Sex, androgens, and whole-organism performance in an Australian lizard

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Understanding underlying physiological differences between the sexes in circulating androgens and how hormonal variation affects morphology–performance relationships may help clarify 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 androgens differed between the sexes and whether they covaried with morphological and performance traits (bite force, sprint speed, endurance). Males had larger head dimensions, stronger bite force, faster sprint speed, and longer endurance compared to females. We found that the sexes did not differ in androgen concentrations and that androgens were weakly associated with both morphological and performance traits. Interestingly, high circulating androgens showed a nonlinear relationship with bite force in males and not females, with this relationship possibly being related to alternative male reproductive tactics. Our results suggest that androgens are not strongly correlated with most performance and morphological traits, although they 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. © 2014 The Linnean Society of London, Biological Journal of the Linnean Society, 2014, ••, ••–••.


INTRODUCTION

Males and females of many species are sexually dimorphic, varying in size, shape, and/or colour (Hedrick & Temeles, 1989; Andersson, 1994; Fairbairn, Blankenhorn & Szekely, 2007). 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, Sawyer & Losos, 2007). Although SD in morphological traits has been studied in numerous taxa (Hedrick & Temeles, 1989; Andersson, 1994; Fairbairn et al., 2007), few studies have attempted to quantify and understand sex differences in performance traits (Lailvaux, Alexander & Whiting, 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 clarify the evolution of SD.

Lizards are model systems for addressing relationships between morphology and performance because of the clearly developed methods for quantifying performance and our understanding of the relationships between performance and 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). 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, play a key role in mediating this interaction
via their organizational and activational effects on morphology and performance (Fig. 1). Androgens can indirectly affect performance by modifying growth rates and organizing the development of morphological traits such as body size and shape (Sinervo et al., 2000; Cox & John-Alder, 2005; Sinervo & Calsbeek, 2006; John-Alder, Cox & Taylor, 2007; Miles et al., 2007b). Androgens may also mediate plastic changes to behaviour and morphology (e.g. muscle mass and fibre type), which can lead to a more active and direct role in shaping variation in morphology and performance (Sinervo et al., 2000; Irshick et al., 2006; Gowan, McBrayer & Rostal, 2010; Huyghe et al., 2010). In addition, androgens are predicted to be higher in males compared to 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, androgens can have complex effects on both male and female morphology, growth, and behaviour (Owens & Short, 1995; Crews, Sakata & Rhen, 1998; Cox & John-Alder, 2005; John-Alder et al., 2007; Starostová et al., 2013). In animal species, including lizards, both circulating levels of androgens and their biological effects can be similar for both sexes, leading to similarities in behaviour, morphology, and performance (Adkins-Regan, 2005; Ketterson, Nolan & Sandell, 2005; Hews, Hara & Anderson, 2012).

Interestingly, much of our understanding about androgen–morphology–performance relationships comes from lizard 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 lower levels of SD or species with reversed sexual dimorphism and whether they exhibit similar patterns. Studying strongly sexually size dimorphic species can make it difficult to understand sex differences in performance because body size often leads to correlated changes in suites of traits, such as limb size, body mass, and lung volume (Van Damme et al., 2008), which may be strongly correlated with performance capacity (Van Damme et al., 2008). In addition, if males and females differ in

Figure 1. Predicted relationships between androgens, morphology [head size, snout–vent length (SVL), mass] and performance (bite force, sprint speed, and endurance) in *Eulamprus quoyii*. Unidirectional arrows indicate that increases in one variable lead to corresponding increases in the connected variable. Grey arrows indicate that the relationships are predicted to be the same for both males and females, whereas black arrows indicate that relationships will either be stronger or only exist among males. Relationships that are supported by our data (**) and those which we did not find support for ‘NS’ are indicated. NS*, no sex differences, although a weak negative relationship was found. NS**, relationship was not sex-dependent.
their sensitivity to androgens, this can make the identification of possible mechanisms contributing to sex-differences more difficult.

Despite the central role lizards have played in addressing questions of functional ecology, few studies have explicitly tested for sex differences in performance (Lailvaux et al., 2003; Lappin, Hamilton & Sullivan, 2006; McBrayer & Anderson, 2007; Miles et al., 2007b; Zajitschek et al., 2012). Of the studies that have done so, males generally perform better than females in performance traits such as biting 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), although this is not always the case (Lailvaux, 2007; Van Damme et al., 2008; Zajitschek et al., 2012) and may be related to the different role of androgens as a result of differential selective pressure between the sexes.

