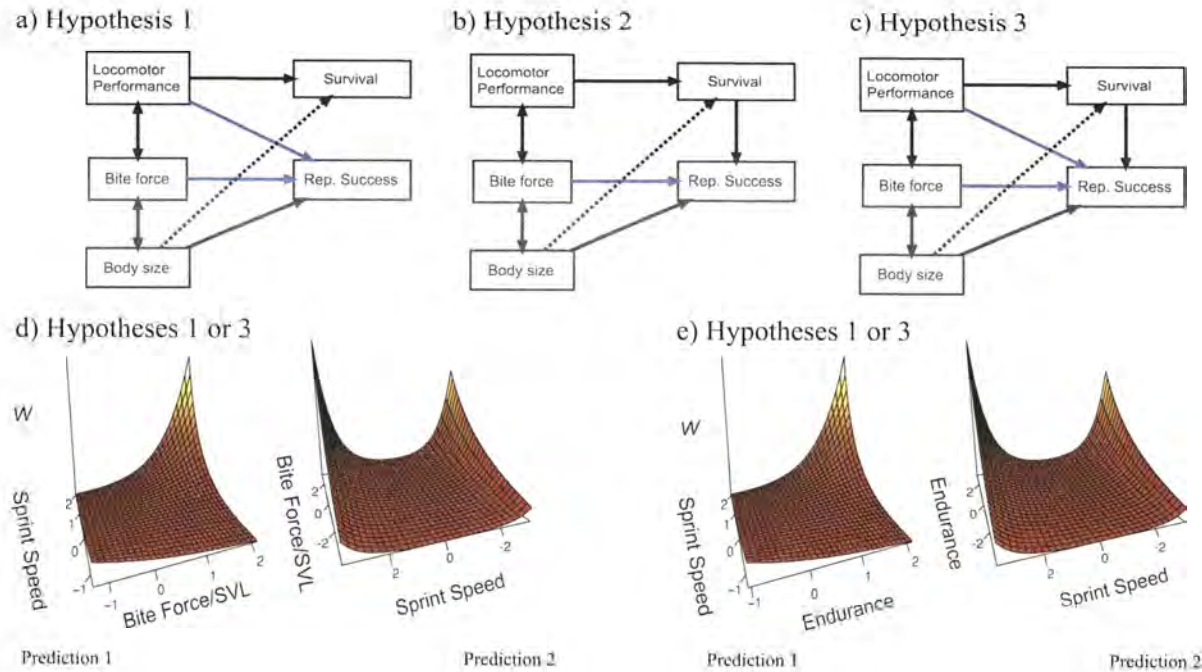


Fig. 1 – (a-c) Three hypotheses depicting the relationship between performance traits and fitness [survival and relative reproductive success ( $W$ )]. Black arrows indicate predicted relationships for both males and females while blue arrows indicate relationships between traits male fitness. Solid arrows indicate the predicted relationship is positive while dotted arrows indicate a negative relationship. (c-d) If hypotheses 1 or 3 are supported then we predict alternative fitness optima between performance traits that would relate to ARTs in *E. quoyii* (Noble et al. 2013c). **Prediction 1** – Predicted fitness surfaces if more dominant, territorial males (large males; high bite force) have high speed, and endurance for defending territories/guarding females/ ensuring dominance hierarchies while floaters have high speed and endurance for moving over large areas and sneaking matings. This result would suggest that the same traits are selected for in both floaters and territorials. **Prediction 2** – Predicted fitness surfaces if large territorial lizards are slower because they do not require speed to monitor smaller territories while large floaters are faster for sneaking mating opportunities with females. We would also predict that floaters would sire offspring across more clutches, but less within clutches. Note that floater and territorial males do not differ significantly in body size (Noble et al. 2013c; Stapley and Keogh 2005).



## MATERIALS AND METHODS

### *Lizard collection*

We collected 216 (108 males and females) adult *E. quoyii* from five sites in the Sydney region (see Noble et al. 2013b for details). Lizards were brought back to the laboratory at Macquarie University campus where they were sexed by everting the hemipenes and measured (head width, length, depth and snout-vent length [SVL] to the nearest 1 mm and mass to the nearest 0.1 g). From each lizard we excised a small (~ 3 mm) tail tip for later genetic analysis. Lizards were then transferred to plastic bins [487 (L) x 350 (W) x 260 mm (H)] in a temperature-controlled room for three days to measure whole-organism performance traits. Each bin had a hide box and a water bowl with newspaper as a substrate. Lizards were maintained at ambient temperatures of ~22–26°C with an elevated basking site of ~28–30°C. Ultraviolet lighting and water were provided at all times and lizards were fed once during the three days with crickets or mealworms.

### *Performance measurements*

We measured lizard bite force, sprint speed, and endurance daily over three consecutive days. Prior to all measurements lizards were heated to their preferred body temperature (~ 28°C; Law and Bradley 1990) by placing lizards in plastic zip-lock bags and floating them in warm water until they reached near optimal body temperature. For each lizard we recorded the time lizards began performance trials and the body temperature of lizards before the start (i.e. prior to bite force) and at the end (i.e. after endurance measurements) of performance measurements using a Miller-Weber cloacal thermometer. We took the maximal measurement for all our performance variables (Losos et al. 2002).

#### *I. Bite force*

Bite force (N) was measured a total of six times on all lizards using a Kistler force transducer (Kistler Inc., Winterthur Switzerland) that was connected to a Kistler charge amplifier (Model 5995, Kistler Inc.). We measured each lizard twice a day, once before our measurement of sprint speed and once after, over three consecutive days. We took a second measurement of bite force after running down the racetrack because lizards appeared to be more motivated to bite after they had run. We induced lizards to bite the two plates by gently pinching the sides of their mouth.

### *II. Sprint speed*

We measured sprint speed along a 2 m racetrack immediately after the first bite force measurement. The running surface of the racetrack was lined with rubber matting and at each 25 cm interval a white line indicated distance. A bucket was placed at the end of the racetrack, which the lizard fell into once the run was complete. Lizards were placed at the starting line and stimulated to run by taping them gently on their tail-base. In most cases lizards ran extremely well to the end of the finish line, with few incidences of stops and reversals. We recorded sprinting lizards using a Panasonic HD video camera (30 fps) and quantified the speed of each of the three runs using MotionPro Motion Analysis Software (<http://www.motionprosoftware.com/>).

### *III. Endurance*

We measured endurance immediately following the lizard's sprint. Lizards were run on a modified human treadmill to measure their maximal endurance. A transparent plexi-glass box with adjustable compartments was placed on top of the treadmill. The middle compartment was adjusted so that the lizards could run unobstructed, while also ensuring that they could not easily turn around. All trials were run at a speed of  $1.0 \text{ km} \cdot \text{hr}^{-1}$ . Lizards were placed on the treadmill and were stimulated to run by gently tapping the base of their tail. After each tap of the tail base we gave the lizard a few seconds to continue running. If the lizard was not stimulated to run we allowed the lizard to move closer towards the end of the treadmill and tapped the lizard again. We continued this until the lizard could no longer run at which point we allowed the lizard to fall into a container at the base of the treadmill. We then placed the

lizard back on the treadmill and continued the same procedure until the lizard had fallen into the container three times, at which point we considered the lizard exhausted. The time when the lizard started running on the treadmill to when it fell in the container the third time was recorded as its time to exhaustion.

### ***Survival and Reproductive success***

#### *Experimental Setup*

Lizards were allocated to one of six semi-natural, experimental enclosures, measuring 16 x 10 m (length x width) located on the campus of Macquarie University. Details on these enclosures can be found in Noble et al. (2013b). Briefly, the enclosures were constructed from color-bond fencing and silicone and foam were used to tightly seal the perimeter of each enclosure to ensure that lizards were not able to escape. Each enclosure contained two piles of large rocks and a line of branches and logs, which connect each rock pile. We also placed roofing tiles, stacked in threes, at 2 m intervals throughout the enclosure to form a grid and provide cover for the lizards. We placed four plastic containers in each enclosure, which were filled with drinking water and the top of each enclosure was open, allowing for predation by birds. In each enclosure we allocated 18 male and female water skinks. We ensured that lizards from each collection site were represented within each enclosure and that there was natural variation in body size among males and females. The densities of lizards in our enclosures fall within the range of natural variation in the wild (D. Noble, 2010 unpublished data and Gerry Swan 2010, personal communication).

Lizards were allowed to mate freely under these semi-natural conditions and all lizards were collected at the end of the breeding season (October 20<sup>th</sup>, 2010) to estimate survival. The enclosures prevented immigration and emigration and we searched enclosures exhaustively for any surviving lizards. Surviving females were brought back into the laboratory and placed in individual bins until parturition. At parturition, female offspring were measured and weighed and a small amount of tail tissue (~ 3 mm) was excised for paternity analysis.

#### *Paternity Assignment*

Whole genomic DNA was extracted from tail tissue using a Blood and Tissue Extraction Kit (Qiagen) according to the manufacturers protocol. We assigned paternity to offspring using 6 microsatellite DNA loci (Ek100, Ek107, Ek8, Ek37; Morrison et al. 2002; Scott et al. 2001) and (GQ20/21, GQ16/17; Sumner et al. 2001). PCR reactions were carried out in 20  $\mu$ L reaction volumes containing 1.0  $\mu$ L of genomic DNA, 10  $\mu$ L of GoTaq® (Promega), 0.5  $\mu$ L (10 pmol  $\cdot$   $\mu$ L<sup>-1</sup>) of forward and reverse primers and 8.0  $\mu$ L of nuclease-free water. PCR conditions for each locus are described in Scott et al. (2001) and Sumner et al. (2001). Forward primers were labeled with different fluorescent dyes (TET, NAD, VIC, FAM) and product from the final PCR reactions were pooled into a single plate, ran on an ABI 3730 DNA analyzer (Applied Biosystems) and scored by the Australian Genomic Research Facility (AGRF) using GENEMAPPER software (Applied Biosystems).

Parentage was assigned using the likelihood-based method in the program CERVUS 3.0 (Kalinowski et al. 2007). We simulated 100,000 offspring with 95% loci typed and 1% mistyped loci, using a strict confidence level of 95% and a relaxed confidence level of 80%. The loci used in our study were highly variable, ranging from 3-34 alleles at a single locus with mean polymorphic information content (PIC) of 0.7014. The combined non-exclusion probability for a parent pair was  $4.46 \times 10^{-6}$ . Paternity was assigned conservatively, and we excluded males as being putative sires if they had one or more mismatches with an offspring. In some cases, males could only be compared at four loci with offspring because of differences in the loci missing between the male and offspring. In these situations we assigned paternity to the male only if he had no mismatches and the trio (male, female and offspring combination) LOD scores were significant.

### *Data Analysis*

We excluded three lizards from analyses (two females; 1 male) because we did not obtain any performance measurements for one female, while one male and female were extreme outliers; being extremely heavy for their body size [greater than 3 standard deviations ( $\sigma_x$ ) from the mean ( $\bar{x}$ )]. We did not obtain bite force measurements for 13 lizards (10 males; 3 females) because they were not motivated to bite the plates. In addition, we were unable to obtain measurements on maximal sprint speed for six lizards (all females) and endurance for one lizard (female). Missing data can bias parameter estimates if data is not missing at random



(Nakagawa and Freckleton 2008; Nakagawa and Freckleton 2011), therefore, we adopted both a complete-case analysis and an analysis with imputed data points for our missing performance traits to test whether our standardized regression coefficients were affected by our missing data. We present the results of our complete-case analysis given that missing data analyses suggested parameter estimates were not strongly affected by the missing individuals. However, missing data patterns and imputation results can be found in the *Supplementary materials*.

We estimated selection gradients using generalized linear models (GLMs) with a Tweedie error distribution (log link) for reproductive success and a binomial error distribution for survival. Tweedie probability distributions contain an index parameter,  $p$ , which permits modeling of a compound Poisson-gamma distribution, allowing us to model zeros and positive, non-integer continuous data (characteristics of relative reproductive success) under a single statistical framework while also controlling for over-dispersion (Noble et al. 2013c). To estimate what value of  $p$  was best for our data we fitted our full models and varied  $p$  between 1.1 – 1.6 in intervals of 0.1 and compared  $AIC_C$  between respective models. Models containing the value of  $p$  with the lowest  $AIC_C$  were deemed the best fit and this value of  $p$  was used for all candidate models in the same analysis.

We present selection gradients from our multivariate GLMs in a similar fashion to LeBas et al. (2004) and Chaine and Lyon (2008) for reproductive success, while we corrected our partial regression coefficients from our logistic regression model for survival (Janzen and Stern 1998). Linear selection gradients ( $\beta_i$ ) indicate selection that changes the population mean while nonlinear selection gradients ( $\gamma_{ii}$ ; quadratic selection gradients or  $\gamma_{ij}$ ; correlational selection gradients) describe how the phenotypic variance of a trait is changed (Brodie III 1992; Brodie III et al. 1995; Lande and Arnold 1983). We converted the number of offspring sired to relative reproductive success (i.e. the number of offspring sired divided by the male and female population mean within each of the six enclosures) and standardized each of the traits by subtracting each value from the trait mean,  $\bar{x}$ , and dividing by its standard deviation,  $\sigma_x$  (Brodie III et al. 1995; Lande and Arnold 1983). We calculated relative reproductive success for the sexes separately in each of the enclosures because of the differences in the mean number of offspring between the sexes. We present linear selection gradients ( $\beta_i$ ) from models without the quadratic and cross products, whereas quadratic and correlational selection gradients come from our full model (Brodie III et al. 1995; Lande and Arnold 1983; Mitchell-

Olds and Shaw 1987). In addition, quadratic terms and their standard errors were doubled (Stinchcombe et al. 2008).

We generated a candidate set of models based on our *a priori* hypotheses (Fig. 1; see Introduction for details). Given that selection is predicted to act differently on performance traits in relation to reproductive success in males and females, we modeled the sexes separately. However, in survival models we pooled the sexes and included sex as a covariate given that we predicted performance traits to be important to survival for both sexes. We have previously shown that body size (SVL) and condition strongly affect reproductive success in males and females (Noble et al. 2013b; Noble et al. 2013c) and there is good reason to believe that these traits are also linked to survival (Civantos and Forsman 2000). Therefore, we included SVL and condition (residuals from regression between log mass and log SVL) in all models. Directional selection gradients were estimated for sprint speed, bite force and endurance in models of reproductive success and survival. However, we included quadratics for SVL, sprint speed and bite force, and interactions between sprint speed and bite force along with bite force and endurance in models of reproductive success (Fig. 2). We calculated the  $\Delta AIC_C$  between our best approximating model and our set of competing candidate models, along with each models Akaike weight ( $w$ ) and evidence ratio (ER) (Burnham and Anderson 2002; Symonds and Moussalli 2011). We avoided discounting models that were within 6  $\Delta AIC_C$  units of each other because simple models are often ranked as the best approximating model when the sample size is small and because we were interested in seeing how predictions from models containing correlational and non-linear parameters related to our hypotheses (Richards 2005; Symonds and Moussalli 2011). We assessed the fit of our top-models by plotting residuals against each predictor variable to test for non-linearity (Zuur et al. 2009). We also checked that residuals were centered on the mean. Since we predicted that ARTs were present in our system and because the systematic structure of our model maybe complex, any predictors showing evidence for non-linearity were carefully scrutinized and we re-fit models containing non-linear parameters to test whether it improved model fit (Zuur et al. 2009).

In situations where there was substantial model uncertainty (i.e.  $< 0.90$  Akaike weight for top model) we model-averaged our coefficients (Grueber et al. 2011; Symonds and Moussalli 2011). Two different model-averaging techniques exist, 'natural' model-averaging (Burnham and Anderson 2002), where coefficients are averaged over the models in which they occur and full model averaging (Lukacs et al. 2010), where coefficients are averaged across

all models in the model set with models not containing the coefficients assumed to have zero estimate. Although it is recommended to use ‘natural averaging’ when there is strong support for a single model (i.e. Akaike weight > 0.90), Symonds and Moussalli (2011) suggest the use of natural averaging when the goal is to understand how predictor variables relate to the response. Since our estimates seemed weak, but potentially biologically important in relation to our predictions, we adopted the natural averaging method (Grueber et al. 2011). Following Symonds and Moussalli (2011), model-averaged estimates and standard errors can be obtained as follows:

$$\beta_{avg} = \frac{\sum_{i=1}^m \omega_i \beta_i}{\sum_{i=1}^m \omega_i}$$

$$se(\beta_{avg}) = \sum \omega_i \sqrt{var(\beta_i) + (\beta_i - \beta_{avg})^2}$$

where  $\beta_i$  is the coefficient estimated in model  $i$  and  $w_i$  is the model Akaike weight. For calculation of the standard error,  $var(\beta_i)$  is the variance estimate for  $\beta_i$  in model  $i$  and all other variables are defined as above. We averaged estimates from models with 6  $\Delta AIC_C$  units of each other using the ‘MuMIn’ package in R (Bartoń 2013). We used our standardized traits ( $\bar{x} = 0$ ,  $\sigma_x = 1$ ).

We visualized top models (within 2  $\Delta AIC_C$ ) using the *vis.gam* function in the R package ‘mgcv’ (Wood 2006). This allowed us to plot the effects of parametric model coefficients on reproductive success while controlling for all other variables in the model. In addition to our parametric model, we fit non-parametric cubic regression splines to the data (Wood 2006). For our top parametric model we fit a smoothing function involving all the input variables in our top parametric model while also including enclosure as a parametric coefficient. We compared model predictions from our smoothed fitness landscape to predictions made from parametric models (Schluter 1988). The smoothing function for each input variable is estimated automatically by minimizing the generalized cross validation (GCV) score. We fit two smoothed models of the form:

$$E(w|z) = EN + f(z_1) \dots + f(z_i) + e$$

$$E(w|z) = EN + f(z_1, \dots, z_i) + e$$

where mean relative reproductive success  $[E(w|z)]$  is a smoothed non-parametric cubic spline of standardized input variables,  $z$ , 1 to  $i$  with error  $e$  controlling for enclosure (EN). In all cases, we avoided extrapolating the fitness landscape too far beyond the bounds of our data (i.e. within 0.08 – 0.10 units of a known data point).

