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Original Article

Multiple mating in a lizard increases fecundity but provides no evidence for genetic benefits

Daniel W.A. Noble,[a] J. Scott Keogh,[b] and Martin J. Whiting[a]

[a]Department of Biological Sciences, Macquarie University, 209 Culloden Road, Sydney, New South Wales 2109, Australia and [b]Division of Evolution, Ecology and Genetics, Research School of Biology, The Australian National University, Building 116, Dailey Road, Canberra, Australian Capital Territory 0200, Australia

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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 males and 108 females) *E. quoyii* in 6 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 or multiply sired using 6 polymorphic microsatellite DNA loci. To test whether females gain genetic benefits, we measured 5 fitness traits on offspring over their first active season and compared these traits between single paternity and multiple paternity clutches. Contrary to predictions from genetic benefits models, offspring from multiple paternity and single paternity clutches did not differ significantly in snout–vent length, mass, growth rate, sprint speed, or endurance. Although multiple paternity and single paternity females did not differ significantly in body size, condition, or mass, multiple paternity females invested more heavily in reproduction when body size, mass, and condition were controlled, producing significantly more offspring compared with single paternity 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.

Key words: **direct fitness benefit, *Eulamprus quoyii*, indirect fitness, multiple mating, multiple paternity, performance, polyandry.**

INTRODUCTION

Explaining the widespread occurrence of multiple paternity (MP) 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). Although 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 increasing 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 3 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 3 hypotheses predict that offspring from multiply mated females have increased fitness, on average, compared with 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 unmated 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

Address correspondence to D.W.A. Noble. E-mail: daniel.wa.noble@gmail.com.

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, 2011; Byrne and Whiting 2011), much of our knowledge comes from studies on birds (Griffith et al. 2002; Westneat and Stewart 2003), frogs (Byrne and Roberts 2012; Byrne and Whiting 2011; Roberts and Byrne 2011), fish (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 suggests 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, 2004; Olsson and Madsen 2001; Olsson et al. 2005, 2011; Lancaster et al. 2009); 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 major histocompatibility complex 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 immunosuppressive 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 overshadowed 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 MP (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 data set to determine paternity, we tested whether single paternity (SP) and 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 with 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 5 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 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, seminatural enclosures (details below).

Experimental setup

Lizards were allocated to 1 of 6 outdoor enclosures (10 × 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 (Swann G, personal communication; Wechmann K, 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 2 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 3 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 to 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 × 45 × 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 and 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 reweighed and measured, marked by toe-clipping (Ferner 2007), and then released into 1 of 4 large (10 × 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 remeasured and weighed.

Offspring sprint speed

The sprint speed of each offspring was measured once/day for more than 3 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 of more than 1 m for analysis because individuals are known to perform suboptimally 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 taping them on their tailbase. We kept lizards running by gently tapping them each time they stopped for more than 2 s. We continued this until each lizard was tapped 5 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 for 3 consecutive days. We used the maximum time to exhaustion for our analysis (Losos et al. 2002).

Offspring growth rate

We calculated offspring growth rates during 2 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 seminatural 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: growth rate = $(SVLt_2 - SVLt_1)/(\Delta t)$, where $SVLt_2$ is the SVL at marking (period 1) or recapture (period 2), $SVLt_1$ is the SVL at either birth (period 1) or marking (period 2), and Δt 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 manufacturer's protocol. We assigned paternity to offspring using 6 microsatellite DNA loci (Ek100, Ek107, Ek8, Ek37; Scott et al. 2001; Morrison et al. 2002) and (GQ20/21, GQ16/17; Sumner et al. 2001). Polymerase chain reactions (PCRs) were carried out in 20 μL reaction volumes containing 1.0 μL of genomic DNA, 10 μL of GoTaq® (Promega), 0.5 μL (10 pmol μL^{-1}) of forward and reverse primers, and 8.0 μL 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, and FAM), and product from the final PCR reactions was 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 to 34 alleles at a single locus with mean polymorphic information content of 0.7014. The combined nonexclusion probability for a parent pair was 4.46×10^{-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 4 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) likelihood ratio (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 3 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 analysis of variance (Anova). Differences in clutch size and relative clutch mass between SP and MP clutches were compared using analysis of covariance (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 standard deviation (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 generalized linear model 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 data set.