Eastern water skinks (Eulamprus quoyii) are a unique system for exploring sex-dependent differences in androgen–morphology–performance relationships. This species is not sexually dichromatic and males and females do not differ in snout–vent length (SVL). Males have larger heads, differ in limb length and are heavier than females (Schwarzkopf, 2005). Eulamprus quoyii has a short breeding period and a polygynandrous mating system with high levels of multiple paternity (approximately 65% of clutches are sired by multiple males; Noble, Keogh & Whiting, 2013a). Males exhibit alternative reproductive tactics, with some males adopting ‘resident’ or ‘territorial’ tactics, having small home ranges, and being more active, whereas other males adopt ‘floater’ tactics, are scarcely observed, and have large home ranges (Noble et al., 2013b). Eulamprus quoyii forms dominance hierarchies, where more dominant individuals have greater access to food and basking opportunities compared to subordinate individuals and a hierarchy is generated where progressively less dominant individuals miss out on food and are the first to be supplanted from basking sites (Done & Heatwole, 1977). Although overt aggression is not as common as in other lizards species, males and females do engage in aggressive displays (Done & Heatwole, 1977). The similarity in size between the sexes and the lack of sexual dichromatism provides an excellent opportunity to examine the androgen–morphology–performance paradigm given that most previous work has been carried out in species where the sexes vary in numerous morphological traits (e.g. body size, mass, coloration, androgens). Comparisons with species showing less sexual dimorphism may help test the generality of sex differences in performance and the role that androgens play in mediating variation in these functional traits.

The present study aimed to investigate: (1) whether there are sex differences in performance (sprint speed, endurance, bite force); (2) whether male and female E. quoyii differ in circulating androgen levels; (3) whether circulating androgens co-vary with performance; and, if so, (4) does the relationship between androgens and performance vary between the sexes? Given our understanding of morphological differences between the sexes, we made explicit predictions about how morphology would influence performance traits and how androgens (assuming sexes differ in circulating androgen levels) would influence performance (Fig. 1). We predicted that the relationship between body size (SVL) and performance will be similar for males and females given that the sexes do not differ in SVL (Fig. 1, grey lines). On the other hand, males are expected to exhibit a correlation between androgens and performance, assuming that males have higher circulating levels of androgens, and this is predicted to explain sex differences in performance (Fig. 1; black lines). The directionality is positive because androgens are expected to promote muscle development and growth. It has been shown in other lizards that androgens increase endurance (Sinervo et al., 2000), bite force (Cox et al., 2009), and sprint speed (Mills et al., 2008), and are hypothesized to positively influence head size (Irschick et al., 2006).

MATERIALS AND METHODS

LIZARD COLLECTION

We captured 216 adult E. quoyii (108 males and 108 females) from five sites in the Sydney region (Noble et al., 2013a) from 12 August to 22 September 2010 prior to female ovulation (early October). Female E. quoyii become vitellogenic in September; however, follicle size does not reach an advanced stage until female ovulation occurs (Vernon, 1969). Females are live-bearing and give birth to offspring in December to January (Noble et al., 2013a). Male testis weight is largest in September and spermatogenesis continues into October and November until testis size begins to decrease (Vernon, 1969; D. Noble, unpublished data).

We collected approximately 30–50 µL of blood from the suborbital sinus within minutes (mean = 5.26 ± 0.45 min; median = 4 min, N = 201) of capture for hormone analysis. Blood was placed on ice until it could be processed in the laboratory. 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 (SVL: from snout of lizard to beginning of cloaca; inter-limb length: length between insertion point between front arm and back leg), body mass, tail length (length from tip of tail to the posterior...
point of the cloacal opening), and head dimensions (head width: distance between widest points of head; head length: distance from the tip of the snout to the anterior edge of tympanum; head depth: maximal height in the occiput region of the head) using a transparent ruler and digital calipers. To keep the time between hormone collection and performance measurements to a minimum, lizards were then transferred to plastic bins (Keiji; 487 mm (length) × 350 mm (width) × 260 mm (height)) in a temperature-controlled room for 3 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 approximately 22–26 °C with an elevated basking side of approximately 28–30 °C under ultraviolet lighting. Water was provided at all times and lizards were fed once during the 3 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 measurement was: (1) bite force, (2) sprint speed, and (3) endurance. We adopted this protocol for measuring performance because (1) of the logistical constraints in measuring the large sample of lizards, which prevented us from holding lizards for longer than 3 days; (2) we wanted the repeated measures of sprint speed, endurance and bite force to be as independent as possible, and (3) bite force and sprint speed were non-exhaustive for lizards, allowing us to also measure endurance on the same day. *Eulamprus quoyii* was highly motivated to sprint and run down the treadmill and careful inspection of model residuals did not reveal strong outlying data points in our final analysis, which would suggest that final measurements were submaximal. Although this may impose stronger correlation between performance measurements, our data strongly suggest this was not a problem.