## RESULTS

### *Relationship between morphology, performance and reproductive success in males*

Fifty-six males did not sire any offspring (56/97 = 58%), while 41 males sired at least one offspring (42%). The number of offspring sired by males ranged from 1 – 17, and offspring were sired across 1 – 6 clutches. Relative reproductive success ranged from 0 – 7.65 across individuals. Male body size was strongly correlated with bite force ( $r = 0.62$ ,  $P < 0.001$ ,  $n = 97$ ; Fig. 2b) and endurance ( $r = 0.33$ ,  $P < 0.001$ ,  $n = 97$ ; Fig. 2c). Bite force was also significantly correlated with endurance ( $r = 0.40$ ,  $P < 0.001$ ,  $n = 97$ ; Fig. 2f) however; there were weak, non-significant correlations between all other variables (Table S1; Fig. 2).

Inspection of the distribution of males with above average reproductive success (Fig. 2) showed that large males with high bite force had high reproductive success (Fig. 2b). In contrast, large ( $> 1 \sigma_x$  above the  $\bar{x}$ ) and medium ( $\sim 1 \sigma_x$  above the  $\bar{x}$ ) males that exhibited high and low speeds had the highest reproductive success (Fig. 2a). This pattern was also reflected in the bite force-sprint speed plots where males with high and medium bite force that had low and high sprint speed, respectively, sired the majority of offspring (Fig. 2e). There was no clear pattern between traits and relative reproductive success in the other plots (Fig. 2c, d, f, g).

There was substantial model uncertainty among our candidate model set, with 7 models being within 6  $\Delta AIC_C$  units of each other (Table 1; 'Males') and three models within 2  $\Delta AIC_C$  units of each other (Table 1; 'Males'). Inspection of residuals from our top-model (modelA1.10; Table 1) revealed evidence for non-linearity in body size and body condition with specification tests suggesting significant evidence for curvature ( $P < 0.04$  for body size



and condition). We generated a second set of candidate models that incorporated quadratic and interaction parameters involving only sprint speed, condition and body size to test whether this improved model fit (Table 3). Although the inclusion of non-linear terms improved model fit, model uncertainty remained (Table 3) with almost equal support for five candidate models (bold models in Table 3). We present predictions for our top supported model for simplicity, but other models made qualitatively similar predictions. Our top model predicted a wide fitness peak in plots of sprint speed and body size, with fast-large males (i.e.  $1 \sigma_x$  above the  $\bar{x}$ ) and slow-large males (i.e.  $2 \sigma_x$  above the  $\bar{x}$ ) predicted to have equal reproductive success (Fig. 3a). This wide fitness peak was also evident in plots of sprint speed and body condition (Fig. 3c & d) with slow-good body condition and fast-average body condition individuals predicted to have the highest reproductive success. Predicting the entire fitness surface (Fig. 3d) revealed two fitness peaks with faster-medium condition individuals and slower-good condition individuals predicted to have the highest reproductive success. A single male in poor body condition with high sprint speed (Fig. 2d) appeared to sire a substantial number of offspring. Removing this male and re-running the top model changed inferences slightly (see Fig. S1). Most notably, the model no longer predicted two fitness peaks but rather stronger stabilizing selection for fast males  $1 \sigma_x$  above  $\bar{x}$  (Supplementary materials; Fig. S1c). Our non-parametric cubic regression spline models made similar predictions to our parametric models (Fig. 4). Model-averaged parameter estimates from relative reproductive success models within  $6 \Delta AIC_c$  units of each other can be found in Table S3. Irrespective of the model, directional selection gradients for SVL, body condition and sprint speed were strong while gradients for bite force and endurance were weak (Table 4).

We hypothesized that sprint speed is used by floater males to increase their chances of successfully sneaking matings and if this were the case we would predict that faster sprinters would sire offspring across a larger number of females. There was similar model uncertainty in our models of clutch number (Table 5), however the top model (modelA2.3; Table 5) predicted that medium sized males that were in lower body condition but faster sprinters sired offspring across slightly more clutches (Fig. 5a & b). Model-averaged parameter estimates for clutch number models can be found in Table S3.

*Relationship between morphology, performance and reproductive success in females*

Forty-four females (44/96 = 56%) did not produce offspring, while 52 females (54%) produced from 1 – 9 offspring. Relative reproductive success ranged from 0 – 3.86 for individual females. Female body size was also strongly correlated with bite force ( $r = 0.61$ ,  $P < 0.001$ ,  $n = 96$ ) and endurance ( $r = 0.46$ ,  $P < 0.001$ ,  $n = 96$ ). Female endurance was significantly correlated with body condition ( $r = -0.27$ ,  $P < 0.01$ ,  $n = 96$ ) and bite force ( $r = 0.41$ ,  $P < 0.001$ ,  $n = 96$ ). Maximal sprint speed was also positively correlated with female body condition ( $r = 0.30$ ,  $P < 0.01$ ,  $n = 96$ ). All other variables were not correlated in females (Table S2).

The top supported female model had an Akaike weight of 0.90 and was greater than 6  $\Delta AIC_C$  units from all other candidate models (Table 1; ‘Females’). The top model included only female body size and body condition (Table 4).

*Morphology, performance and survival*

Twenty-eight males (29 %) did not survive over the breeding period while 69 survived (71%;  $n = 97$ ). In contrast, 22 females did not survive (23%) while 74 females survived (77 %;  $n = 96$ ). Although there was a tendency for males to have a reduced survival probability over the breeding period (Table 6), there was weak evidence for sex-dependent survival (Table 6). Body size and endurance were negatively related to the log odds of survival, while increased sprint speed and body condition positively influenced the log odds of survival. However, in all cases there was weak evidence that these traits explained variance in survival across the breeding season (Best supported model was the null; Table 6).

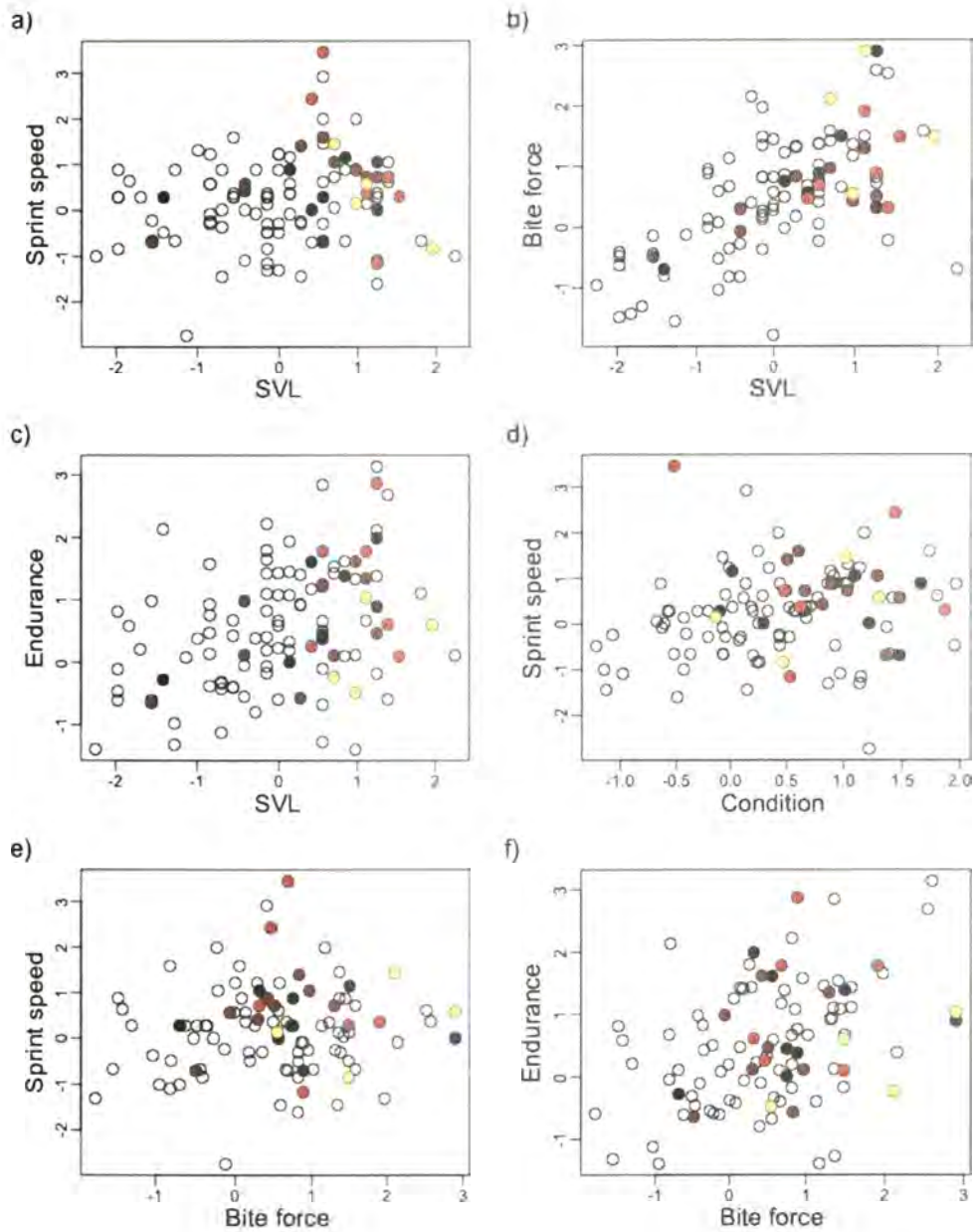


Fig. 2 – Relationship between morphological traits [body size (SVL), body condition] and performance traits (sprint speed, bite force and endurance) for males. Relative reproductive success (RRS) of each male is overlaid on these plots. Open circles:  $RRS \leq 1$ , solid black circles:  $RRS = 1 - 2$ , Solid brown circles:  $RRS = 2 - 4$ , Solid red circles:  $RRS = 4 - 6$  and Solid yellow circles:  $RRS \geq 6$ .

Table 1 – Candidate models evaluated for the relationship between relative reproductive success, morphology and performance for both males and females. For each model we present their AIC<sub>C</sub>, ΔAIC<sub>C</sub>, Akaike *w*, and evidence ratio (ER). Sample size (*n*) and the number of parameters estimated (*k*) are also provided. “NL” indicates that the model is “not likely”. All phenotypic traits are standardized ( $\bar{x} = 0$ ,  $\sigma_x = 1$ ). Models in bold are those within 6 ΔAIC<sub>C</sub> units of each other (Richards 2005; Symonds and Moussalli 2011). See Table 2 for model formula details. Sample sizes for males was *n* = 97 and females *n* = 96.

<b>Males</b>					
<b>Model</b>	<b>k</b>	<b>AIC<sub>C</sub></b>	<b>ΔAIC<sub>C</sub></b>	<b>Akaike <i>w</i></b>	<b>ER</b>
modelA1.1	16	289.98	14.98	0	1790.05
<b>modelA1.2</b>	<b>11</b>	<b>279.86</b>	<b>4.86</b>	<b>0.031</b>	<b>11.36</b>
modelA1.3	13	282.55	7.55	0.008	43.6
modelA1.4	13	284.86	9.86	0.003	138.38
modelA1.5	14	287.58	12.58	0.001	539.15
modelA1.6	14	284.83	9.83	0.003	136.32
<b>modelA1.7</b>	<b>12</b>	<b>280.70</b>	<b>5.70</b>	<b>0.020</b>	<b>17.29</b>
<b>modelA1.8</b>	<b>11</b>	<b>278.12</b>	<b>3.12</b>	<b>0.074</b>	<b>4.76</b>
<b>modelA1.9</b>	<b>10</b>	<b>277.32</b>	<b>2.32</b>	<b>0.111</b>	<b>3.19</b>
<b>modelA1.10</b>	<b>9</b>	<b>275.00</b>	<b>0</b>	<b>0.353</b>	-
modelA1.11	6	304.77	29.77	0	NL
<b>modelA1.12</b>	<b>8</b>	<b>275.71</b>	<b>0.71</b>	<b>0.247</b>	<b>1.43</b>
<b>modelA1.13</b>	<b>10</b>	<b>276.72</b>	<b>1.72</b>	<b>0.149</b>	<b>2.36</b>

<b>Females</b>					
<b>Model</b>	<b>k</b>	<b>AIC<sub>C</sub></b>	<b>ΔAIC<sub>C</sub></b>	<b>Akaike <i>w</i></b>	<b>ER</b>
modelA3.1	16	279.57	13.12	0.001	706.27
modelA3.2	11	273.30	6.85	0.029	30.72
modelA3.3	13	276.01	9.56	0.008	119.10
modelA3.4	13	276.54	10.09	0.006	155.24
modelA3.5	14	276.81	10.36	0.005	177.68
modelA3.6	14	276.90	10.45	0.005	185.86
modelA3.7	12	275.60	9.15	0.009	97.03
modelA3.8	11	273.07	6.62	0.033	27.39
<b>modelA3.9</b>	<b>8</b>	<b>266.45</b>	<b>0</b>	<b>0.904</b>	-
modelA3.10	6	286.07	19.62	0	NL



Table 2 – Model formula for candidate models evaluated in Table 1.

<b>Males</b>	
<b>Model</b>	<b>Formula</b>
modelA1.1	RelRS ~ EN + SVL + COND + END + END <sup>2</sup> + SS + SS <sup>2</sup> + BF + BF <sup>2</sup> + BF*END +
modelA1.2	RelRS ~ EN + SVL + COND + END + SS + BF
modelA1.3	RelRS ~ EN + SVL + COND + END + SS + BF + BF <sup>2</sup> + BF*SS
modelA1.4	RelRS ~ EN + SVL + COND + END + SS + BF + BF <sup>2</sup> + BF*END
modelA1.5	RelRS ~ EN + SVL + COND + END + END <sup>2</sup> + SS + BF + BF <sup>2</sup> + BF*END
modelA1.6	RelRS ~ EN + SVL + COND + END + SS + SS <sup>2</sup> + BF + BF <sup>2</sup> + BF*SS
modelA1.7	RelRS ~ EN + SVL + COND + END + SS + BF + BF*SS
modelA1.8	RelRS ~ EN + SVL + COND + SS + BF + BF*SS
modelA1.9	RelRS ~ EN + SVL + COND + SS + BF
modelA1.10	RelRS ~ EN + SVL + COND + SS
modelA1.11	RelRS ~ EN
modelA1.12	RelRS ~ EN + SVL + COND
<b>Females</b>	
<b>Model</b>	<b>Formula</b>
modelA3.1	RelRS ~ EN + SVL + COND + END + END <sup>2</sup> + SS + SS <sup>2</sup> + BF + BF <sup>2</sup> + BF*END +
modelA3.2	RelRS ~ EN + SVL + COND + END + SS + BF
modelA3.3	RelRS ~ EN + SVL + COND + END + SS + BF + BF <sup>2</sup> + BF*SS
modelA3.4	RelRS ~ EN + SVL + COND + END + SS + BF + BF <sup>2</sup> + BF*END
modelA3.5	RelRS ~ EN + SVL + COND + END + END <sup>2</sup> + SS + BF + BF <sup>2</sup> + BF*END
modelA3.6	RelRS ~ EN + SVL + COND + END + SS + SS <sup>2</sup> + BF + BF <sup>2</sup> + BF*SS
modelA3.7	RelRS ~ EN + SVL + COND + END + SS + BF + BF*SS
modelA3.8	RelRS ~ EN + SVL + COND + SS + BF + BF*SS
modelA3.9	RelRS ~ EN + SVL + COND
modelA3.10	RelRS ~ EN

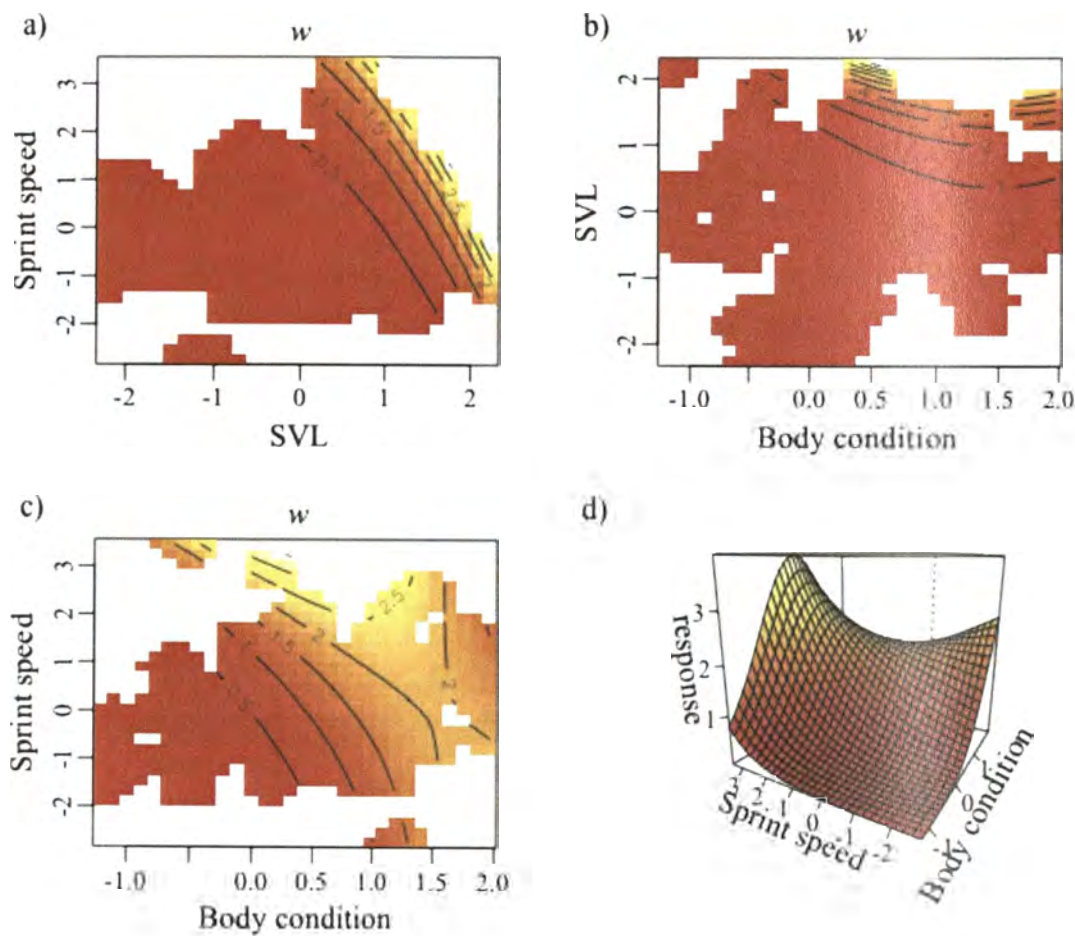


Fig. 3 – Contour plots (a – c) and surface plot (d) of predicted relative reproductive success ( $w$ ; ‘response’). a) Predicted relative reproductive success as a function of sprint speed and body size (SVL) while controlling for body condition ( $\bar{x} = 0$ ); b) Predicted relative reproductive success as a function of body size (SVL) and condition while controlling for sprint speed ( $\bar{x} = 0$ ); c & d) Predicted relative reproductive success as a function of sprint speed and body condition while controlling for body size ( $SVL = 1 \sigma_x$ ). Note that model predictions are only relevant for larger males ( $> 1 \sigma_x$  above  $\bar{x}$ ). Predictions are made from modelA1.5 (Table 3).