We analyzed our data on offspring fitness traits and clutch type with generalized linear mixed models (GLMMs) using the "lme4" package (Bates et al. 2012) 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 sample size corrected Akaike's information criterion (AIC_C) between our candidate set of models and calculated ΔAIC_C, Akaike weights (ω_i), and evidence ratios (ER). Akaike weights sum to 1 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, models with ΔAIC_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 that 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 identifications were included as random effects to account for nonindependence 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 reran 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 GLMMs 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 female 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 and offspring alleles from 7 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 1 male and 41 (65%) were sired by multiple males. Of the multiply sired clutches, 27 were sired by at least 2 males, 14 by at least 3 males, and 1 by 4 males. The distribution of birthing dates between SP and MP females overlapped extensively (Figure 1). Females produced anywhere from 1 to 9 offspring in a single clutch. Multiply sired females had a tendency to bias offspring sex toward males; however, this was not significantly different to singly sired females (odds of producing males was 1.62 times that of singly sired clutch: $\hat{\zeta} = 1.80$, $P = 0.07$). 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 ± 0.25 offspring, whereas singly sired females gave birth to an average of 3.64 ± 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 3 females, which gave birth to only a single baby (mean ± SE of singly sired clutches = 4.05 ± 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).

Offspring mass and body size

Mean offspring mass at birth was 1.09 ± 0.02 and 1.05 ± 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 GLMMs allowed us to incorporate offspring predictors in analyses and we found 3 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 ($\beta_{male} = 0.02 \pm 0.01$, $t = 1.50$).

Mean offspring SVL at birth was 37.78 ± 0.26 and 37.39 ± 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 contained offspring sex, maternal SVL, maternal

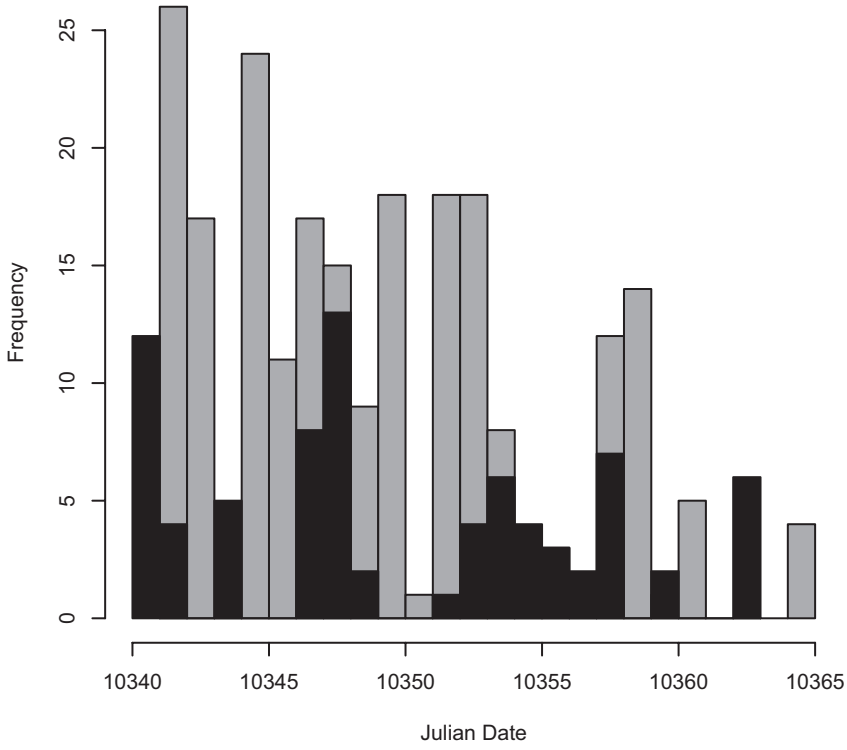

Figure 1
Distribution of offspring birth dates (Julian dates) between SP (black bars) and MP (gray bars) clutches of female *Eulamprus quoyii*.