Prior to all measurements, lizards were warmed to their preferred body temperature (approximately 28 °C; Law & Bradley, 1990) by placing lizards in plastic zip-lock bags and floating them in warm water. 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 individual 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 all analyses (Losos, Creer & Schulte, 2002).

**Bite force**

Bite force [Newtons (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.) that was connected to a Kistler charge amplifier (Model 5995; Kistler Inc.). We induced lizards to bite two plastic plates by gently pinching the sides of their mouth.

**Sprint speed**

Sprint speed was measured directly after the first bite force measurement using a 2-m racetrack. This was measured once per day over the 3-day period for a total of three measurements. 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 (http://www.motionprosoftware.com/).

**Endurance**

Endurance was measured immediately following sprinting. Lizards were run on a modified human treadmill to measure their maximal endurance. A transparent plexiglass box with adjustable compartments was placed on top of the treadmill. The middle compartment was adjusted so that the lizards could run unobstructed, while ensuring that they could not easily turn around. All trails were run at a speed of 1.0 km h−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. We took one measurement of endurance a day over the 3-day measurement period.

**Androgen assay**

We measured plasma androgens using a double-antibody enzyme-immunoassay. 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 steroids: testosterone 100%, 5α-dihydrotestosterone (DHT) 24%, 5β-DHT 12%, 4-androstene-3β,17β-diol 8%, and 5α-androstane-3α,17β-diol 6% (Hirschenhauser, Mostl & Kotrschal, 1999). Assay sensitivity was 0.006 ng mL⁻¹. The assay was biochemically validated in our laboratory by demonstrating parallelism between a serially diluted plasma pool and the standard curve.

Assay procedures were similar to those described previously (Palme & Möstl, 1994; Touma et al., 2003). Briefly, we coated microtiter plates (Nunc maxisorp) with 250 μL of protein A solution (Sigma P7837; 2 μg mL⁻¹) and incubated overnight at room temperature. Wells were emptied and blocked with 300 μL of a second coating buffer for at least 3 h. Plates were washed and loaded in duplicate with 25 μL of standard, control or diluted plasma sample (1 : 10 in phosphate buffer) immediately before 100 μL 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 with shaking. After washing, we added 250 μL of streptavidin-peroxidase conjugate (Sigma S2438; 0.2 μg mL⁻¹) to each well. Plates were incubated for 45 min at 4 °C with shaking and then washed again. Substrate solution (250 μL well⁻¹, tetramethylbenzidine; Sigma 87748; 66.7 μg mL⁻¹) was added to the plate and incubated for 45 min as above. The enzymatic reaction was stopped by adding 50 μL of sulphuric acid (2 mol L⁻¹) to each well and absorbance was measured at a wavelength of 450 nm (reference filter = 630 nm) using a ELx808 microplate reader (BioTek Instruments). To monitor precision and reproducibility, low (approximately 70% binding) and high (approximately 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 dilution of 1 : 100. For samples with low androgen 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, version 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. Because of insufficient plasma, it was not possible to obtain circulating androgen data for all lizards; therefore, for each of the analyses, we indicate the final sample size used.

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 sample-size-corrected Akaike information criteria (AICc) (Burnham & Anderson, 2002). We also calculated Akaike weights (ω), which are a measure of the probability of each model out of the candidate set (Symonds & Moussalli, 2011). For models within 2 ΔIC units of one another, we chose the most parsimonious model, even though models within 2 ΔIC units are considered equally plausible (Burnham & Anderson, 2002).