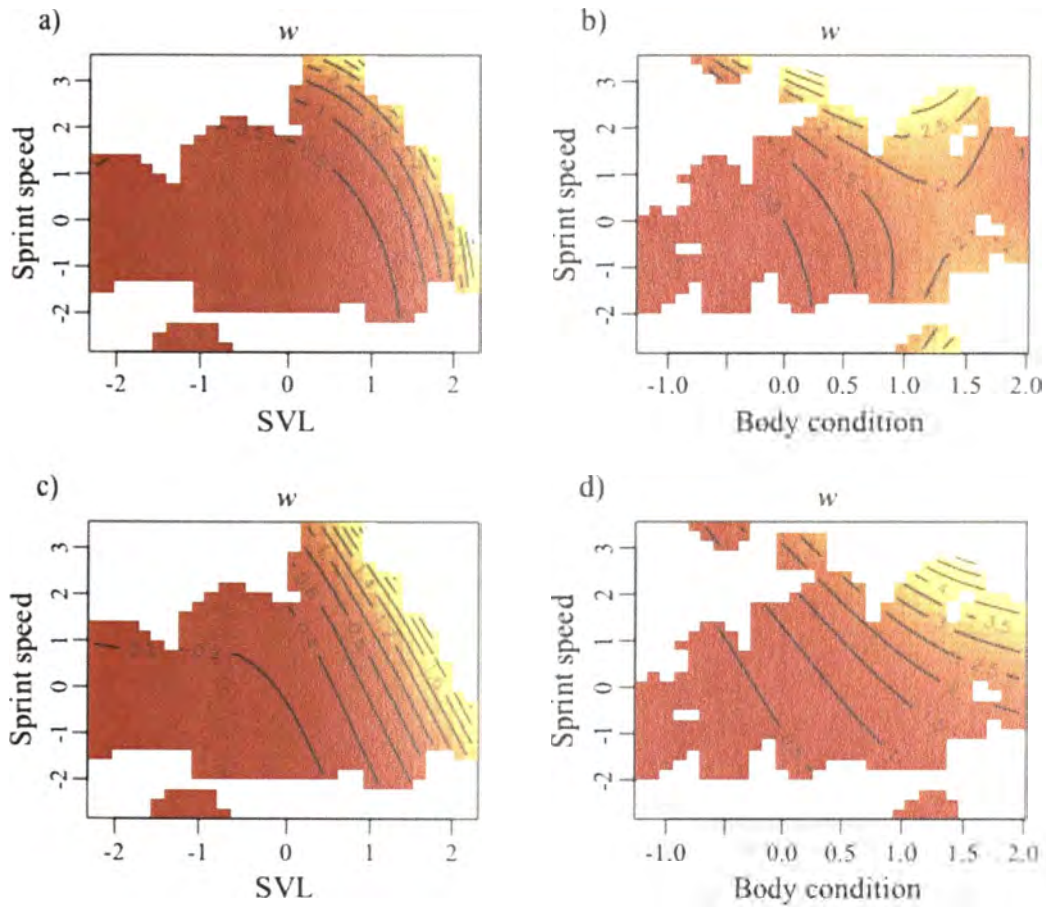


Fig. 4 – Contour plots of predicted relative reproductive success for males ( $w$ ) from non-parametric cubic spline regression. Two different models were fit to the data: a & b)  $E(w|z) = EN + f(z_1 \dots z_i) + e$ ; c & d)  $E(w|z) = EN + f(z_1) \dots f(z_i) + e$ . See methods for details. a & c) Predicted relative reproductive success as a function of sprint speed and body size (SVL) while controlling for body condition ( $\bar{x} = 0$ ); b & d) Predicted relative reproductive success as a function of sprint speed and body condition while controlling for large males ( $\bar{x} = 1$ ).

Table 3 – Candidate set of models evaluated to correct for non-linearity in model residuals. The top model from our original candidate set is listed for comparison to alternative models (modelA1.9). Bolded models are those with 2  $\Delta AIC_C$  units of each other and are considered equally plausible. Final sample size for models,  $n = 97$ .

Model	k	$AIC_C$	$\Delta AIC_C$	Akaike $w$	ER	Model Formula
modelA1.1	11	275.67	1.33	0.101	1.94	RelRS ~ EN + SVL + COND + COND <sup>2</sup> + SS + SS <sup>2</sup>
modelA1.2	12	277.16	2.82	0.048	4.10	RelRS ~ EN + SVL + COND + COND <sup>2</sup> + SS + SS <sup>2</sup> + COND*SS
<b>modelA1.3</b>	<b>11</b>	<b>274.84</b>	<b>0.50</b>	<b>0.153</b>	<b>1.28</b>	<b>RelRS ~ EN + SVL + COND + COND<sup>2</sup> + SS + COND*SS</b>
modelA1.4	11	277.55	3.21	0.039	4.98	RelRS ~ EN + SVL + COND + SS + SS <sup>2</sup> + COND*SS
<b>modelA1.5</b>	<b>12</b>	<b>274.34</b>	<b>0</b>	<b>0.196</b>	<b>1</b>	<b>RelRS ~ EN + SVL + SVL<sup>2</sup> + COND + COND<sup>2</sup> + SS + COND*SS</b>
modelA1.6	13	276.90	2.56	0.055	3.60	RelRS ~ EN + SVL + SVL <sup>2</sup> + COND + COND <sup>2</sup> + SS + COND*SS + COND*SVL
<b>modelA1.7</b>	<b>11</b>	<b>274.95</b>	<b>0.61</b>	<b>0.145</b>	<b>1.36</b>	<b>RelRS ~ EN + SVL + SVL<sup>2</sup> + COND + SS + COND*SS</b>
<b>modelA1.8</b>	<b>10</b>	<b>275.29</b>	<b>0.95</b>	<b>0.122</b>	<b>1.61</b>	<b>RelRS ~ EN + SVL + SVL<sup>2</sup> + COND + SS</b>
<b>modelA1.9</b>	<b>9</b>	<b>275.00</b>	<b>0.66</b>	<b>0.141</b>	<b>1.39</b>	<b>RelRS ~ EN + SVL + COND + SS</b>



Model	k	AIC <sub>C</sub>	ΔAIC <sub>C</sub>	Akaike <i>w</i>	ER	Model Formula
<b>modelA2.1</b>	<b>9</b>	<b>227.55</b>	<b>1.72</b>	<b>0.137</b>	<b>2.36</b>	NC ~ EN + SVL + COND + SS
modelA2.2	10	229.01	3.18	0.066	4.90	NC ~ EN + SVL + COND + SS + SS <sup>2</sup>
<b>modelA2.3</b>	<b>10</b>	<b>225.83</b>	<b>0</b>	<b>0.323</b>	<b>1</b>	NC ~ EN + SVL + COND + COND <sup>2</sup> + SS
<b>modelA2.4</b>	<b>11</b>	<b>227.34</b>	<b>1.51</b>	<b>0.152</b>	<b>2.13</b>	NC ~ EN + SVL + SVL <sup>2</sup> + COND + COND <sup>2</sup> + SS
<b>modelA2.5</b>	<b>11</b>	<b>225.84</b>	<b>0.01</b>	<b>0.322</b>	<b>1.01</b>	NC ~ EN + SVL + COND + COND <sup>2</sup> + SS + SS*COND

Table 5 – Candidate models evaluated for predictors of the number of clutches a male sired. Model formula abbreviations are as follows: NC: number of clutches sired across; EN: enclosure; SVL: Body size; COND: Body condition; SS: Sprint speed. Final sample size for model evaluation, *n* = 97.

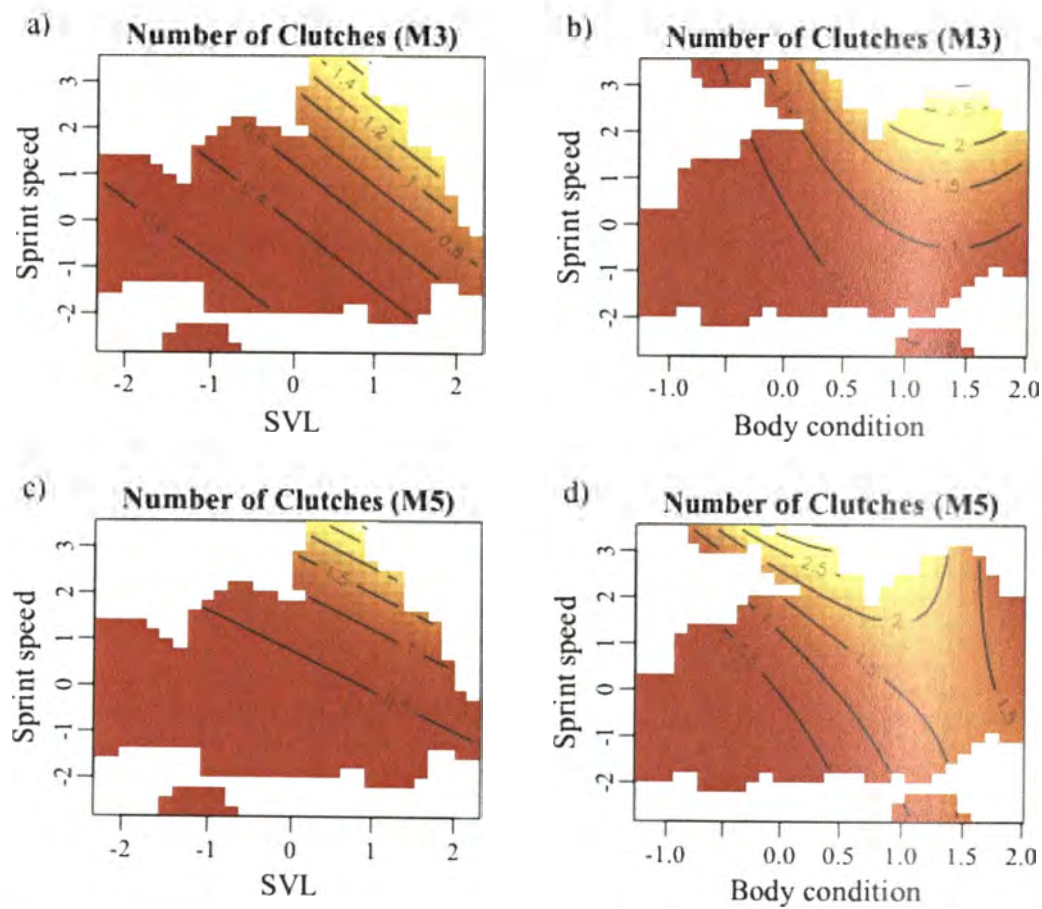


Fig. 5 – Contour plots of the predicted number of clutches as a function of sprint speed and body condition (a & c) and sprint speed and body size (b & d) from modelA2.3 (M3; Table 5) and modelA2.5 (M5; Table 5).

Table 4 – Parameter estimates, standard errors (se) and upper (U) and lower (L) 95% confidence intervals from GLMs with relative reproductive success (RRS) [males and females] and number of clutches sired (males) as dependent variables. Note that all input variables are standardized to ( $\bar{x} = 0, \sigma_x = 1$ ). Parameter estimates for directional selection gradients for bite force and endurance are also provided for reference even though there was weak support for the inclusion of the parameters in final models. Model averaged parameter estimates are also presented in Table S3 for the full set of models within 6  $\Delta AIC_C$  units of each other. The model number estimates come from are provided. Note that enclosure is controlled for in all models, but is not presented for simplicity. Directional selection gradients are presented from models with only main effects and quadratic parameters and standard errors are doubled. Upper and lower confidence intervals are calculated by multiplying standard errors by 1.96. Note that all parameters are taken directly from our GLMs.

Parameter	Est.	se	RRS (Males)		Model	Est.	se	RRS (Females)		Model	Est.	se	Number of clutches (Males)		Model
			U	L				U	L				U	L	
Body size	0.79	0.19	1.16	0.42	ModA1.10; T2	0.67	0.13	0.92	0.42	ModA3.9	0.48	0.16	0.79	0.17	ModA2.1
Body condition	0.77	0.22	1.20	0.34	ModA1.10; T2	0.36	0.13	0.62	0.11	ModA3.9	0.62	0.19	0.99	0.25	ModA2.1
Sprint speed	0.28	0.16	0.59	-0.03	ModA1.10; T2	-	-	-	-	ModA3.2	0.31	0.14	0.58	0.04	ModA2.1
Body size <sup>2</sup>	0.66	0.34	1.33	-0.01	ModA1.5; T3	-	-	-	-	-	-	-	-	-	-
Body condition <sup>2</sup>	-1.12	0.62	0.10	-2.34	ModA1.5; T3	-	-	-	-	-	-0.57	0.29	-0.002	-1.14	ModA2.3
BC * SS	-0.35	0.22	0.08	-0.78	ModA1.5; T3	-	-	-	-	-	-	-	-	-	-
<b>Parameters</b>															
Bite Force	0.09	0.21	0.50	-0.32	ModA1.2; T2	-0.06	0.22	0.37	-0.49	ModA3.2	-	-	-	-	-
Endurance	-0.01	0.19	0.36	-0.38	ModA1.2; T2	0.06	0.23	0.51	-0.39	ModA3.2	-	-	-	-	-

Table 6 – Log odds and standardized (Bavggrad; Janzen and Stern 1998) selection differentials for the effects of body size (SVL), body condition, sprint speed and endurance on survival probability during the breeding period. Each variable was dropped from the full model to assess impact of its removal on model explanatory power. All models were compared with a null model (intercept only). Estimates are presented from the full model, which contained enclosure. All phenotypic traits were standardized ( $\bar{x} = 0$ ,  $\sigma_x = 1$ ) prior to analysis. Final sample size for the analysis included 97 males and 96 females ( $n = 193$ ) for which we had complete data.

Coefficient	Logistic regression		Bavggrad
	Estimate	SE	Estimate
Intercept	0.68	0.44	NA
Sex (M)	-0.34	0.47	NA
Body condition	0.16	0.21	0.040
SVL	-0.03	0.18	-0.009
Sprint Speed	0.06	0.19	0.020
Endurance	-0.14	0.22	-0.030

Single term deletion	AIC <sub>C</sub>	ΔAIC <sub>C</sub>
Full model	233.29	10.45
Enclosure	229.49	6.65
Sex	231.56	8.72
Body condition	231.62	8.78
SVL	231.08	8.24
Sprint Speed	231.15	8.31
Endurance	231.44	8.60
Null	222.84	0



## DISCUSSION

We found support for the hypothesis that locomotor traits are positively related to reproductive success in males and that these do not indirectly affect reproductive success through decreased survival during the breeding season (Hypothesis 1; Fig. 1). As predicted, the effect of locomotor performance on reproductive success was sex-dependent. Fast and slow males had high fitness while there was no evidence that sprint speed increased reproductive success in females. Contrary to our predictions, after controlling for body size and condition, neither bite force nor endurance were directly related to male reproductive success even though larger males tended to have higher bite force and endurance. Interestingly, we did find some support for prediction 2 (Fig. 1d), with larger males that were both fast and slow sprinters predicted to have the highest reproductive success. Unexpectedly, there was also evidence for non-linear/correlational selection between sprint speed and body condition in large males with average body condition/fast sprinters and high body condition/slow sprinters being predicted to have the highest reproductive success. We did not find evidence that body size or locomotor performance was related to survival during the breeding season.

### *Alternative reproductive tactics and a role for behaviour in understanding patterns of selection on whole-organism performance*

Sexual selection often leads to males adopting status-dependent alternative reproductive tactics (Andersson 1994; Gross 1996). The integration of behavioural, physiological and performance traits permit males to acquire paternity through subtly different mechanisms and such behavioural variation has important consequences for our understanding of selection on performance in nature (Irschick 2002). In *E. quoyii*, sprint speed is a sexually selected trait, while endurance and bite force were indirectly selected through selection for large body size. Sprint speed results are congruent with other lizard species, which have shown positive relationships between male dominance/reproductive success and sprint speed (Husak et al. 2006a; Husak et al. 2008; Robson and Miles 2000). However, there are notable exceptions in territorial species (Perry et al. 2004) and predictions will likely depend strongly on an understanding of the different behavioural tactics used by individual males and the specifics of the mating system. Dominant lizards that defend large territories may benefit by being faster

through better defense of territories or greater efficiency in guarding females (Husak et al. 2006a; Husak et al. 2008; Noble et al. 2013a). However, an equally plausible hypothesis is that sprint speed may be more important for sub-ordinate males for sneaking copulations from females (Husak et al. 2008). Indeed, male *E. quoyii* with low sprint speed were also predicted to have similar reproductive success to males with high sprint speed likely reflecting the different behavioral tactics in this species (Noble et al. 2013c). Males with slower speeds were larger than males adopting faster speeds, suggesting they were more dominant males given the positive correlations between body size, bite force and endurance and the strong associations between these traits and dominance in other lizards. The wide fitness peak spanning from fast sprinters to slow sprinters among large males is consistent with our understanding of ARTs in this system (Noble et al. 2013c). Body size is the most important determinant of reproductive success in *E. quoyii*, however, large males have been shown to adopt both floater and territorial/resident tactics, whereby floaters have large home ranges, are observed little, but move frequently and territorials have small home ranges, move little but are commonly observed active (Noble et al. 2013c; Stapley and Keogh 2004; Stapley and Keogh 2005). Although we caution labeling the tactics along this fitness peak given our limited understanding of dominance and territoriality in *E. quoyii*, these results do suggest that sprint speed maybe less important for territorial lizards given they are generally considered to have smaller home ranges (Morrison et al. 2002; Stapley and Keogh 2004; Stapley and Keogh 2005). Speed might be more important for floaters if they are sneaking copulations or pursuing females over large distances. Indeed, we found support that sprint speed was associated with siring a larger number of clutches, an important feature thought to be associated with floater males (Noble et al. 2013c).

Interestingly, both parametric and non-parametric models identified evidence for non-linear and correlational selection between sprint speed and body condition that resulted in two fitness peaks among large males. Although this result is still preliminary, given model uncertainty, this does provide clues to tactic adoption in *E. quoyii*. Average body condition-fast sprinters and high body condition-slow sprinters are predicted to have the highest reproductive success suggesting that tactic adoption in *E. quoyii* may depend both on male body size and condition. Status-dependent tactics are often the result of differences in size, condition and/or age between dominant and subordinate males (Oliviera et al. 2008) and future

experimental work manipulating body condition for different sized males will provide insight into how these traits interact to influence both behavior and performance.

*Sex-specific selection and the evolution of sex differences in performance*

The sexes of many species differ in whole-organism functional performance (Van Damme et al. 2008). In lizards, males most often have higher sprinting speed, endurance and bite force (Lappin et al. 2006; Van Damme et al. 2008). Sex differences have been attributed to sexual dimorphism in body size and head dimensions as a result of sexual and natural selection on morphology or performance (Van Damme et al. 2008). In *E. quoyii*, males and females do not differ in body size, but they do differ in head and limb dimensions (Noble et al. 2013a; Schwarzkopf 2005) and exhibit sex differences in bite force, endurance and sprinting speed (Noble et al. 2013a). We show strong sexual selection for large body size in males and females, which may explain the lack of sexual dimorphism we see in this species. We also found selection on sprinting speed in males but not females providing a possible evolutionary explanation for sex differences in sprinting performance in *E. quoyii*. In contrast, there was no evidence for sexual selection on maximal endurance or bite force despite sex-differences (Noble et al. 2013a). Sex-differences in endurance may be a result of indirect selection for higher sprint speeds and possibly longer limbs (Husak et al. 2006a), which permit males to run more efficiently. Alternatively, differences in mass (either due to muscle mass or energy stores) and muscle and body physiology may all contribute to males being able to run for longer periods (Van Damme et al. 2008). Reasons for bite force differences between the sexes are unclear. Noble et al. (2013a) showed that an important anabolic steroid, testosterone, did not differ between male and female *E. quoyii*, however there were differences in head dimensions. Bite force is closely linked to head dimensions (Herrel et al. 2007) and different ontogenetic trajectories in head shape or muscle development, which might be mediated by androgens may result in differences in bite force between the sexes. Differences in aggression levels or dietary preferences between the sexes might also help build jaw musculature and lead to increased bite force in males.



*Contrasting sexual and natural selection on locomotor performance*

Sexual and natural selection can act in similar or opposing directions on phenotypic traits (Andersson and Iwasa 1996), yet it is predicted that both sexual and natural selection act in unison on locomotor traits (Irschick et al. 2008). Better performing individuals are predicted to be more dominant during intra-sexual encounters (Lailvaux and Irschick 2006; Robson and Miles 2000) while also having a higher probability of escaping predatory attacks. The breeding season is a period of high male activity, given the need to defend territories and resources, which are essential in securing females. High levels of activity and conspicuousness can also make males more susceptible to predation (Marler and Moore 1988) and thus natural selection is predicted to be strong during this period. We found little evidence for natural selection on locomotor traits in *E. quoyii*, during the breeding season. This contrasts, with other studies showing increased probability of survival for higher performers (Irschick et al. 2008; Le Galliard and Ferrière 2008) and maybe the result of the very short breeding season in *Eulamprus* (Head et al. 2005; Vernon 1969) or may simply be an artifact of our experimental design given the limited number of predators in enclosures. Nonetheless, our results suggest that sexual selection is strong on sprint speed, while natural selection is weak and may explain why there is such a wide fitness peak from high sprinting to low sprinting males in this species and may aid in the evolution of ARTs in this system. Long-term studies on the role of natural selection on locomotor traits along with an understanding of plasticity in these traits through the season will be necessary to clarify the role natural selection plays in shaping the adaptive landscape.

*Conclusions*

Evolutionary biologists have expressed great interest in understanding how selection acts on whole-organism performance traits given their more direct relationship with fitness (Arnold 1983; Irschick et al. 2008), yet most work has focused solely on either natural or sexual selection and on a subset of performance traits (Irschick et al. 2008). Indeed, there have only been a few studies quantifying sexual selection (i.e. reproductive success) on performance traits that we are aware of (Husak et al. 2006a; Le Galliard and Ferrière 2008) and only one study has explicitly examined how behavioural attributes of individuals relate to patterns of



selection on functional performance traits (Husak et al. 2006a; Husak et al. 2008). Our results suggest that bite force and endurance were not direct targets of selection and although sprint speed is a sexually selected trait in *E. quoyii*, individuals with both high and low speed can achieve high fitness possibly through different behavioural mechanisms. Indeed, sexual selection on speed may help explain the evolution of sexual dimorphism in traits and performance between the sexes. Our study is the first to quantify both natural and sexual selection on multiple performance traits in a single species. We suggest that sexual selection on performance capacity has been underappreciated in lizards and as a result has rarely been quantified in nature, providing considerable scope to improve our understanding of these integrated traits that may be direct targets of selection.

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## SUPPLEMENTARY MATERIALS

### *Missing Data Patterns and Multiple imputation*

Missing data mechanisms can be classified into one of three different types: 1) Missing completely at random (MCAR); 2) Missing at random (MAR) and 3) Missing not at random (MNAR) (Nakagawa and Freckleton 2008; Nakagawa and Freckleton 2011). When missing data is not MCAR this can bias parameter estimation and influence model selection (Nakagawa and Freckleton 2011). Missing data imputation is a suitable tool when the pattern of missing data is MCAR or MAR (i.e. missing observations are systematically related to a known variable in the dataset) (Nakagawa and Freckleton 2011). Detection of whether data follows one of the above missing data mechanisms can be achieved by: 1) testing for mean differences in variable  $x_i$  [where  $i = 1 \dots n$  (i.e. number of variables)] with the groups being defined by the ‘observed’ and ‘missing’ values for variable  $z$  (Little 1988); 2) conducting logistic regressions between a binary coded missing variable,  $R$ , and other variables in the dataset to test whether they can predict patterns of observed or missing values (Nakagawa and Freckleton 2011); 3) Graphing missing data patterns to understand how ‘missing-ness’ in one variable relates to known values in a second variable (van Buuren and Groothuis-Oudshoorn 2011). Under the assumption of MAR, data imputation occurs by imputing missing data using either a model-based (e.g. multivariate normal model) or MCMC method with variables known to explain variation in the missing variable used as predictors in the model. This imputation step is done repeatedly to create  $x$  independent datasets. Generally, 3 – 5 datasets are sufficient for robust estimation (van Buuren and Groothuis-Oudshoorn 2011). These imputed datasets are then re-analyzed and an average parameter estimate can be computed along with the variability of these estimates across the data sets as follows (Nakagawa and Freckleton 2011):

$$\beta_{pooled} = \frac{1}{M} \sum_{i=1}^M \beta_i$$



$$var_{withinimp} = \frac{1}{M} \sum_{i=1}^M var_i$$

$$var_{acrossimp} = \frac{1}{M-1} \sum_{i=1}^M (\beta_i - \beta_{pooled})^2$$

$$Tot_{var} = var_{withinimp} + \left(1 + \frac{1}{M}\right) var_{acrossimp}$$

where  $\beta_{pooled}$  is the average parameter estimate calculated from each estimate from imputed data set  $M_i$  ( $i = 1 \dots n$ ),  $var_{withinimp}$  is the variance within a single imputation around the  $\beta_i$  estimate (i.e. standard error of estimate squared),  $var_{acrossimp}$  is the variance between estimates from different imputed data sets and  $Tot_{var}$  is the total variance around the pooled estimate.

We excluded a total of 23 lizards from our analysis because they were missing one or more performance measure or because they were extreme outliers (2 lizards). Although the proportion of missing data is low ( $23/216 = 11\%$ ) it is easy to envisage these data missing systematically. For example, suppose that faster sprinters are less motivated to bite while lizards that bite harder are less likely to sprint. In this case our missing data is related to our observed data in a systematic manor and may have important consequences for parameter estimation. This is possible given that we had sprint speed data for 10 males for which we did not have bite force data and sprint speed appears to influence reproductive success in males and be related to body size and presumably bite force. Given the high variance in male reproductive success these 10 males may have important consequences on inferences. This might be particularly true give that our response variable of models is related in a non-linear fashion to our predictors. Given the importance of sprint speed to fitness, we ran a second selection analysis using imputed data to understand whether our missing values affected our inferences. This was made possible given the strong relationships between some of our morphological variables and performance traits and also sex-based differences in performance (Noble et al. 2013). For example, Table S1 and S2 provide correlations between

morphological and performance traits for both males and females and can be used to inform imputation models. Furthermore, Noble et al. (2013) shows links between head dimensions and temperature in explaining variation in bite force, all of which can be used to generate predictive models of missing bite force data. Using this information, we generated a prediction matrix (Table S4) defining the traits used to impute missing data in the variables of interest.

To impute missing data we used the R package ‘mice’ (Multiple Imputation using Chained Equations) (van Buuren and Groothuis-Oudshoorn 2011). This is a highly flexible imputation package, which allows for the specification of a multitude of different univariate imputation models, while also permitting control over the predictors used to impute missing data. We used predictive mean matching (pmm), which is a form of semi-parametric imputation model. This is a good imputation model for numeric data because it can preserve non-linear relations even with uncertainty around the systematic structure of model (van Buuren and Groothuis-Oudshoorn 2011). Using our standardized variables we imputed each of our missing data following TableS4. We created 5 independent datasets with 50 iterations for each imputation. We excluded the two individuals, which were extreme outliers in mass for all analyses (1 male; 1 female) as they remained problematic for imputations. For all other individuals we had complete morphological data and we used the relationships between the variables to impute performance traits ( $n = 107$  males and females; 214 total). We graphically assessed missing data patterns (Fig. S2). There was no strong evidence that the 10 males with missing bite force differed drastically from observed values in SVL, sprint speed or endurance (Fig. S2) and in all cases the frequency of missing information,  $\gamma$ , was well below 0.10 suggesting that there was little evidence that our data followed a MNAR mechanism (Nakagawa and Freckleton 2011). We therefore assumed our data to be MAR. For simplicity we re-ran only our top supported models for both males and females to understand how parameter estimates changed with the addition of the missing data. Independent imputations showed little variance in estimates with almost all variance being the result of within imputation variance in males. The variance around estimates in the top male model increased with the addition of the extra individuals, however, the model predicted a similar outcome to models containing only  $n = 97$  individuals, suggesting little bias in parameter estimates as a result of our 10 missing males (Table S5). This pattern was the same for models of the number of clutches a male sired (Table S5). There was greater variation in parameter estimates in

female models (Table S5), but there was again little change when including the seven females with missing sprint speed data.

**Table S1** – Pearson’s correlation coefficients between body size, body condition, sprint speed, bite force and endurance in males. Below diagonal are coefficients and above diagonal is the significance of coefficients. Sample size for all correlations is  $n = 97$ . ‘\*\*\*\*’  $P < 0.001$ ; ‘\*\*\*’  $P < 0.01$ ; ‘NS’ not significant.

	SVL	Body condition	Sprint speed	Bite force	Endurance
SVL	-	NS	NS	***	***
Body condition	0.10	-	NS	NS	NS
Sprint speed	0.18	0.20	-	NS	NS
Bite force	0.62	0.16	0.09	-	***
Endurance	0.33	-0.06	0.08	0.40	-

**Table S2** – Pearson’s correlation coefficients between body size, body condition, sprint speed, bite force and endurance in females. Below diagonal are coefficients and above diagonal is the significance of coefficients. Sample size for all correlations is  $n = 96$ . ‘\*\*\*\*’  $P < 0.001$ ; ‘\*\*\*’  $P < 0.01$ ; ‘NS’ not significant.

	SVL	Body condition	Sprint speed	Bite force	Endurance
SVL	-	NS	NS	***	***
Body condition	-0.11	-	**	NS	**
Sprint speed	0.14	0.30	-	NS	NS
Bite force	0.61	-0.08	0.06	-	***
Endurance	0.46	-0.27	0.11	0.41	-

**Table S3** – Model-averaged coefficients for models of relative reproductive success and number of clutches, within 6  $\Delta\text{AIC}_C$  units of each other. For details on models see Tables 2 – 4 in main manuscript. Model coefficients are from models of  $n = 97$  male *E. quoyii* with standardized traits ( $\bar{x} = 0$ ,  $\sigma_x = 1$ ). Natural model-averaging was used (see main manuscript for more details).



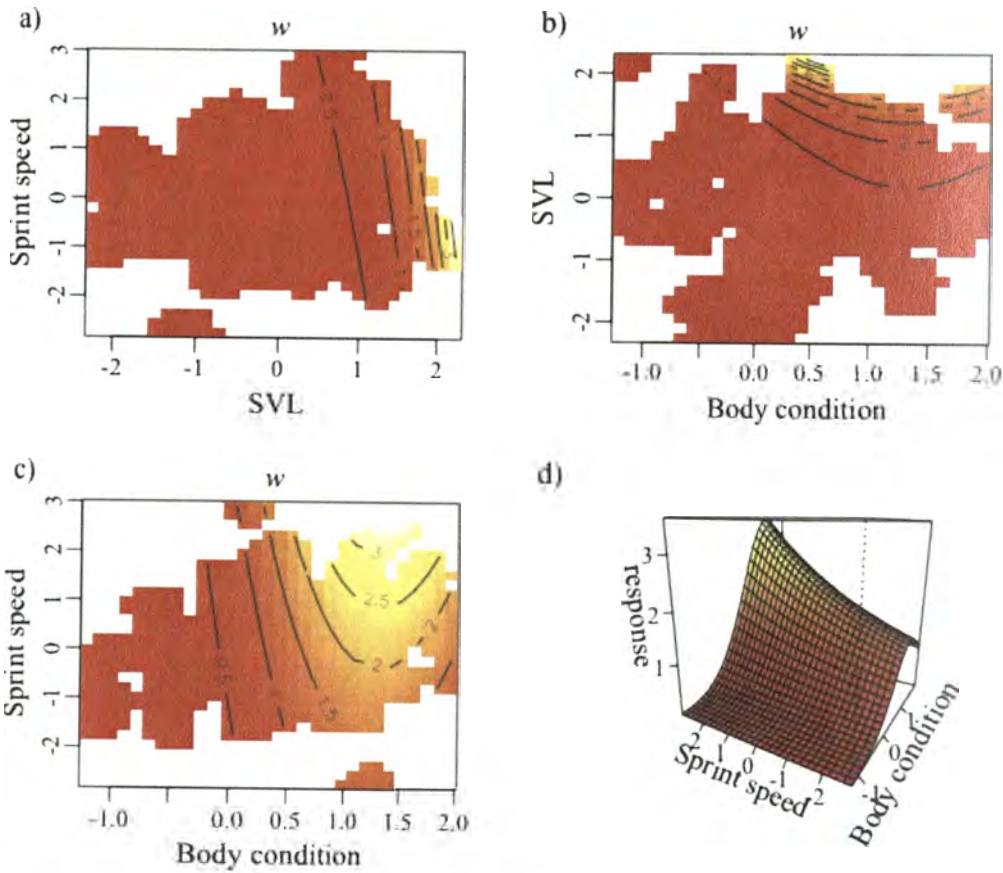
Coefficient	Relative RS		Number of clutches	
	$\beta_{avg}$	$se_{avg}$	$\beta_{avg}$	$se_{avg}$
SVL	0.75	0.20	0.43	0.17
SVL <sup>2</sup>	0.30	0.17	0.17	0.15
Body condition	1.20	0.56	1.35	0.59
Body condition <sup>2</sup>	-0.55	0.31	-0.59	0.30
Sprint speed	0.38	0.24	0.38	0.19
Body condition * Sprint speed	-0.33	0.22	-0.33	0.19
Sprint speed <sup>2</sup>	0.08	0.10	0.08	0.07
Body condition * SVL	-0.09	0.27	-	-
Bite force	0.07	0.22	-	-
Bite force * Sprint speed	0.29	0.20	-	-
Endurance	0.02	0.20	-	-
Bite force <sup>2</sup>	-0.10	0.15	-	-
Bite force * Endurance	-0.08	0.21	-	-
Endurance <sup>2</sup>	-0.03	0.15	-	-

**Table S4** – Predictor matrix used for multiple imputations in ‘mice’. ‘0’ in the matrix indicates that predictors were not used to impute values of the row variables, while ‘1’ in the matrix indicates that predictors were used to impute row variable values. For example, ‘cenMass’ was imputed using predictive mean matching with the variables Sex (1), cenSVL (12), cenTail Length (13), cenTail width (14), cenInter-limb length (ITLL; 18), cenSprintSpeed (20), cenEndurance(21). The abbreviation ‘cen’ means that variables are standardized ( $\bar{x} = 0, \sigma_x = 1$ ).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	Specimen.ID	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Sex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Enclosure	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Temp.BiteForce	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
5	Temp.Endur	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
6	Temp.SS.2m	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
7	Survival	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	NumOffspring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	Num.Clutches	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	RelRS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	cenMass	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1	1	0
12	cenSVL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	cenTailLen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	cenTailWidth	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
15	cenHeadwidth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	cenHeadlen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	cenHeadDep	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	cenITLL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	cenBiteForce	0	1	0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	1	1	0
20	cenSprintSpeed	0	1	0	1	0	1	0	0	0	1	1	1	1	1	0	0	0	1	1	0	1	0
21	cenEndurance	0	1	0	0	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	0
22	cenCondition	0	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0

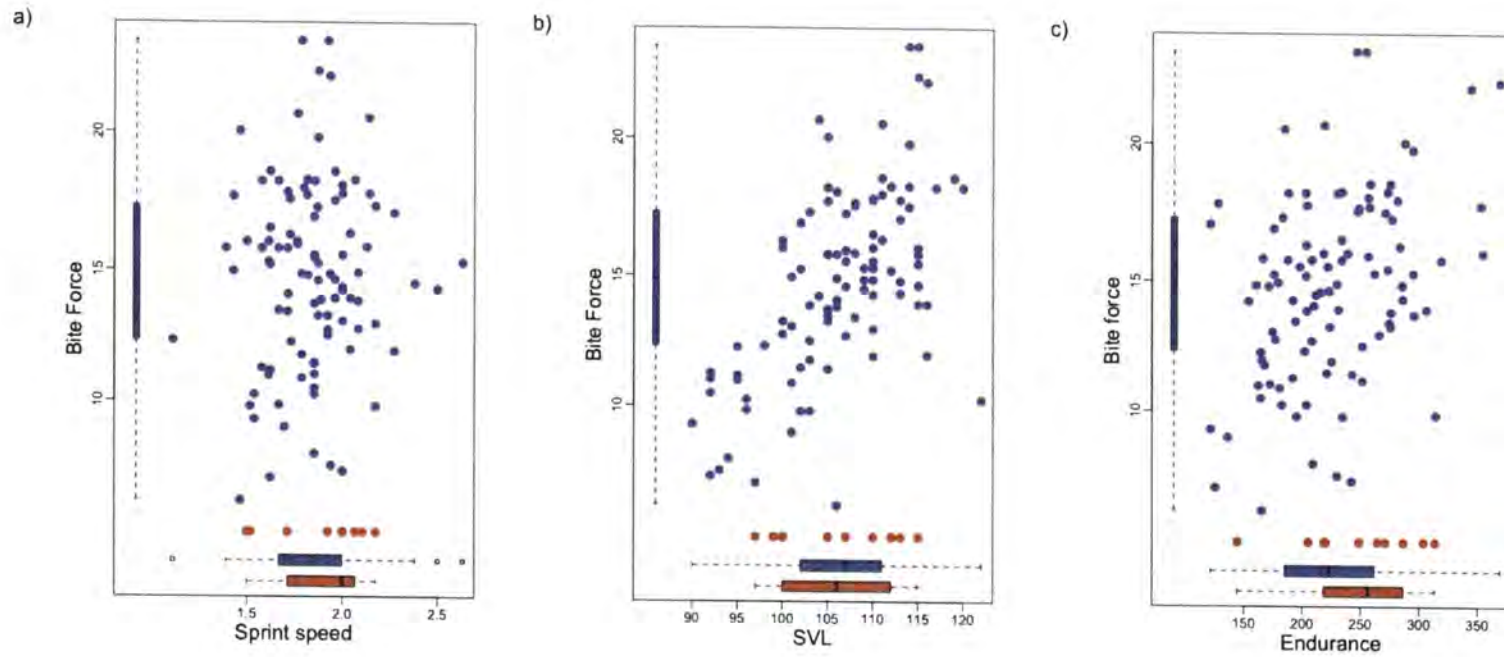
**Table S5** – Pooled model parameter estimates and standard errors for  $n = 5$  imputed data sets using  $n = 107$  male and female lizards. Bite force and endurance are given from the main effects model, which includes only directional selection gradients for SVL, body condition, and sprint speed. The R functions used to generate pooled estimates can be found in the Appendix. We do not report the frequency of missing data values (Nakagawa and Freckleton 2011) because all were extremely low  $\gamma < 0.10$  in all cases.