To test for sex differences in androgens, 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 variables (Cade & Noon, 2003). To make coefficients more comparable, we mean centred and standardized each trait by dividing by 2 SDs prior to analysis, making continuous and binary categorical predictors more comparable (Gelman, 2008).

Changes in muscle mass may cause plasticity in some morphological measurements, particularly head width and depth, as well as body mass. These traits have been shown to change in some species through the season, presumably as a result of plasma androgens (Irschick et al., 2006). To test whether circulating androgens are associated with larger head dimensions and body mass, we added androgen concentration to our top supported models for these morphological traits (above), controlling for collection date by including it as a covariate.

To test for sex differences in performance and to examine whether morphological traits and circulating androgens 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 colinearity 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 bite force. In our sprint speed and endurance models, we included main effects of SVL, residual mass, sex, androgen concentration (log androgens + 1), collection date, researcher, and cloacal temperature at the time of performance. Because we were also interested in whether there might be differences in the relationship between androgens, SVL, and residual mass 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 led to strong colinearity between these parameters (variance inflation factors 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, androgen concentration, 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 nonlinearity 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 nonlinearly related to performance measurements in males (Morrison, Keogh & Scott, 2002; Stapley & Keogh, 2004, 2005; Keogh et al., 2012; Noble et al., 2013b). If quadratics were included in models, we compared the AICc between our main effects model and our new model to test whether it improved model fit. In bite force models, there were three strongly influential points with extremely low bite force residuals. We assumed that this might be the result of 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 (Losos et al., 2002). Coefficients were again standardized for these analyses (Gelman, 2008). We present coefficients and their SEs from main effect models along with ΔAICc 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 androgen concentration in sprint speed models given that performance traits needed to have a balanced sample size, and including androgens 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

SVL was not different between males and females (GLM comparing null model with model containing sex: ΔAICc = 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 (Figs 2C, 3D, Table 1). Males and females differed in head dimensions and there was significant heterogeneity of slopes between body size and head width (Fig. 3A, Table 1), head length (Fig. 3B, Table 1), and head depth (Fig. 3C, Table 1). In all cases, males had larger head dimensions compared to females (Fig. 3A, B, C). Many lizards had broken or re-growing tails (low points; Fig. 3F) and this made the relationship between tail length and body size complicated. We therefore only present 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. 3F). Females had significantly larger inter-limb length compared to males after controlling for body size (Fig. 3E, Table 1). 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.

SEX DIFFERENCES IN CIRCULATING ANDROGENS

Median plasma androgen concentration increased with collection date [β0.5 = 0.02, confidence interval (CI) = 0.01–0.04] and males had a tendency to have lower median plasma androgens than females, although these distributions largely overlapped [Fig. 4; Intercept: β0.5 = 0.87, CI = 0.50–0.98; Sex (M): β0.5 = −0.34, CI = −0.61–0.05]. Median plasma androgens were not related strongly to either SVL
We found only weak evidence that androgens positively influenced head width, depth or mass directly (Table 2). Collection date was positively associated with mass and head width and negatively associated with head depth (Table 2). However, the inclusion of collection date explained little variation (1–2%; Table 2) and effect sizes were weak.

**Effect of morphology and androgens on performance across the sexes**

We found weak evidence that androgens were linearly related to sprint speed, endurance and bite force in both males and females (Fig. 5); however, it did appear to show a nonlinear relationship with bite force in males (Fig. 5E). Males with nondetectable androgen concentrations and males with high androgen concentrations had both high bite force, whereas males with intermediate androgen concentrations had lower bite forces (Fig. 5E). Increased androgen concentrations had a tendency to be associated with lower levels of endurance (Fig. 5C) but appeared to be driven by a single male. The top-supported model for sprint speed and endurance included only the main effect parameters and there was no evidence for heterogeneity of slopes in morphology and performance traits between the sexes (Tables 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). Androgens did not strongly influence endurance and there was no evidence for nonlinearity in androgens after controlling for all other variables. Residual mass and SVL were positively related to sprint speed (Table 3). Males had higher sprint speed compared to females and increased androgen concentrations decreased maximal sprint speed independently of sex and there was no evidence for a significant interaction between androgens and sex (Table 3). Body size and residual head width both strongly affected bite force in males and females, whereas androgens did not appear to be linearly related to bite force after controlling for all other variables in the model (Table 4). Residual plots identified nonlinearity between androgens 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 improve model fit, although both models were almost equally supported (ΔAICc between main effects model in Table 4 and model with quadratics for androgens and residual head width = 1.76).