Parameter	RRS (Males)		RRS (Females)		# of Clutches (Males)	
	Bavg	se_pooled	Bavg	se_pooled	Bavg	se_pooled
SVL	0.68	0.17	0.68	0.13	0.42	0.16
Condition	1.49	0.47	0.37	0.12	1.20	0.44
Sprint speed	0.56	0.19	0.10	0.14	0.29	0.14
SVL <sup>2</sup>	0.32	0.17	-	-	-	-
Condition <sup>2</sup>	-0.43	0.28	-	-	-0.45	0.14
Condition*Sprint speed	-0.34	0.21	-	-	-	-
<b>Parameter</b>						
Bite Force	0.08	0.21	-0.10	0.21	-	-
Endurance	-0.10	0.18	0.17	0.22	-	-



**Fig. S1** – Contour plots (a – c) and surface plot (d) of predicted relative reproductive success ( $w$ ; ‘response’) after removing male with sprint speed greater than  $3 \sigma_x$  from  $\bar{x}$ . a) Predicted relative reproductive success as a function of sprint speed and body size (SVL) while controlling for body condition ( $\bar{x} = 0$ ); b) Predicted relative reproductive success as a function of body size (SVL) and condition while controlling for sprint speed ( $\bar{x} = 0$ ); c & d) Predicted relative reproductive success as a function of sprint speed and body condition while controlling for body size (SVL =  $1 \sigma_x$ ). Note that model predictions are only relevant for larger males ( $> 1 \sigma_x$  above  $\bar{x}$ ).





**Fig. S2** – Margin plots depicting distributions of observed (blue) and missing (red) data for males between bite force and (a) sprint speed, (b) SVL and (c) Endurance. Red dots on lower margin indicate data points where bite force is missing but for which we have either sprint speed, SVL or endurance while red boxplot shows the distribution of these data points in relation to the observed data (blue boxplot). The corner margin numbers provide the sum of missing points. Under a MAR and MCAR assumption these distributions should largely overlap.

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## Appendix – R script and functions used for post processing imputed datasets

### Function 1 – Trait standardization and relative reproductive success

Calculating relative reproductive success, body condition and standardizing all continuous predictors to  $\bar{x} = 0$ ,  $\sigma_x = 1$ . Note that this function requires the 'plyr' package.

```
selection.caics <- function(x, variables){  
  ## Calculate condition  
  x["condition"] <- residuals(lm(log(x$Mass)~log(x$SVL)))  
  ## Scale predictors  
  x[paste("cen", names(x[,variables]), sep = "")] <- as.data.frame(sapply(x[,variables],  
function(x){scale(x, center = TRUE, scale = TRUE)}))  
  ## Calculate mean RS within sex and by enclosure.  
  meanRS1 <- ddpby(x, .(Sex, Enclosure), summarise, meanRS = mean(NumOffspring, na.rm  
= TRUE))  
  ## Merge this dataframe with 'data' based on both the Sex and Enclosure columns  
  x <- merge(x, meanRS1, by = c("Sex", "Enclosure"))  
  ## Calculate Relative RS for each individual  
  x["RelRS"] <- x$NumOffspring/x$meanRS  
  x  
}
```

### Function 2 – Extracting model parameters and standard errors

Extracts coefficients and standard errors from gam model object

```

model.matrix <- function(x, coefs = TRUE, se = TRUE){
  if(coefs == TRUE & se == TRUE){
    mods_par <- lapply(x, function(x){data.frame(coefs = coef(x), se = sqrt(diag(vcov(x, freq =
TRUE))))})
  }else if(coefs == TRUE & se == FALSE){
    mods_par <- lapply(x, function(x){data.frame(coefs = coef(x))})
  }else{
    mods_par <- lapply(x, function(x){data.frame(se = sqrt(diag(vcov(x, freq = TRUE))))})
  }
  mods_par
}

```

```

coefficients <- t(data.frame(model.matrix(models, coef = TRUE, se = FALSE)))
se <- t(data.frame(model.matrix(models, coef = FALSE, se = TRUE)))

```

### Function 3 – Pooled estimates of model parameters and standard errors

Takes transposed estimates for each coefficient and se in the model and calculates pooled estimates across the M imputed data sets. Equations are the same as those listed in the methods. Function arguments are: x = data frame containing coefficients; y = data frame containing standard errors; n = the number of imputed datasets.

```

pooled_est <- function(x, y, n){
  est_mean <- colMeans(x)
  est_var_within <- (colMeans(y))^2
  est_var_across <- 0
  for(i in 1:length(est_mean)){
    est_var_across[i] <- (sum((x[,i] - est_mean[i])^2))/(n-1)
  }
  tot_var <- est_var_within + (1+(1/n))*est_var_across
  dat <- data.frame(Est_mean = est_mean, Est_se = sqrt(tot_var))
  dat
}

```

```
pooled_est(x = coefficients, y = se, n = 5)
```

### Function 4 – Calculating the frequency of missing information, $\gamma$

Function that calculates corrected sample size df

```
mice.df <- function(n, lambda, dfcom){
```

```
dfold <- (n - 1)/lambda^2
dfobs <- (dfcom + 1)/(dfcom + 3) * dfcom * (1 - lambda)
df <- dfold * dfobs/(dfold + dfobs)
return(df)
}
```

Calculating,  $\gamma$

```
r      <- (1 + 1/n)*(est_var_across/est_var_within)
lambda <- (1 + 1/n)*(est_var_across/tot_var)
dfcom  <- df.residual(model)
dfsmallsamp <- mice.df(n, lambda, dfcom)
fmi    <- (r + 2/(dfsmallsamp + 3))/(r + 1)
```



## CONCLUSIONS AND FUTURE DIRECTIONS

The work presented in this thesis has focused on characterizing and understanding variability in important behavioural and phenotypic traits and their effect on fitness, either directly through survival and reproductive success or indirectly through their influence on cognition. This work also provides insight into how sexual and natural selection may act on behavioural traits to promote the evolution of alternative male mating tactics and female polyandry, while also providing insight into how behavioural variation may influence success at spatial cognitive tasks.

Patterns of selection on behavioural, performance and morphological traits coincide with the theoretical expectations of alternative male mating tactics in this system (Morrison *et al.*, 2002; Stapley & Keogh, 2004; 2005; Keogh *et al.*, 2012) where large, presumably dominant males, adopt different tactics to acquire paternity (Noble *et al.*, 2013). The largest males appear to adopt a resident- or territorial-like tactic remaining active over many days while guarding a small home range or group of resident females (Morrison *et al.*, 2002; Stapley & Keogh, 2005). In contrast, slightly smaller large males, which are presumably less dominant relative to larger males, tend to adopt a wide-ranging floater strategy that involves being less active while occupying a large home range. These males are predicted to be faster runners because of their need to move over larger areas and possibly sneak matings with females or intercept wide-ranging females. Indeed my results suggest that this is the case. However, territorial males do not seem to require faster speeds to obtain paternity as is seen in other lizards (Husak *et al.*, 2006) and this is probably because of their smaller home range/territory size. Reproductive tactics are also predicted to differ in their behavioural type (Stapley & Keogh, 2004) and the work in this thesis suggests that the behavioral types associated with alternative reproductive tactics (ARTs) appear to have similar spatial learning abilities. Furthermore, ARTs in this system and likely other behavioural types may be controlled in part by circulating levels of plasma testosterone.

My empirical work has allowed me to develop a theoretical framework for understanding the mating system of *E. quoyii*, which can be used in future experiments on this species. Although a formidable challenge, it will no doubt inform on the importance of behaviour and performance to reproductive success, while also allowing empirical testing of

detailed mechanistic hypotheses about *how* and *why* these phenotypes have evolved and their influence on individual fitness.

### **The future of *Eulamprus*: Where to next?**

When I began my thesis there was little known about how natural and sexual selection acted on phenotypic variability in *E. quoyii*. Consequently, much of the work presented in this thesis has been observational in nature. However, experimentation is essential to establish cause-and-effect relationships and to more rigorously test the theoretical foundation established in this thesis. There are a number of important research directions, which still need exploration to fully reconcile the patterns uncovered in this thesis. Below is a short list of important missing pieces of information, which will be fruitful areas to pursue in the future.

- 1) *Dominance hierarchies and male-male competition* – We know very little about what determines the outcome of male-male contests and what phenotypes predict dominance in *E. quoyii*. Controlled laboratory contests, whereby performance and morphological measurements on individually paired males are quantified and assessed for their predictive power in explaining contest outcome will be necessary to understand these patterns. A particularly fruitful experiment would involve quantifying sprint speed, bite force and endurance on size-matched males to determine how performance traits influence contest outcome.
- 2) *The role of steroid hormones in explaining variability in male and female behaviour, performance and cognition* – Water skinks are an interesting system compared to many lizards because males and females are of similar size and morphology (aside from shape), exhibit similar behavioural phenotypes and have similar levels of circulating testosterone. An extremely exciting area to pursue in *E. quoyii* would be to manipulate circulating levels of testosterone and corticosterone and understand how this affects their behaviour (particularly those traits related to ARTs) and performance. Such an experiment will shed light on the proximate causes of plasticity in behavioural tactics and is easily achieved in this species. The conceptual framework developed in this thesis will provide an excellent opportunity for making clear predictions in such experiments. Manipulating across the sexes will be essential in terms of understanding

why females exhibit similar behaviours to males (both in terms of ARTs and aggression) but also in understanding how hormones affect cognitive differences between the sexes. Indeed, such manipulations may also help explain patterns of polyandry in this species.

- 3) *Ontogeny of heritability and the long-term consequences of maternal effects* – Maternal effects are strong in *E. quoyii* for both morphological and performance traits in young lizards. Although this may not necessarily be too surprising given that *E. quoyii* is live-bearing, it does provide some interesting questions surrounding evolutionary responses on such traits given that selection on body size and sprint speed is strong. Does the estimate of heritability and maternal effects for body size and sprint speed change during development? In other words, if we compare body size and sprint speed of offspring born from known parents on or after sexual maturity do they show stronger estimates compared to when they are young? Understanding these questions in *E. quoyii* will no doubt be difficult given their long lifespan, but will be important in fully appreciating the role of selection in explaining phenotypic variability in this species.
- 4) *Territoriality and resource defense in E. quoyii* – *E. quoyii* shows lower levels of aggression compared to other lizards, yet still seem to exhibit similar defense of resources. Understanding the nature of these resources will be important. Do resident/territorial males actively defend a specific area for resources such as shelter or do they defend harems of sedentary females?
- 5) *Post-copulatory sexual selection* – My thesis explores male-male-competition in the context of pre-copulatory sexual selection, however, we have a very poor understanding of post-copulatory processes. Understanding sperm competition and possibly cryptic female choice may provide important insight into understanding the patterns of variability in reproductive success in *E. quoyii*. We still do not know how many times females will actually mate (although we know at least 4 times from paternity data) and answers to this question will help understand both female polyandry and the level of post-copulatory sexual selection in this system.

- 6) *Phenotypic constraints and exploration of the adaptive landscape* – Trade-offs among different phenotypic traits are the norm and understanding these trade-offs will be necessary to fully comprehend the role of constraint in impeding exploration of adaptive peaks in fitness landscapes. Trade-offs among behavioural, physiological and performance traits will obviously be very important to understand in *E. quoyii* and may be mediated somewhat by differences in body condition. Experimental manipulations of body condition and their effects on physiology (testosterone, corticosterone), performance (sprint speed, endurance, bite force) and behaviour (home range area, activity etc.) will be useful future studies to conduct.

In summary, while my thesis has addressed a wide range of questions and hypotheses regarding sexual and natural selection on phenotypic traits including cognition in the Eastern Water Skink *Eulamprus quoyii*, it has also generated interesting questions and novel future directions which promise to make a significant contribution to evolutionary theory.

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## APPENDIX I

**Appendix I** contains papers that I contributed to during my PhD.

- J. Scott Keogh, Daniel W.A. Noble, Eleanor E. Wilson and Martin J. Whiting (2012). Activity predicts male reproductive success in a polygynous lizard. PLoS ONE, 7(7): e38856. doi:10.1371/journal.pone.0038856.
- Qi Yin, Daniel W.A. Noble, Jinzhong Fu and Martin J. Whiting (2012). Spatial and social organization in a burrow-dwelling lizard from China (*Phrynocephalus vlangalii*). PLoS ONE, 7(7): e41130. doi:10.1371/journal.pone.0041130.

# Activity Predicts Male Reproductive Success in a Polygynous Lizard

J. Scott Keogh<sup>1\*</sup>, Daniel W. A. Noble<sup>2</sup>, Eleanor E. Wilson<sup>1</sup>, Martin J. Whiting<sup>2</sup>

<sup>1</sup> Division of Evolution, Ecology and Genetics, Research School of Biology, The Australian National University, Canberra, Australia, <sup>2</sup> Department of Biological Sciences, Division of Brain, Behaviour and Evolution, Macquarie University, Sydney, Australia

## Abstract

Activity patterns and social interactions play a key role in determining reproductive success, although this is poorly understood for species that lack overt social behaviour. We used genetic paternity analysis to quantify both multiple paternity and the relative roles of activity and social behaviour in determining reproductive success in a nondescript Australian lizard. During the breeding season we intensively followed and recorded the behaviour of a group of seven males and 13 females in a naturalistic outdoor enclosure to examine the relative roles of body size, activity and social interactions in determining male fertilization success. We found multiple paternity in 42% of clutches. No single behaviour was a significant predictor of male fertilization success in isolation, but male-female association, interactions and courtship explained 41% of the variation in male fertilization success. Males with the highest number of offspring sired invested heavily in interacting with females but spent very little time in interactions with males. These same males also sired offspring from more clutches. When taken collectively, an index of overall male activity, including locomotion and all social interactions, significantly explained 81% of the variation in the total number of offspring sired and 90% of the variation in the number of clutches in which males sired offspring. We suggest that the most successful male strategy is a form of endurance rivalry in which active mate searching and interactions with females have the greatest fitness benefits.

**Citation:** Keogh JS, Noble DWA, Wilson EE, Whiting MJ (2012) Activity Predicts Male Reproductive Success in a Polygynous Lizard. PLoS ONE 7(7): e38856. doi:10.1371/journal.pone.0038856

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\* E-mail: scott.keogh@anu.edu.au

## Introduction

According to sexual selection theory males are predicted to maximize their reproductive success by mating with many females [1]. The success of different males in obtaining mates depends strongly on the resources required by receptive females and their spatial and temporal distribution [2,3,4]. In polygynous mating systems, males can achieve high reproductive success by defending resources required by females for mating (resource defence polygyny), defending groups of females (female defence polygyny) or by exhibiting showy, sexual ornaments or displays such as in lekking species. For example, in some species males that display more vigorously at a lek are more likely to be chosen by females [5], whereas in other systems large aggressive males are expected to outcompete rival males for resources or females [1]. Therefore, specific morphological and behavioural traits that enhance a male's ability to acquire females, and/or the resources that they require, are expected to be targets of selection.

Much of our knowledge about the predictors of male reproductive success come from studies on insects, birds and frogs [1,6]. In many cases morphological predictors such as male body size or armaments are correlated with high reproductive success and are most common in mating systems where males monopolise resources used by females or females themselves [7]. As a consequence, many studies target species in which males are elaborately ornamented or show clear sexual dimorphism [1]. We know much less about species lacking clear sexual dimorphism and

which may use less obvious tactics to secure paternities. Furthermore, in mating systems where females are dispersed, are sexually receptive for short periods, and where resources are less important for them, male behavioural attributes may be more important contributors to reproductive success [8,9,10,11]. In such situations, selection for behavioural attributes that allow males to persist at a breeding site for long periods of time (endurance rivalry) or that promote increased interactions with females when they are receptive, are predicted to be under selection.

The Southern Water Skink (*Eulamprus heatwolei*) is distributed widely across southern Australia, is viviparous, and females give birth to 1–5 offspring per litter. The mating system of *E. heatwolei* is highly polygynous. Many adult females establish home ranges close to river edges where there is an abundance of large logs and fallen debris and the home ranges of males overlap an average of 2.29 females [12]. However, both males and females exhibit alternative reproductive tactics (territorial or floater) that form part of a behavioural syndrome [12,13,14]. In novel environments in the lab, floater males are more active than territorial males and spend more time feeding [13]. In an anti-predator context, floater males are more likely to flee into a refuge and have a longer latency to emerge [13]. In the wild, larger territorial males were more likely to father an entire clutch or share paternity with fewer other sires than smaller territorial males but floater males tended to father heavier offspring [14]. However, both small and large floater and territorial males sire offspring with neither strategy

clearly advantageous over the other, and many males sire no offspring [12,14]. The Southern Water Skink (*Eulamprus heatwolei*) is a good species in which to test the relative importance of behaviour in sexual selection because females display high levels of multiple paternity [12,14], there is high variation in male reproductive success [12,14], and males and females have different behavioural phenotypes [14]. It was not possible in our previous studies on a wild population to assemble highly detailed information on social interactions and activity levels relative to mating success. Here we combined intensive behavioural observation of an adult group of lizards in a single large naturalistic enclosure with genetic paternity data to test the hypothesis that the proportion of time a male is observed in active behaviours predicts male fertilization success.

Results

We established a breeding population of 13 female and seven male lizards in a single large semi-natural enclosure (10×10 m) where we could conduct detailed behavioural observations on each of the lizards during the breeding period. All 13 females from our large outdoor enclosure were collected at the end of the breeding period. Twelve of the thirteen females gave birth to a total of 37 offspring. Litter size ranged from one to four offspring (mean=3.1, SE=0.23). Paternity was assigned with 100% certainty to all offspring. Seven out of 12 litters had one father and multiple paternity was identified in the remaining five (42% of clutches). Of these, four had two fathers and one had three fathers in a litter of three offspring. Male reproductive success was not significantly related to male body size (Table 1). A small male sired the most offspring: 14 out of 37. Apart from this small male, there was a trend for large males to sire more offspring than small males. Only one small male failed to sire any offspring in this experiment. Seven copulations were observed and four of these resulted in offspring.

During the breeding season we recorded 1119 behavioural observations for the 7 males in our enclosure (range, 115–261 observations) that could be divided into four behavioural categories (Figure 1). All categories of behaviour were examined as possible predictors of male fertilization success but none of them individually were significant predictors (Table 1). However, interactions with females explained 41% of the variation in male fertilization success (Table 1). When all active male behaviours were taken into account, including both social interactions and general locomotion, there was a strong and significant positive correlation that explained 81% of the variation in male fertilization success (Table 1; Figure 2). Males that exhibited more active behaviours also sired offspring from more clutches (Figure 2;  $R^2 = 0.9$ ;  $F_{1,7} = 56.12$ ;  $P = 0.002$ ). Conversely, there was a negative

relationship between the proportion of behaviours males devoted to interacting aggressively with other males and male fertilization success (Table 1). Therefore, males that had the highest fertilization success invested heavily in interacting with females but comparatively little in interactions with males (Figure 3).

Discussion

We have quantified in a very direct way the activity level of individual males, how they divide total activity between aggressive interactions with other males and interactions with females, and how that activity relates to fitness. We show that males exhibiting a greater proportion of active behaviours during the breeding season sired more offspring in a greater number of clutches, irrespective of male body size.

The correlation between reproductive success and activity could be explained by two subtly different (and not necessarily exclusive) processes. First, more active males may increase their reproductive success by remaining active over a greater number of days increasing the number of receptive females they interact and copulate with (endurance rivalry). Attendance at breeding sites has been shown to be an important determinant of reproductive success in many vertebrate species. Salvador *et al.* [10] followed a population of Cyren’s Rock Lizard (*Iberolacerta cyreni*) for two consecutive years and found that activity level (number of times observed during the breeding season) strongly predicted male reproductive success. More active males gained access to mates by having more females within their home range [10]. In the Galapagos sea lion (*Zalophus wollebaeki*) male reproductive success was predicted by male attendance at breeding sites and this is due to the long reproductive period (five or more months) in this species [9]. This relationship was also independent of male body size [9] and suggests that body size *per se* may not always be as important in governing male reproductive success as is often assumed. This hypothesis should be particularly prominent in systems where females are temporally variable in their receptivity and the breeding season lasts for a long time [2]. This is unlikely to be the case for *Eulamprus heatwolei* because females are known to have a relatively short receptive period of only one to two weeks during mid October [15]. Such a restrictive receptive period suggests that male attendance is unlikely to increase a male’s reproductive success although copulations before the breeding period and sperm storage may play an important role and this hypothesis cannot be ruled out.

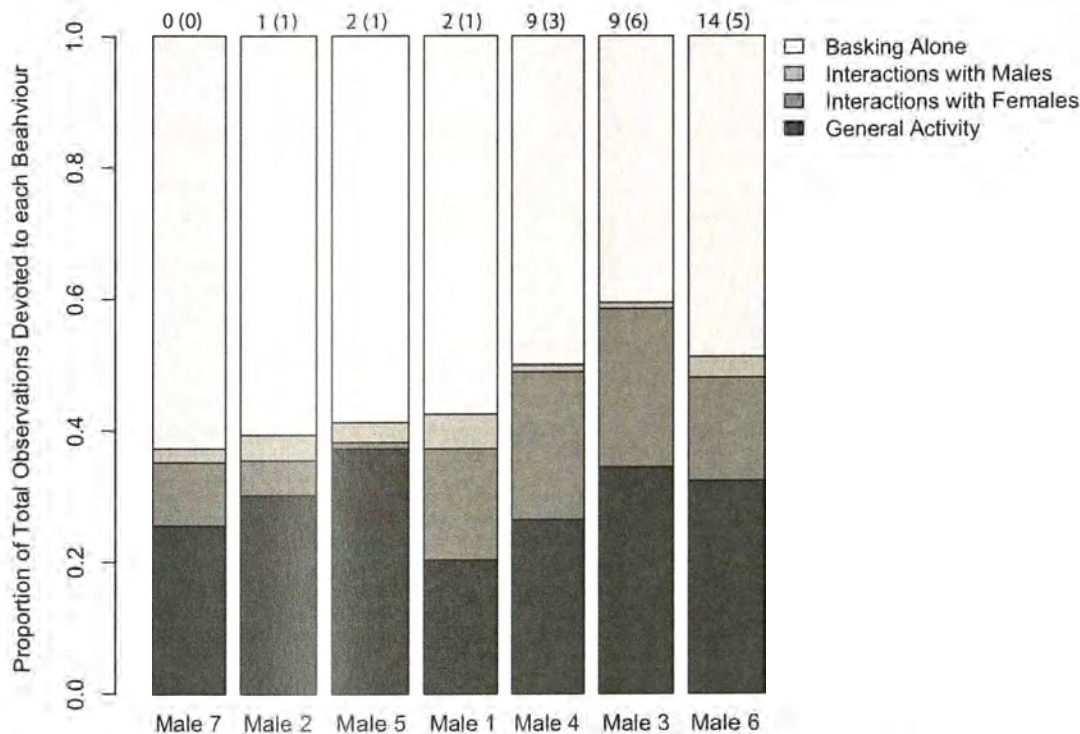
The alternative hypothesis is that more active males are likely to traverse their environment and increase their probability of encountering receptive females, providing males with a greater number of mating opportunities. Male Common Lizards (*Lacerta agilis*) with elevated testosterone levels have been shown to move greater distances than control males and mate with more females [16]. In the North American Red Squirrel (*Tamiasciurus hudsonicus*) male search effort (home range area) and search ability (number of receptive females encountered during the mating season) both correlated with the number of matings a male obtained and his actual reproductive success [8]. Our data provides support for the latter hypothesis as males that gained more offspring interacted with more females and sired offspring from more clutches. This is also consistent with the short and near simultaneous female receptive period in the wild [15]. Finally, males clustered into two groups: four males with low activity and low numbers of offspring sired and three males with high levels of activity and higher numbers of offspring sired. A larger sample size is needed to test whether this bimodal distribution correlates with discreet alternate reproductive tactics, but this is entirely possible given that previous

**Table 1.** Regression analyses of the active behaviour categories, all active behaviours combined and male body size, relative to the total number of offspring sired.

Behaviour	R <sup>2</sup>	F <sub>1,7</sub>	P
General activity	0.09	0.471	0.5229
Interactions with females	0.41	3.484	0.1209
Interactions with males	−0.17	1.000	0.3632
All active behaviours	0.81	21.202	0.0154
Snout-vent length	0.09	0.515	0.5054

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**Figure 1. Summary of the proportion of activity for each behavioural category for each male.** Total number of offspring sired by each male is shown above each bar with the number of clutches in parentheses.  
doi:10.1371/journal.pone.0038856.g001

work on this species has documented floater and resident males in the same population [12,14].

In our study 42% of litters had more than one sire. Paternity analysis of offspring born from wild collected females from the same population showed the proportion of multiple paternity to range from 57–64.7% [12,14], which suggests that many females are promiscuous. There are a number of adaptive hypotheses as to why females mate multiply. First, females may benefit directly by ensuring against sperm limitation [17,18]. This can result from a high rate of infertility or sperm deformations among males or due to the inadequate transfer of sperm during copulation [17]. Alternatively, females may gain indirect fitness benefits by mating with more than one male if they gain ‘good genes’ through the promotion of sperm competition or disease resistance [19,20] or by increasing the probability of being fertilized by a male that is genetically compatible with herself [21]. However, multiple mating may not always be adaptive to females and their tendency to do so could be a result of direct selection on male mating behaviour followed by correlated indirect selection on female mating rates [22]. Females are able to easily reject males [15] but are also promiscuous [12]. The benefits of multiple mating for females in this species are as yet unclear, but in other lizard species such as the Sand Lizard (*Lacerta agilis*), mating with multiple males results in higher offspring viability [23]. Furthermore, reproductive success in *L. agilis* is tied to mate searching and encounter rates. During warmer periods male *L. agilis* are able to spend more time actively searching for females and thereby potentially increasing rates of multiple paternity [24]. Similarly, the high reproductive success (both in total numbers and number of clutches) of more active male *E. heatwolei* suggests that selection on male activity levels is strong.

In conclusion, our results demonstrate the importance of activity related behaviours in sexual selection in a species that lacks visual ornamentation and which has low display rates. Dynamic behavioural traits may play a more important role in intrasexual competition than previously thought in reptiles and future work quantifying the relative roles of different behavioural traits to male reproductive success promises to be a fruitful area of study.

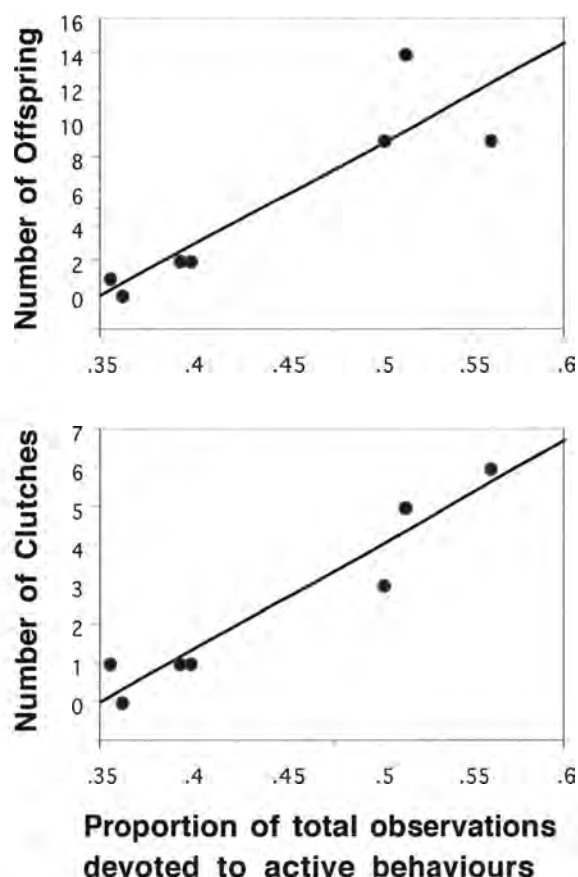
## Methods

### Study Animal

We collected adult *E. heatwolei* from 24 September until 5 October, immediately after spring emergence, from a large population in the Tidbinbilla Nature Reserve, 25 km southwest of Canberra in the Australian Capital Territory (elevation 800 m). We measured snout-vent length (SVL) and tail length to the nearest mm, head length and head width to the nearest 0.1 mm, and weight to the nearest 0.1 g. Individuals were sexed by checking for hemipenes. Lizards were marked individually with a unique toe-clip combination and the toes and 5 mm of tail tip were retained for genotyping. Natural toe loss is common in *E. heatwolei* (JSK pers. obs.) and toe-clipping has been shown to have no effect on lizard behaviour or fitness in a closely related species [25]. All individuals used in this study had complete or fully regrown tails, were free of visible parasites, and appeared to be in good health at the onset of the experiment.

### Experimental Enclosure and Lizard Husbandry

We used a single large (10×10 m) enclosure with high quality habitat (an abundance of logs and refugia) where we recorded detailed data on all social interactions and other behaviours of males to compare with paternity data. The large size of the



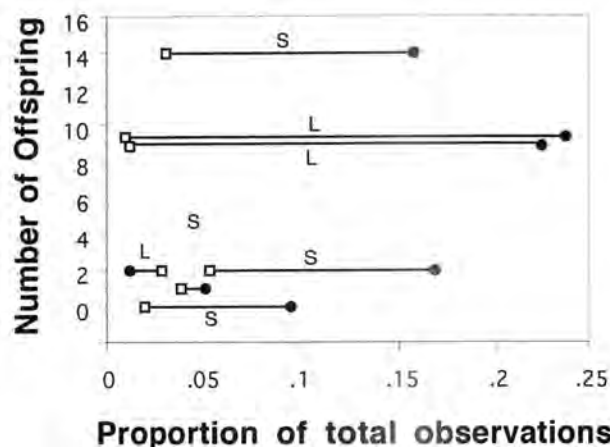
**Figure 2. The relationship between active behaviours with other lizards relative to the number of offspring sired (top) and the number of clutches in which males sired offspring (bottom).**

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enclosure allowed natural home-range establishment and behaviour but it was small enough to allow us to observe the entire enclosure at once. The density of lizards in the enclosure approximates natural densities in high quality habitat during the mating season (Morrison *et al.* 2002).

Thirteen adult females (96–104 mm SVL) and seven adult males (88–96 mm SVL) were introduced into the enclosure on 6 October. For ease of identification, we painted a unique number on the dorsum of each lizard using non-toxic, xylene-free paint pens. We observed the enclosure from 7 October – 21 November, including two weeks after the mating season ended and lizard behaviour had obviously decreased.

Lizards in the enclosures always had access to water and fed on insects that naturally occurred in the enclosures. We supplemented their diet with wet dog food twice per week. Females were brought into the laboratory on 8 January to give birth and housed in individual snap-lock containers (30L×21W×9H cm), in a temperature-controlled environment (18°C) with a natural light cycle (12 h light: dark). The lizards were provided with bark chip bedding, a cardboard retreat site, and heat tape for basking (30°C) eight hours a day to allow natural thermoregulation. The lizards were provided with fresh water *ad libitum* and dog food and mealworms every second day. The females were checked twice daily until they gave birth. Neonates were removed upon discovery and housed separately.



**Figure 3. The proportion of behaviour related to aggressive interactions with other males (open squares) relative to active interactions with females (filled circles) for each male as a function of the number of offspring sired.** Male body size is noted where “S” refers to small males (88–89 mm) and “L” refers to large males (95–96 mm). Males that spent a greater proportion of time interacting with females also tended to spend comparatively little time interacting with rival males.

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### Behavioural Sampling

During observation periods one person (EEW) walked around the perimeter of the enclosure and continuously scanned for all activity related behaviours and social interactions. Observations were recorded from 1000–1600 h when lizards are most active and only on days when conditions favoured lizard activity (warm, sunny, calm). In order to facilitate recording observations on multiple lizards at once, we broadly divided activity into four basic categories, but because we had a manageable number of lizards, it was rarely the case that multiple interactions were happening at the exact same time. The first category was general activity related behaviours with no obvious receiver. These behaviours involved locomotion (movement of an individual greater than 2 cm from its initial position) and head-bobbing (stereotypical up and down movement of the head and neck) where no conspecific was observed. We also recorded interactions between two individuals. Interactions involving a male and female included courtship, chasing (when a male actively chased a female), copulations or when a male was observed within 5 cm of the female while basking (stationary in the sun) or being stationary (stationary in the shade). Interactions involving two males included fighting (biting) and chasing when one individual actively displaced the other by chasing him through the enclosure. In each case we recorded the type of behaviour, the individuals involved, and the onset time of interactions. Because behaviours were performed rapidly and generally lasted only a few seconds, we recorded occurrence instead of duration. If multiple behaviours were displayed in rapid succession by a single individual, we scored only the most dominant behaviour exhibited (for example, courtship or fighting over chasing).

### Paternity Assignment

Neonates were individually toe-clipped and approximately 5 mm of tail tip was removed for genotyping. All offspring, mothers and potential sires were genotyped for three highly polymorphic microsatellite loci, Ek37, Ek100, Ek107, as described in Scott *et al.* [26] and Morrison *et al.* [12]. We assigned paternity

to offspring manually by first matching maternal alleles in the offspring and then going through the alleles of all the potential fathers until there was a match at all three paternal alleles.

## Data Analyses

We divided the 6.5 week observation period into three time periods: acclimatisation, the mating period, and post-mating period. The mating period is relatively short and we demonstrated in an earlier study that social interactions during this period are very different than before and after the mating period [15]. Therefore, we excluded all of the behavioural data before and after the mating period and focused our analyses on the 19-day mating period only (October 20 – November 8). We calculated the total number of behavioural events for each male and then expressed each behavioural category as a proportion of his total, thereby controlling for unequal sampling duration. We performed a series of linear regressions between the total number of offspring sired and the proportion of the total number of observations invested in each category of behaviour. We also examined these behaviours in the context of male body size (snout-vent length presented by results similar if mass was used instead) and the number of clutches in which a male sired offspring. For each male

we also examined the difference between the relative number of social interactions with females and aggressive interactions with males.