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

Residual bite force, sprint speed, and endurance were weakly correlated in males (Table 5) and females (Table 5).
DISCUSSION

We found that male *E. quoyii* were faster and ran for longer than females and had a stronger bite force, which may be the result of differences in mass and body shape between the sexes. Plasma androgen concentrations did not differ between the sexes and there was no evidence that they co-varied with morphological or performance traits, other than weakly with sprint speed (Fig. 1). Interestingly, high circulating androgens showed a nonlinear relationship with bite force in males and not females, and this relationship may be related to male alternative reproductive tactics (Noble et al., 2013b). Strong differences in the sexes with respect to 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 link between these performance traits and fitness in lizards (Sinervo et al., 2000; Husak, 2006; Husak et al., 2006).

The higher performance capacity (sprint speed, endurance and bite force) of male compared to female *E. quoyii* is consistent with patterns in a wide range of taxa, in which males generally perform better than females (Lailvaux, 2007; Van Damme et al., 2008). Our results are interesting in that these differences may map more closely to shape and energy store/mass differences between the sexes rather than corresponding changes in suites of traits that scale with body length (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. Limb size (Losos, 1990; Bonine & Garland, 1999) and increased energy stores and/or muscle mass are considered 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). Additionally, differences in reproductive state between males and females may also contribute to differences in running performance (Miles, Sinervo & Frankino, 2000). However, we collected males and females at the beginning of the reproductive season and prior to female ovulation. Female follicle size is still small during this period (Vernon, 1969) and many females did not reproduce in 2010 (Noble et al., 2013a). It is therefore unlikely that differences in reproductive state alone could explain the differences in performance that we observed. Such strong differences in performance between the sexes suggest that there may be differential selection on

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**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) (LJL)

<table>
<thead>
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<th>Model</th>
<th>Log (head width)</th>
<th>Log (head length)</th>
<th>Log (head depth)</th>
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<th>Log (mass)</th>
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<td>Δ AICC</td>
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<td>0.28</td>
<td>0.16</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Sample-size corrected Akaike information criteria (AICc), the difference in AICc between the best-supported model and model i (ΔAICc), and the Akaike weight (w) of each model are provided. Bolded values are from the top-supported models. Sample sizes for each analysis = 213. SVL, snout–vent length; UCI, upper confidence interval; LCI, lower confidence interval.
these functional traits (Lailvaux, 2007; Van Damme et al., 2008), which is plausible given the important link between these performance traits and fitness (Sinervo et al., 2000; Husak, 2006; Husak et al., 2006). Male *E. quoyii* emerge earlier in spring than females and are more active and conspicuous compared to females (D. Noble, unpubl. data). If males have higher predation rates during this time, we

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**Figure 3.** Relationships between log snout–vent length (SVL) and morphological measurements for males (black) and females (grey). A, log head width. B, log head length. C, log head depth. D, log mass. E, log inter-limb length. F, log tail length. Solid lines are best-fit lines from the models in Table 1. Dashed lines indicate the upper and lower 95% confidence intervals. SVL, snout–vent length.

would predict selection for faster sprinters. By contrast, we have shown that body size, time spent active, and home range area are sexually-selected traits in males and these may be correlated with bite force, endurance and/or sprint speed, which are predicted to be important for maintaining dominance (Robson & Miles, 2000) and remaining active/having large home ranges.

Plasma androgens did not differ between male and female *E. quoyii*, although there was substantial variation within each sex. A lack of androgen differences between the sexes has been observed in other taxa (Ketterson et al., 2005), including lizards (Hews et al., 2012). Ketterson et al. (2005), provides evidence that testosterone is higher in females of sexually monomorphic bird species, and, although the data in lizards are limited, comparisons between two Sceloporus species that differ in female aggression and degree of sexual dimorphism have revealed similar findings (Hews et al., 2012). Testosterone is known to be important in sexual signal development and in affecting home range, movement rates, and aggression in lizards (Sinervo et al., 2000; Cox et al., 2005, 2009; Whiting et al., 2006; Miles et al., 2007b). Both male and female *E. quoyii* are aggressive and males do not develop obvious sexual signals commonly observed in many lizard taxa (D. Noble, pers. observ.). In addition, male *E. quoyii* exhibit alternative reproductive tactics (ARTs) that are linked to differences in movement rates, home range size, and activity (Noble et al., 2013b). This variation in home range and activity appears to also manifest itself in females in *E. heatwolei*, a congeneric species (Stapley & Keogh, 2005), and may in part explain why the sexes have similar androgen concentrations.