## Ethics Statement

All work carried out as part of this project was done under the approval of the Australian National University Animal Experimentation Ethics Committee (F.BTZ.01.99) and with research permits from Environment ACT (permit number LT1999008). Tidbinbilla Nature Reserve gave us permission to capture the animals used in this study.

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## Author Contributions

Conceived and designed the experiments: JSK. Performed the experiments: EEW JSK. Analyzed the data: JSK DWAN EEW MJW. Contributed reagents/materials/analysis tools: JSK. Wrote the paper: JSK DWAN EEW MJW.

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# Spatial and Social Organization in a Burrow-Dwelling Lizard (*Phrynocephalus vlanguaii*) from China

Yin Qi<sup>1\*</sup>, Daniel W. A. Noble<sup>2,3\*</sup>, Jinzhong Fu<sup>1,3</sup>, Martin J. Whiting<sup>2</sup>

**1** Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, Sichuan, China, **2** Department of Biological Sciences, Macquarie University, Sydney, New South Wales, Australia, **3** Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada

## Abstract

Shared ecological resources such as burrow complexes can set the stage for social groupings and the evolution of more complex social behavior such as parental care. Paternity testing is increasingly revealing cases of kin-based groupings, and lizards may be a good system to inform on the early evolution of sociality. We examined spatial and social organization in the lizard *Phrynocephalus vlanguaii* from China and tested genetic relatedness (based on eight microsatellite DNA loci) between offspring and parents that shared burrow complexes. Adult males and females had similar spatial patterns: they overlapped most with members of the opposite sex and least with their own sex. Males in better body condition overlapped with more females, and both sexes showed high site fidelity. Most lizards used a single burrow, but some individuals used two or three burrows. While high site fidelity is consistent with sociality in lizards, juveniles did not preferentially share burrows with parents, and we documented only a few cases of parent-offspring associations through burrow sharing. We suggest that *P. vlanguaii* conforms to a classical polygynous mating system in which the burrow forms the core of the male's territory and may be offered as an important resource for females, but this remains to be determined.

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\* E-mail: qiyin@cib.ac.cn

† These authors contributed equally to this work.

## Introduction

Sociality (long-term stable groups with overlapping generations) in vertebrates has traditionally been thought to be restricted primarily to birds and mammals, which clearly demonstrate long-term cohesive groups [1,2]. Complex social behavior is predicted to evolve when related and/or unrelated groups of individuals merge [2,3], setting the scene for the evolution of cooperative and altruistic behavior, parental care and group foraging, all commonly observed in birds and mammals [1,2]. Recent work, however, in other 'less' social vertebrates, such as lizards, has revealed simple forms of sociality [4,5,6,7]. Lizards exhibit less complex forms of sociality compared with mammals and birds, involving aggregations of kin and non-kin groups [5,8] that are sometimes associated with rudimentary forms of parental care [9]. For example, desert night lizards (*Xantusia vigilis*) aggregate under logs in groups of 2–18 individuals [5]. Juvenile aggregations with adults tend to be genetically related and most individuals belong to the same nuclear family. Such aggregations result from delayed dispersal of juvenile lizards, generating simple kin-based sociality in *Xantusia* [5]. Similar kin-based associations have been recorded in multiple species in the *Egernia*-*Liopholis* clade of Australian skinks, which typically form nuclear families [4,10]. More recently, the Australian lizard *Liopholis kintorei*, which lives in burrows excavated in sandy deserts, has been documented to live in nuclear families in which group members 'cooperate' to maintain the burrow system [7]. These systems provide a unique opportunity to

explore the early stages and evolution of sociality. However, the paucity of ecological and genetic studies in the vast majority of lizard clades precludes statements regarding the general form of sociality in lizards.

Parental care is a particularly prevalent form of social behavior in birds and mammals; however, it is rare among lizards [1,11]. Although maternal care in lizards has evolved multiple times, it generally takes on a rudimentary form [1]. For example, Taiwanese long-tailed skinks (*Mabuia longicaudata*) show simple forms of maternal care where females brood and actively defend clutches of eggs from egg-eating snakes [6,12]. Similar egg brooding behaviors have been recorded in the North American skinks of the *Plestiodon* [formally *Eumeces*] complex [13] and pythons [14]. However, studies in live-bearing (viviparous) skink species suggest that maternal care can manifest itself in more complex forms [9]. In *Egernia ichthii*, offspring born to aggressive females have higher survival compared with offspring born to less aggressive females and this may be the result of decreased infanticide [9]. Similarly, the black rock skink, *Egernia saxatilis*, lives in family groups in which the presence of a parent significantly reduces the likelihood of infanticide [10,15]. More complex forms of maternal care in reptiles may be associated with the evolution of viviparity because it provides greater opportunity for interaction between parents and offspring [5].

Defending key resources (territoriality) necessary for both adult and offspring survival may be an important stepping-stone for the



evolution of parental care [16]. For example, defending a burrow or crevice that is limited may provide a direct fitness benefit to adults and aggressive behaviors necessary for resource defense may cross functional contexts and promote the protection of offspring from conspecifics [12,17]. Alternatively, sheltering sites within a territory may provide protection from predators or stochastic climatic conditions [18]. Systems where all of the above criteria are met may provide an opportunity to test for the presence of parental care and provide important insight into the diversity of social systems in lizards.

The Qinghai Toad-headed Agama (*Phrynocephalus vlangalii*; Figure 1) is a high-elevation, viviparous lizard found in the northern part of the Tibetan Plateau [19]. Both males and females are highly aggressive and use complex tail displays during social interactions [20]. Tail curling may function in establishing male social rank by signaling individual body condition [21]. Lizards also have a tail-tip badge that is sexually dimorphic: orange in females and black in males. *Phrynocephalus vlangalii* excavate burrows to approximately 70 cm in loose sand [22] and these burrows are essential for over-winter survival because temperatures can drop to well below zero during the winter months ( $-10.3 \pm 1.9^\circ\text{C}$  to  $-2.4 \pm 1.46^\circ\text{C}$ ; Monthly average temperatures from November–March). Our observations of a population of *P. vlangalii* in Xiamen Nature Reserve revealed that adults and offspring sometimes occupied excavated burrows together (Figure 1). Here, we combine two years of data on the spatial and social organization of *P. vlangalii* with molecular estimation of individual relatedness to test the hypothesis that offspring found within adult burrows are part of a parent-offspring relationship and suggestive of sociality. We also explore morphological differences between the sexes because of its implications in understanding lizard mating systems and territoriality. Testing the parent-offspring hypothesis is a first step in identifying whether kin-based sociality and parental care may be present in this taxonomically differentiated group of lizards. We first address patterns of burrow use by adult males and females and then test whether adult lizards may be sharing burrows with their offspring during the winter, a critical period of their life.

## Results

### Morphology

We tested for significant differences between males and females in morphological traits that typically correlate with lizard mating



**Figure 1. Female *Phrynocephalus vlangalii* in a burrow with a young juvenile.**

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systems. When year was controlled for, mean male snout-vent length (SVL) was significantly greater than mean female SVL (Table 1; ANCOVA; Sex:  $F_{1, 168} = 11.85$ ,  $p < 0.001$ ; year (covariate):  $F_{1, 168} = 4.702$ ,  $p = 0.03$ ; Sex\*Year:  $F_{1, 168} = 2.35$ ,  $p = 0.13$ ). When both SVL and year were controlled for, mean male head length was significantly greater than mean female head length (Table 1; ANCOVA; Sex:  $F_{1, 167} = 27.45$ ,  $p < 0.001$ ; SVL (covariate):  $F_{1, 167} = 91.48$ ,  $p < 0.001$ ; Year (covariate):  $F_{1, 167} = 9.04$ ,  $p < 0.01$ ; Sex\*Year:  $F_{1, 167} = 2.01$ ,  $p = 0.16$ ). Mean male head width was significantly greater than mean female head width (Table 1; ANCOVA; Sex:  $F_{1, 167} = 7.60$ ,  $p < 0.01$ ; SVL (covariate):  $F_{1, 167} = 90.57$ ,  $p < 0.001$ ; year (covariate):  $F_{1, 167} = 128.64$ ,  $p < 0.001$ ; Sex\*Year:  $F_{1, 167} = 1.85$ ,  $p = 0.18$ ).

### Use of burrows and space

Fifty-three (87%) male lizards were classified as residents (used the same burrow for ten or more days) during the breeding season and 10 (12.5%) males were located in both years. The average number of burrows each male occupied was  $1.41 \pm 0.11$  ( $n = 29$  from 2010; Table 2). Of these, 66% occupied one burrow, 27% occupied two burrows and 7% occupied three burrows. Each male was re-sighted with a mean frequency of  $6.88 \pm 0.49$  ( $n = 53$ ) over  $18.43 \pm 0.82$  days ( $n = 53$  during our 30-day census period). We also classified 62 (77%) female lizards as residents during the breeding season. The average number of burrows each female occupied was  $1.30 \pm 0.09$  ( $n = 30$ ; Table 2). Of these, 71% occupied one burrow and 29% occupied two burrows. Each female was re-sighted with a mean frequency of  $6.46 \pm 0.40$  ( $n = 62$ ) over  $18.02 \pm 0.62$  days ( $n = 62$ ) during our 30-day census period (Figure 2, Table 2). We recaptured 11 females in 2010.

Male home range area averaged  $43.75 \pm 9.29 \text{ m}^2$ , while females averaged  $32.01 \pm 8.79 \text{ m}^2$  (Table 2; 2009–2010 combined). The mean maximum distance a male moved from his burrow was  $13.8 \pm 3.09 \text{ m}$  ( $n = 14$ ) while females moved a mean maximum distance of  $8.41 \pm 1.66 \text{ m}$  ( $n = 17$ ), and was not significantly different between the sexes ( $W = 152$ ,  $p = 0.19$ ; Figure 2). Home range area was not significantly different between the sexes and was not related to SVL (ANCOVA; Sex:  $F_{1, 32} = 3.29$ ,  $p = 0.08$ ; SVL (covariate):  $F_{1, 32} = 1.09$ ,  $p = 0.30$ ). Body condition was not significantly correlated with home range area in males ( $r = 0.05$ ,  $p = 0.86$ ,  $n = 15$ ). Male home range overlapped with an average of  $1.27 \pm 0.33$  other males ( $n = 15$ , range = 1–4) and  $1.67 \pm 0.43$  females ( $n = 15$ , range = 1–5). Female home range overlapped with an average of  $1.00 \pm 0.29$  females ( $n = 19$ , range = 1–4) and  $1.84 \pm 0.53$  males ( $n = 19$ , range = 1–8) (Table 3). Male body condition was significantly positively correlated with the number of females overlapped ( $r_s = 0.61$ ,  $p = 0.02$ ,  $n = 15$ ) and with overlap pressure on females ( $r_s = 0.65$ ,  $p = 0.01$ ,  $n = 15$ ).

### Burrow sharing and relatedness in adults and offspring

A total of 97 lizards were found in 54 (68%) of the 80 burrows we excavated. Seventy-one (73%) of these lizards were offspring, seven (7%) were adult males and 19 (20%) were adult females. Offspring were found on their own or with other young in 28 burrows while they were found with adult males and/or females in 15 burrows. A single adult female was found with 1–3 offspring in eight instances while a single adult male was found with 1–5 offspring on five occasions. An adult male and female were found together in the same burrow only once, along with one baby. Ten females and one male were collected from burrows with no other individuals.

Coefficient of relatedness ( $R$ ) estimates ranged from  $-0.57$  to  $0.77$  among all possible pairs of individuals in the sample. The population level mean relatedness estimate was  $-0.012 \pm 0.003$ .



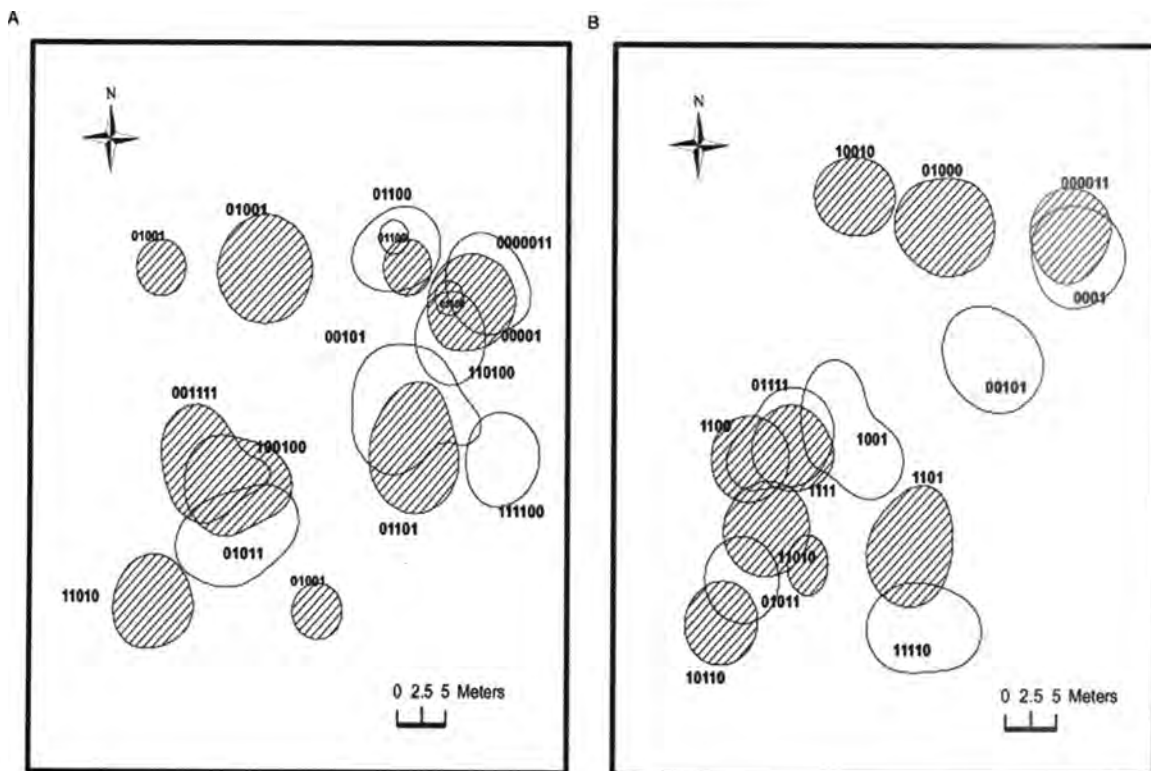
**Table 1.** Mean  $\pm$  SE (N) snout-vent length (mm), head length (mm) and head width (mm) of male and female *P. vlanguilii* captured in 2009 and 2010 in Xiamen Nature Reserve.

	2009		2010		Combined (2009–2010)	
	Male	Female	Male	Female	Male	Female
Snout vent length (SVL)	57.29 ± 0.40 (35)	56.47 ± 0.52 (41)	57.11 ± 0.49 (35)	54.68 ± 0.52 (61)	57.20 ± 0.31 (70)	55.40 ± 0.38 (102)
Head length	16.76 ± 0.15 (35)	16.44 ± 0.18 (41)	16.52 ± 0.23 (35)	15.46 ± 0.15 (61)	16.64 ± 0.13 (70)	15.85 ± 0.13 (102)
Head width	15.30 ± 0.12 (35)	15.42 ± 0.21 (41)	13.94 ± 0.14 (35)	13.45 ± 0.12 (61)	14.62 ± 0.13 (70)	14.24 ± 0.15 (102)

The combined (2009–2010) is the mean of the morphological measurements for 2009 and 2010 combined. Parentheses indicate sample sizes.  
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Of the 54 burrows, three (5.5%; Burrows 3, 24, 46) showed evidence of significant parent-offspring relationships. Burrow 3 contained three offspring and a single adult female. Two of the offspring showed significant parent-offspring relationships with the adult female ( $R = 0.71$ – $0.77$ ,  $p < 0.001$ ), while the third baby was unrelated. Burrow 24 contained one adult female and a single baby that were significantly related at the parent-offspring level ( $R = 0.39$ ,  $p = 0.001$ ) while burrow 46 contained one adult male and a single related offspring ( $R = 0.54$ ,  $p < 0.001$ ). In all of these cases the adults and offspring shared alleles at 100% of their loci. In total, seven (32%) of the 22 burrows with multiple individuals had higher relatedness than the population burrow mean (Figure 3).

We identified 30 burrow groups that contained from 1–10 (mean  $\pm$  SE =  $3.23 \pm 0.40$ ) burrows within 2 m of each other. We compared parent-offspring relationships among the individuals found in nearby burrows because we know that some lizards will occupy multiple burrows and the maximum distance between these burrows is very small (2 m). We identified one more parent-offspring relationship (in addition to the burrows identified as having significant parent-offspring relationships above) in a single burrow group. Burrow group 9 contained an adult female in burrow 20 and her offspring in burrow 19 where the primary hypothesis of parent-offspring relatedness could not be rejected ( $R = 0.41$ ,  $p = 0.0023$ ). The parent and offspring in this instance also shared alleles at 100% of their loci. Burrows 20 and 19 were 1.5 m apart.