Although plasma androgens were negatively related to sprint speed, the relationship between androgens, morphology and performance traits was generally weak in *E. quoyii*. Androgens, such as testosterone, have been implicated as a proximate explanation for differences in performance both between (Lailvau, 2007; Van Damme et al., 2008) and within the sexes (Irshick et al., 2006; Miles et al., 2007b). For example, testosterone can affect performance by increasing muscle mass and aggressiveness or through changes in metabolic rate, which is predicted to explain variation in speed, endurance, and bite force between the sexes (Lailvau, 2007; Van Damme et al., 2008). The fact that there were differences in performance between the sexes and yet there are no body length or androgen differences between them suggests that, if androgens such as testosterone do affect morphology–performance relationships, they do so during ontogeny through organizational effects on body shape. However, experimental manipulation of androgens to investigate whether it affects morphology and performance will be necessary. Concentrations of androgen receptor proteins and aromatizing enzymes may also play a role in how tissues, such as muscle, respond to circulating androgens, and

![Figure 4.](image-url)
Table 2. The relationships between androgens (A) and head width, head depth, and body mass in *Eulamprus quoyii*

<table>
<thead>
<tr>
<th>Model</th>
<th>Log (head width) (N = 193)</th>
<th>Log (head depth) (N = 193)</th>
<th>Log (mass) (N = 193)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AICC</td>
<td>Δ AICC</td>
<td>w</td>
</tr>
<tr>
<td>Top model from Table 1 or null (SVL)</td>
<td>−709.63</td>
<td>15.74</td>
<td>0.00</td>
</tr>
<tr>
<td>Top model + log A</td>
<td>−709.03</td>
<td>16.34</td>
<td>0.00</td>
</tr>
<tr>
<td>Top model + CD</td>
<td><strong>725.37</strong></td>
<td>0</td>
<td><strong>0.63</strong></td>
</tr>
<tr>
<td>Top model + log A + CD</td>
<td>−723.30</td>
<td>2.07</td>
<td>0.22</td>
</tr>
<tr>
<td>Top model + log A + CD + Sex × log A</td>
<td>−722.42</td>
<td>2.95</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>LCI</th>
<th>UCI</th>
<th>Estimate</th>
<th>LCI</th>
<th>UCI</th>
<th>Estimate</th>
<th>LCI</th>
<th>UCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.68</td>
<td>2.678</td>
<td>2.688</td>
<td>2.42</td>
<td>2.41</td>
<td>2.42</td>
<td>3.23</td>
<td>3.22</td>
<td>3.25</td>
</tr>
<tr>
<td>Log (androgens + 1)</td>
<td>0.002</td>
<td>−0.01</td>
<td>0.012</td>
<td>−0.0005</td>
<td>−0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>−0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>Log (SVL)</td>
<td><strong>0.16</strong></td>
<td><strong>0.15</strong></td>
<td><strong>0.17</strong></td>
<td><strong>0.14</strong></td>
<td><strong>0.13</strong></td>
<td><strong>0.15</strong></td>
<td><strong>0.45</strong></td>
<td><strong>0.42</strong></td>
<td><strong>0.47</strong></td>
</tr>
<tr>
<td>Sex (M)</td>
<td>0.12</td>
<td>0.11</td>
<td>0.13</td>
<td>0.09</td>
<td>0.08</td>
<td>0.11</td>
<td>0.15</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Sex × log(SVL)</td>
<td>0.05</td>
<td>0.03</td>
<td>0.08</td>
<td>0.04</td>
<td>0.01</td>
<td>0.06</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Collection date</td>
<td>0.02</td>
<td>0.01</td>
<td>0.04</td>
<td>−0.02</td>
<td>−0.04</td>
<td>−0.01</td>
<td>0.07</td>
<td>0.04</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Top models are the best-supported models from Table 1. The change in corrected Akaike information criteria (AIC<sub>c</sub>) between the top model and the models containing log transformed androgen concentration and Julian collection date are provided. Samples sizes for each analysis are provided in parenthesis. We present the full main effects models along with their lower and upper 95% confidence intervals (‘LCI’ and ‘UCI’). R<sup>2</sup> = adjusted R<sup>2</sup>. All estimates are standardized (mean = 0, SD = 2). Bolded values indicate top-supported model. CD, collection date; SVL, snout–vent length.
Figure 5. Relationship between circulating androgen concentrations and performance measurements (sprint speed, endurance, and bite force) for males (A, C, E) and females (B, D, F).

this remains to be explored in the future. Variation in morphology and performance could also be explained by other dimensions of physiology, such as corticosterone concentrations (Miles, Calsbeek & Sinervo, 2007a) or mitochondrial enzyme activity (Seebacher et al., 2003). Few studies have directly tested whether androgens, such as testosterone, covary with performance and morphological traits. In an experimental study on Gallotia galloti, Huyghe et al. (2010) found that pre-implant circulating testosterone was positively correlated with bite force but not with sprint speed, body size or any other morphological trait. Treatment with exogenous testosterone affected muscle mass but 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 testosterone 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 testosterone, bite force, and dewlap size no longer existed (Husak et al., 2007). By contrast, exogenous testosterone increased endurance in yellow and blue-throated morphs of Uta stansburiana (Sinervo et al., 2000). Given the results of the present study, as well as those in the literature on other lizards, it is clear that the role of testosterone with respect to enhancing performance is still poorly understood. A consideration of the interacting...
effects of other hormones and levels of androgen receptors and enzymes will be essential for understanding the proximate role that androgens play in shaping individual performance and morphology.

Interestingly, we did find evidence for a nonlinear relationship between androgen concentrations and bite force in males but not females. Model residuals also suggest that this relationship between bite

### Table 3. Full models testing the relationship between performance traits (maximal endurance, sprint speed), sex, and morphology (SVL, tail length, residual mass)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Log maximal endurance (N = 193)</th>
<th>Maximal speed (N = 190)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate  SE  Std. Est. SE</td>
<td>Estimate  SE  Std. Est. SE</td>
</tr>
<tr>
<td>Intercept</td>
<td>37.973  23.063  5.256  0.015</td>
<td>-49.7  43.9  1.79  0.04</td>
</tr>
<tr>
<td>Sex (M)</td>
<td>0.297  0.039  0.297  0.039</td>
<td>0.11  0.04  0.11  0.04</td>
</tr>
<tr>
<td>SVL</td>
<td>0.015  0.002  0.215  0.031</td>
<td>0.006  0.002  0.08  0.03</td>
</tr>
<tr>
<td>Res. Mass</td>
<td>-0.064  0.156 -0.016  0.039</td>
<td>0.45  0.159  0.11  0.04</td>
</tr>
<tr>
<td>Tail length</td>
<td>-0.002  0.001 -0.106  0.033</td>
<td>0.0008  0.0006 0.05  0.03</td>
</tr>
<tr>
<td>Log (androgens +1)</td>
<td>-0.027  0.014 -0.057  0.031</td>
<td>-0.03  0.02  -0.07  0.03</td>
</tr>
<tr>
<td>Collection date</td>
<td>-0.002  0.002 -0.051  0.035</td>
<td>0.003  0.003  0.07  0.07</td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.018  0.013 -0.044  0.032</td>
<td>0.05  0.01  0.11  0.03</td>
</tr>
<tr>
<td>Researcher 1</td>
<td>0.089  0.042  0.089  0.042</td>
<td>-0.09  0.07 -0.09  0.07</td>
</tr>
<tr>
<td>Researcher 2</td>
<td>-  -  -  -</td>
<td>0.11  0.043  0.11  0.04</td>
</tr>
</tbody>
</table>

**Main effects model**

Main effects model $\Delta$ AIC $\Delta$  $\Delta$

| Sex × Androgens     | -48.86  4.45  4.46  0.49 | -52.60  4.19 |
| Sex × Residual mass | -48.79  4.52  4.52  0.52 | -52.43  4.36 |
| Sex × SVL           | -48.86  4.45  4.45  0.51 | -52.64  4.15 |

**Sexes were separated as a result of strong collinearity between head dimensions and sex. Estimates shown in bold are significant at $P < 0.05$. Three lizards were excluded from models because they were extreme outliers in the analysis. Std. Est., standardized regression coefficient. SVL, snout–vent length.**