**Figure 2. Spatial distribution of *Phrynocephalus vlangualii*.** Spatial distribution and 75% Kernel home ranges of a subset of male (n = 12) and female (n = 14) lizards collected during A) 2009 and B) 2010. Cross-hatching refers to females, empty spaces to males. Males (01011 and 00101) and female (11010) were marked in 2009 and recorded at the same position in 2010. Some individuals have two spatially separate areas because the Kernel method calculates areas of intensive use (see Methods).  
doi:10.1371/journal.pone.0041130.g002

**Table 2.** The number of unique lizards captured in 2009 and 2010.

	2009		2010		Combined (2009–2010)	
	Male	Female	Male	Female	Male	Female
Number of lizards marked	35	41	35	61	70	102
Number of lizards resighted	28	32	33	49	61	81
Mark-recapture rate	0.80	0.78	0.94	0.80	0.87	0.79
Lizards captured: 2009 and 2010	–	–	10	11	10	11
Number of residents	25	24	28	38	53	62
Proportion of residents	0.89	0.72	0.85	0.73	0.87	0.77
Number of sightings	7.20 ± 0.78 (25)	7.58 ± 0.65 (24)	8.18 ± 0.81 (28)	5.66 ± 0.38 (38)	6.88 ± 0.49 (53)	6.46 ± 0.40 (62)
Home range area (m <sup>2</sup> )	48.60 ± 14.60 (8)	30.20 ± 10.9 (10)	38.20 ± 11.70 (7)	14.56 ± 5.58 (9)	43.75 ± 9.29 (15)	32.01 ± 8.79 (19)
Maximum distance moved (m)	11.99 ± 4.09 (7)	10.61 ± 2.39 (10)	15.54 ± 4.86 (7)	5.25 ± 1.70 (7)	13.76 ± 3.09 (14)	8.41 ± 1.66 (17)
Number of burrows occupied	–	–	1.41 ± 0.11 (29)	1.29 ± 0.07 (38)	1.30 ± 0.13 (20)	1.30 ± 0.09 (30)

We scored lizards as residents if they used the same burrow for 10 or more days (see text). Summary statistics (means ± 1SE) are reported for the spatial data. Sample sizes are indicated in parentheses. Home range area was estimated using the minimum convex polygon.  
doi:10.1371/journal.pone.0041130.t002

Discussion

Our study revealed remarkably similar patterns in burrow occupancy for males and females. Both sexes showed relatively high site fidelity within a season and typically occupied a single burrow although some (ca. 30%) occupied a second burrow and a small proportion (ca. 7%) a third burrow. Individuals from both sexes were also resighted at almost the same frequency (close to 7 sightings). However, males appeared to move more and were seen as much as 14 m from their burrows while females moved a maximum of just over 8 m from their burrows; males also had larger home ranges although high variance meant this relationship was marginally non-significant. These differences were not explained by body size and likely reflect sex-specific life history tactics. Both sexes also had some spatial overlap in their home ranges. In terms of the number of individuals overlapping in space, spatial overlap was greatest between the sexes, compared to within

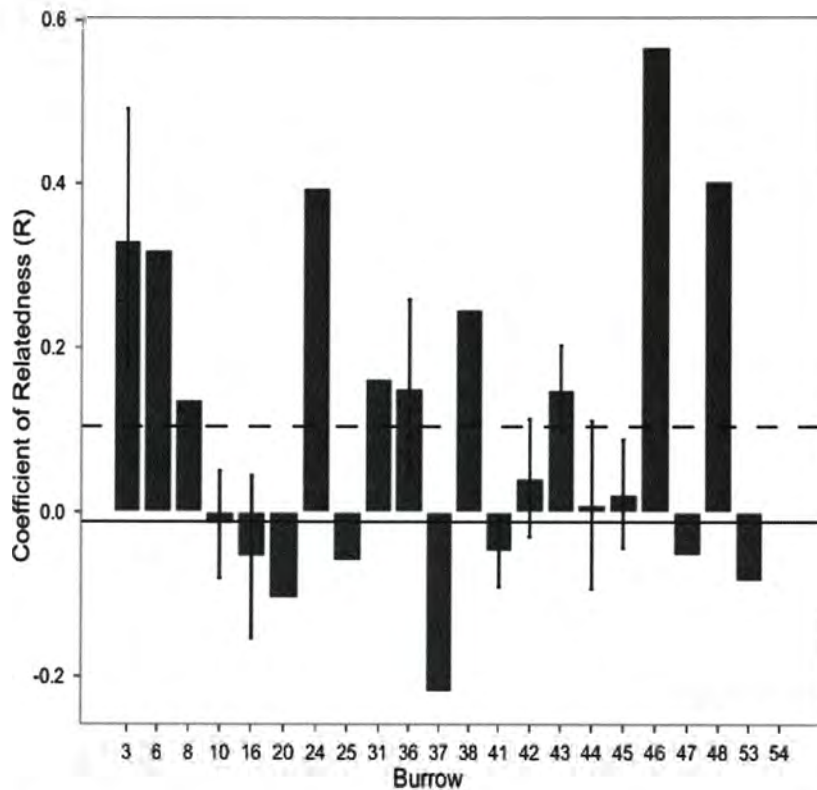
the sexes. That is, males were more likely to overlap with females while females were more likely to overlap with males than other females. Also, males in better body condition overlapped with more females. In terms of overlap pressure—the amount of space shared with another individual, there were no clear patterns and the values were similar for both sexes albeit that a relatively small part of their home range was shared space. Finally, offspring did not show a strong preference for sharing burrows with adult genetic relatives and when they did, only a third of the cases showed higher than average levels of relatedness with only three burrows supporting parent-offspring relationships.

*Phrynocephalus vlangalii* conforms to the typical pattern of space use seen in territorial lizards, which is an exclusive core area that overlaps with members of the opposite sex [18,23]. Males display frequently, are aggressive, and the core of their territory is their burrow entrance. This corresponds to a resource-specific site

**Table 3.** Measures of home range overlap in *P. vlangalii* for adult males with at least 11 sightings (n = 14) and females with at least 8 sightings (n = 13).

	Number overlapped (mean ± SE (n))	Overlap pressure (mean ± SE (n))
♂ on ♀	1.67 ± 0.43 (15)	0.08 ± 0.03 (15)
♂ on ♂	1.27 ± 0.33 (15)	0.11 ± 0.04 (15)
♀ on ♂	1.84 ± 0.53 (19)	0.10 ± 0.04 (19)
♀ on ♀	1.00 ± 0.29 (19)	0.11 ± 0.03 (19)

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**Figure 3. Relatedness of individuals within burrows.** Mean ( $\pm 1$ SE) relatedness coefficient,  $R$  [43], for 22 burrows containing two or more individual *P. vlanguaii*. The dashed line indicates the overall mean burrow relatedness while the solid line is the population mean relatedness. doi:10.1371/journal.pone.0041130.g003

defence [18], which has also been documented in the burrow-dwelling barking gecko (*Ptenopus g. garrulus*; [24]). However, whether females choose mates based on individual quality or some aspect of burrow quality is unknown. Interestingly, females are also highly aggressive, and maintain territories that largely exclude other females, which is the case for many iguanids and agamids (reviewed in [23]). As a consequence, females do not aggregate and males are unable to defend multiple mates and do not appear to mate guard individual females. In resource-based polygynous systems, males that monopolise high quality resources have higher fitness than males in low quality habitats [25]. We did not see any evidence of variation in territory quality because the study area appeared uniform in vegetation structure and food availability. However, males in better body condition were able to overlap more females, suggesting that variance in reproductive success is linked to the control of space. There is also the intriguing prospect that females may benefit from occupying male burrows, which males actively defend. However, there were more burrows than lizards, allowing females the option of avoiding males, and females are capable of digging their own burrows (YQ, unpubl. data). The extent to which females use self-constructed burrows versus other burrows is not known. Finally, male *P. vlanguaii* were larger in body size and had larger head dimensions compared to females, which is typical in polygynous mating systems [26].

The social organisation for this species, coupled with the shared use of burrows, raises the possibility of a cryptic kin-based social system. However, for convincing evidence of parent-offspring associations we predicted frequent burrow sharing between parents and offspring or at the very least, that they would occupy neighbouring burrow systems just a few metres away. Given that

adult lizards defend burrows, a key refuge for young lizards, it could be adaptive for offspring to associate with their parents for several reasons. First, a simple association with a parent could be sufficient to prevent infanticide or aggression from an unrelated adult [10]. Second, parents may even directly intervene in the case of a predation threat. For example, female long-tailed skinks (*Eutropis longicaudata*) actively deter snake predators from their nests [12] and an adult King's skink (*Egernia kingii*) was once observed chasing off a snake that ventured too close to the family refuge [27]. Although we did find evidence that some burrows contained parent-offspring relationships, our observations largely did not support this prediction. First, there were many instances of either solitary, or multiple, offspring in burrows where there was no adult present. Second, the relatively low number of offspring that we did find with at least one parent could be explained by chance given that young lizards are born within the home range of their mother. Even if these few individuals delayed dispersal to remain in the presence of a parent, this is likely to be a weak selective force for the evolution of sociality. Delayed dispersal and social groupings are being documented in more lizard species [4,5,6,7] with the realisation that aggregations of individuals may be the product of social selection. The role of key resources as a potential trigger for the evolution of sociality is as yet poorly understood, but could be a fruitful line of future research.

In summary, we first focused on burrow and space use to establish the potential for social groupings that might influence sociality through parent-offspring associations. Burrows are a key resource necessary for offspring survival, particularly over winter, and young juveniles in their first year of life seem to be dependent on abandoned burrows or in rare cases, the burrows of their



parents. Patterns in spatial overlap largely conformed to predictions of sexual selection theory for males in a polygynous mating system: higher overlap with females than with other males [23]. Female *P. vlangalii* also tended to avoid each other, were observed to be aggressive towards one another, and were more likely to overlap in space with males than females. We did not find evidence of strong spatial and/or pair bonds between males and females that might set the stage for sociality. We suggest that *P. vlangalii* conforms to a classical polygynous mating system tied to resource defence, although detailed studies are required to test the potential value of male-defended burrows to females, and to determine the degree of polygyny.

## Methods

### Ethics statement

All handling and processing of lizards followed approved protocols from the Chengdu Institute of Biology of the Chinese Academy of Sciences. We also followed the ABS (Animal Behavior Society)/ASAB (Association for the Study of Animal Behaviour) "Guidelines for the treatment of animals in behavioural research and teaching". The Chengdu Institute of Biology approved this work and all fieldwork was approved by the Forestry Department of the Sichuan Provincial Government and the Management Office of the Zoige Nature Reserve.

### Study area

We conducted fieldwork at Xiamen Conservation Station in the Zoige Wetland Nature Reserve, southwestern China (33°43'25"N, 102°29'4"E; elev. ca. 3464 m) during the breeding season (May–June of 2009 and 2010). *Phrynosoma vlangalii* occur at a density of 0.19 lizards/m<sup>2</sup> on large, sparsely vegetated sand dunes that are patchy across the landscape [22]. The vegetation on and around these sand dunes is predominantly composed of the grasses *Kobresia humilis*, *Kobresia pratii* and *Elymus nutans* and a shrub, *Salix sclerophylla*.

### Morphological measurements and the use of burrows and space

A 0.2 ha plot (50×40 m) was marked out and divided into 20 quadrants, each 10×10 m [28]. Lizards in the plot were caught by noosing or by pitfall traps. Pitfall traps were simply small holes (ca. 30 cm deep×15 cm diameter) that were dug at the entrance to their burrows and which we filled in after catching the lizard or at the end of the day if we were unsuccessful. All individuals greater than 45 mm snout-vent-length (SVL) were considered sexually mature and were processed immediately following capture. Although we do not know when individuals become sexually mature in this population the smallest size we observed females reproducing was 45 mm SVL (YQ, unpubl. data) and all the males we collected were greater than 50 mm SVL. Lizards were marked permanently by toe-clipping and given a unique color code on their dorsum using non-toxic acrylic paint to facilitate later identification. Sex was determined by checking for hemipenial bulges. Mass was measured with a Pesola® spring scale to the nearest 0.1 g. SVL (snout-vent length), head length (snout to neck length) and head width (distance between the posterior end of the right and left mandible) were measured using digital calipers to the nearest 0.1 mm. Body condition was estimated using the residuals from the regression of log body mass on log SVL [29].

We conducted censuses between 09:00–16:30 h during which lizards were located by slowly walking through the study area four times a day. The census route took approximately 60 min and we scan sampled for lizards at each quadrat for a total of three

minutes before moving onto the next quadrat. Upon sighting a lizard, we recorded their locations, and when possible, the locations of their burrows, using x-y coordinates which we later mapped using ArcGis (9.3) software. We started at a different quadrat each time to minimize any potential bias in the number of sightings per individual. We used only sightings of lizards that were separated by at least 2 h. In total, we marked 76 lizards (35 ♂ and 41 ♀) from 11 May to 11 June 2009, and 96 lizards (35 ♂ and 61 ♀) from 18 May to 16 June 2010. Lizards captured in both years were only included once (2009) in any morphological and spatial analyses.

We scored lizards as residents if they used the same burrow for ten or more days, which is a criterion that has been used in other studies [28,30,31]. We estimated home range area and maximum movement distance from a burrow for each lizard using Hawth's Tools ([www.spatialecology.com](http://www.spatialecology.com)), a plug-in for ArcGIS, by calculating the minimum convex polygons (MCP), Kernel and distance between points. MCP has been suggested to be good at estimating the total area of an individual's home range, but is influenced by the number of individual relocations [32]. The Kernel method, however, estimates the utilization distribution within the home range and has been shown to overestimate home range area [32,33,34]. Row and Blouin-Demers [34] suggested that MCP and Kernel methods should be used simultaneously in calculating individual home range area. In our analysis, MCP was used to estimate the area of the Kernel, while Kernel estimation was used to indicate the utilization distribution.

We used the method of Smith [35] to determine the minimum number of sightings needed for home range estimation by running a series of regressions with MCP home range as the dependent variable. We started with all individuals having at least three sightings (47 males and 41 females), the number of sightings necessary for inclusion was increased until there was no longer a statistically significant relationship between home range size and the number of sightings. For males, 11 or more sightings were necessary for MCP home range estimation (sightings ≤10,  $r=0.55$ ,  $n=18$ ,  $p=0.01$ ), while for females only nine or more sightings were required (sightings ≤8,  $r=0.40$ ,  $n=19$ ,  $p=0.08$ ). For the Kernel method, the grid size was set to 1 m and the smoothing factor set to 3. After comparing the two methods, we determined that the 75% Kernel best described lizard home range area because it assigned similar areas with those assigned by the MCP method.

The amount of overlap between home range areas was calculated with ArcGis (9.3) software. For each individual the number of overlapping males and females was calculated as well as overlap pressure between individuals of the same and opposite sex [36]. We calculated overlap pressure by adding all the areas that other individuals shared with the focal individual and then dividing this sum by the focal individual's home range size. This results in a score from 0– $n$  where  $n$  is the degree of overlap pressure. We counted the number of overlapping individuals and calculated overlap pressure because both of them are standard estimations of overlap that have been used in other studies [31,37].

We analyzed the differences in SVL, head length and head width between the sexes using ANCOVA. For the SVL comparison, we include sex as the main factor and year as a covariate. For head length and head width comparisons, we included sex as the main factor with both the year and SVL as covariates. To control for possible differences between years, we also included an interaction between sex and year in all models. In some cases differences between the sexes varied by year (although they were not significant) and we separately provided means and standard errors for each sex by year and combined (Table 1). We

tested for differences in home range area between the sexes using ANCOVA, controlling for SVL. For males, we used both body condition and SVL as a measurement of individual quality, while for females, we only used SVL because we could not account for any influence of reproductive condition which may affect body mass. Spearman rank correlations were used to examine the relationships between body condition, SVL, home range size and home range overlap. All data were analyzed using R (version 2.14 for Windows, freely available at <http://www.r-project.org>).

### Burrow sharing and relatedness in adults and offspring

We excavated 80 burrows during the hibernation period (November 2010) that were marked in September to test whether juveniles over-wintered with their parents. We chose the over-wintering period to address this question because a burrow is essential for over-wintering survival and this would be the most important time for parents to provide burrows for offspring. All lizards collected in their burrows were toe-clipped (for DNA), measured for body size, sexed, and relocated to the Chengdu Institute of Biology for further behavioral study.

Genomic DNA was extracted from 97 tissue samples collected from lizards found within burrows using the DNeasy Tissue extraction kit (QIAGEN) according to the manufacturer's protocol. We used eight microsatellite DNA loci (Phr27, Phr79, Phr63, Phr160, PVMS12, PVMS18, PVMS35 and PVMS38) for which primers were already developed [38,39]. PCR amplification was performed in 25  $\mu$ L reaction volumes containing 1  $\mu$ L of extracted DNA, 12.5  $\mu$ L PCR mix (TransGen), 1  $\mu$ L of each primer (10 pmol  $\mu$ L<sup>-1</sup>) and 9.5  $\mu$ L of dd H<sub>2</sub>O. Forward primers of Phr27, PVMS18 and PVMS38 were labeled with FAM fluorescein, forward primers of Phr79 and PVMS35 were labeled with HEX fluorescein, and forward primers of Phr63, PVMS12 and Phr160 were labeled with TAM fluorescein. Reactions took place in a thermocycler (Mastercycler pro, Eppendorf) with an initial denaturation of 94°C for 5 minutes, then 30 cycles at 94°C for 30 s,  $T_a$  for 30 s, and 72°C for 1 min followed by 72°C for 10 min. The primer specific annealing temperatures ( $T_a$ ) can be found in [39] and [38]. The fluorescent-labeled PCR products were pooled and alleles were separated using an ABI PRISM 3730 capillary sequencer (Applied Biosystems) and scored using GENEMAPPER vers. 1.95.

We used KINGROUP vers. 2.0 (Kononov *et al.* 2004), which makes use of the method developed by Queller and Goodnight [40] and Goodnight and Queller [41], to calculate pairwise

relatedness and test hypothesized relationships among individuals found within the same burrows (or nearby burrows). The program uses likelihood-based methods on genotypic data when both the maternal and paternal alleles are unknown by calculating the average likelihood values for all possible assumptions about the maternal and paternal origin of alleles [41]. The product of the individual likelihoods are then taken over all the loci and support for the primary vs. null hypothesis is evaluated using a likelihood ratio between the two hypotheses [41]. The statistical significance of the hypothesized relationship is calculated by simulating pairs of individuals of known relationship, where alleles are drawn at random from the population allele frequencies for one individual and then according to the null hypothesis to be tested for the second individual. The program proceeds by creating a large number of simulated pairs that are related according to the null hypothesis. The value of the likelihood ratio, which excludes 95% of these pairs in the simulated likelihood ratio distribution, is then used to test statistical significance between pairs of individuals [41].

We tested whether adult lizards found within the same burrows as offspring were parents of those offspring. *Phrynocephalus vlangalii* is known to occupy from 1–3 distinct burrows (this study). Therefore, we also tested the relationship between offspring and adults found in separate burrows if these burrows were within 2 m of each other. A distance of 2 m was chosen because this is the largest distance between burrows used by a single individual. We differentiate between these two analyses by referring to within-burrow comparisons (=burrows) and between-burrow comparisons (=burrow groups). In all hypothesis tests likelihood calculations were done by simulating 10,000 pairs based on the population allele frequencies from the 97 individuals collected in the study. Any pair where a parent-offspring relationship was supported was checked manually to ensure that the individuals shared alleles at all eight loci.

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### Author Contributions

Conceived and designed the experiments: YQ DN JF. Performed the experiments: YQ. Analyzed the data: YQ DN. Contributed reagents/materials/analysis tools: YQ JF. Wrote the paper: YQ DWAN JF MJW.

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## APPENDIX II

**Appendix II** contains approved animal ethics applications for data chapters

Appendix II removed from Open Access version as they may contain sensitive/confidential content.