### Table 4. Full model estimates (unstandardized and standardized) for the effects of morphology and androgens on bite force in males and females

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bite force: males (N = 88)</th>
<th>Bite force: females (N = 90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate  SE  Std. Est. SE</td>
<td>Estimate  SE  Std. Est. SE</td>
</tr>
<tr>
<td>Intercept</td>
<td>1731.94  453.07  14.00  0.71</td>
<td>-420.26  567.83  9.75  0.57</td>
</tr>
<tr>
<td>SVL</td>
<td>0.33  0.03  4.49  0.46</td>
<td>0.28  0.03  4.23  0.46</td>
</tr>
<tr>
<td>Residual mass</td>
<td>0.84  2.77  0.16  0.51</td>
<td>2.24  2.09  0.56  0.52</td>
</tr>
<tr>
<td>Residual head width</td>
<td>16.36  6.27  1.43  0.55</td>
<td>24.96  6.40  1.95  0.50</td>
</tr>
<tr>
<td>Log (androgens +1)</td>
<td>-0.12  0.03  -2.82  0.73</td>
<td>-0.02  0.19  -0.05  0.39</td>
</tr>
<tr>
<td>Collection date</td>
<td>-0.04  0.22  -0.09  0.43</td>
<td>0.05  0.17  0.14  0.43</td>
</tr>
<tr>
<td>Temperature</td>
<td>1.55  0.95  1.55  0.95</td>
<td>2.80  0.84  2.80  0.84</td>
</tr>
<tr>
<td>Researcher 1</td>
<td>0.80  0.73  0.80  0.73</td>
<td>0.77  0.61  0.77  0.61</td>
</tr>
</tbody>
</table>

**Sexes were separated as a result of strong collinearity between head dimensions and sex. Estimates shown in bold are significant at $P < 0.05$. Three lizards were excluded from models because they were extreme outliers in the analysis. Std. Est., standardized regression coefficient. SVL, snout–vent length.**
Table 5. Pearson’s correlation coefficients between residual sprint speed, bite force, and endurance for males (below diagonal) and females (above diagonal)

<table>
<thead>
<tr>
<th></th>
<th>Residual sprint speed</th>
<th>Residual endurance</th>
<th>Residual bite force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual sprint speed</td>
<td>–</td>
<td>0.19 (−0.02 to 0.37)</td>
<td>0.16 (−0.05 to 0.35)</td>
</tr>
<tr>
<td>Residual endurance</td>
<td>0.10 (−0.10 to 0.30)</td>
<td>–</td>
<td>0.11 (−0.10 to 0.30)</td>
</tr>
<tr>
<td>Residual bite force</td>
<td>0.03 (−0.18 to 0.23)</td>
<td>0.09 (−0.12 to 0.28)</td>
<td>–</td>
</tr>
</tbody>
</table>

95% confidence intervals are provided in parenthesis after each estimate. No correlation coefficients were significant at $P < 0.05$.

force and androgen concentrations may be nonlinear in males, although we acknowledge that re-fitting models with quadratics did not improve model fit. Although the pattern in the raw data between bite force and androgens needs to be interpreted with caution, this result is congruent with theoretical expectations of ARTs in E. quoyii (Noble et al., 2013b). Males exhibit different reproductive tactics, with territorial/resident males having small home range areas and lower movement rates but spending more days active, whereas floater males have large home range areas and move a lot but are not observed often (Stapley & Keogh, 2004, 2005; Noble et al., 2013b). Selection has also been shown to favour these alternative behavioural tactics (Noble et al., 2013b). The nonlinear relationship between bite force and androgens may be caused, in part, by differences in circulating androgens between resident and floater males. Because both territorial and floater males are similar in SVL, they are predicted to have similar bite forces because of the strong relationship between bite force and body size. However, this hypothesis will need to be tested experimentally before any firm conclusions can be made.

In conclusion, few studies have explicitly tested for sex differences in performance (Lailvaux, 2007) and fewer studies have explored their proximate underpinnings. Determination of physiology–morphology–performance relationships across taxa that exhibit different degrees of sexual dimorphism will help to clarify whether androgens, such as testosterone, play a role in driving variation in morphology, performance, and fitness, and will also allow us to test the generality of the findings obtained in strongly dimorphic species.

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references
SEX, ANDROGENS, AND WHOLE-ORGANISM PERFORMANCE 15


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