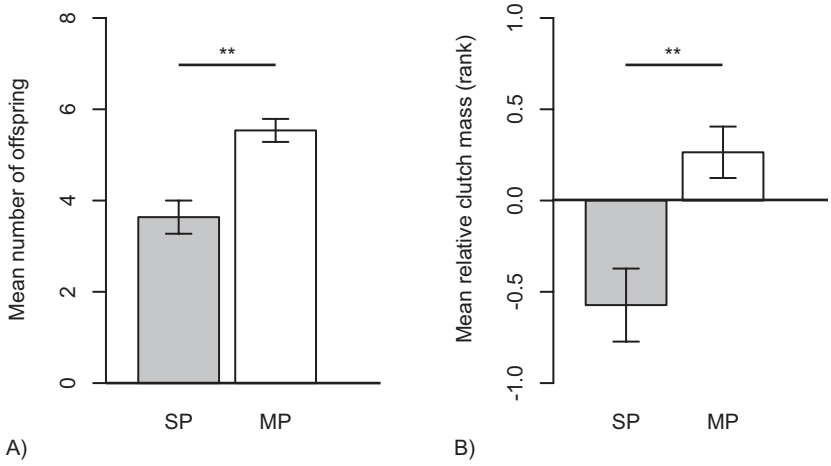

Figure 2
Mean (±SE) number of offspring (A) and relative clutch mass (B) for SP and MP clutches. ** indicates that differences are significant at $P < 0.001$.

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 ($\beta_{male} = -0.21 \pm 0.16$, $t = -1.30$), and 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 ± 0.02 and 0.59 ± 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 ($\beta = 0.03 \pm 0.01$, $t = 2.15$).

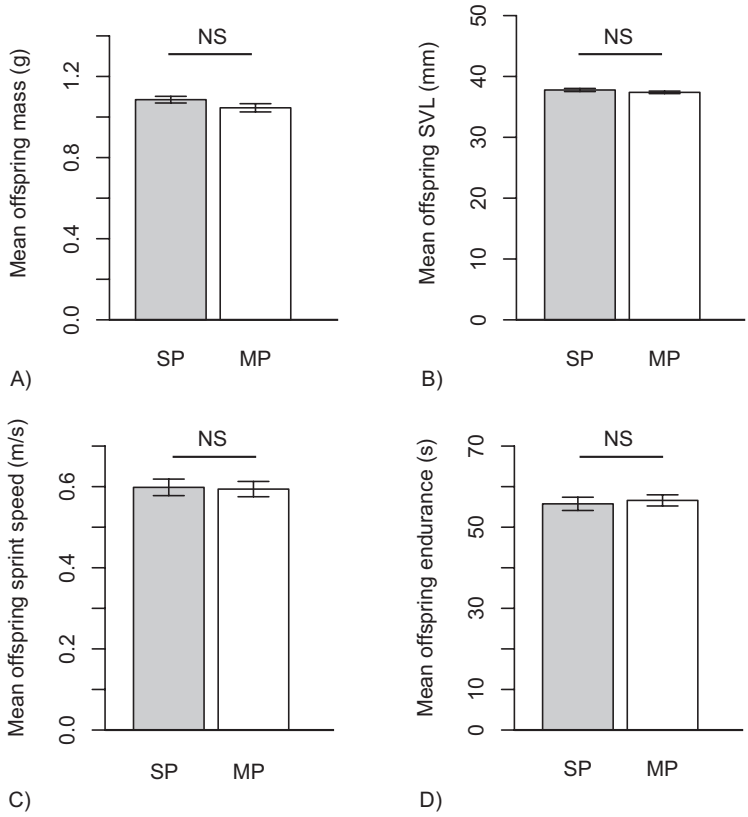

Figure 3
Mean (±SE) offspring mass (A), SVL (B), sprint speed (C), and endurance (D) for SP and MP clutches. NS indicates that differences are not statistically significant at $\alpha = 0.05$.

Mean offspring endurance was 55.76 ± 1.63 and 56.61 ± 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 $\omega_i = 0.39$; $\beta_{TimeCap} = -0.008 \pm 0.003$, $t = -2.34$; Table 1).

Offspring growth rates

We obtained growth rate estimates for 235 ($n = 57$ SP and $n = 178$ MP) offspring between birth and marking and 122 ($n = 29$ SP and $n = 93$ 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 $\omega_i = 0.50$; $\beta_{male} = 0.02 \pm 0.007$, $t = 2.26$; Table 1). Offspring growth rates in our seminatural 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.

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 MP in *E. quoyii*.

Although the incidence of MP 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, *Eulamprus heatwolei*, where offspring from females with experimentally varied mating rates did not have higher growth rates or survival compared with 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

Table 1

Comparison of AIC_C, ΔAIC_C, Akaike weights (ω_i), and ER for our candidate models in analyses of offspring fitness traits (SVL, mass, log(sprint speed), log(endurance), and growth rates)

Model	AIC_C	ΔAIC_C	ω_i	ER
Offspring SVL (n = 205 offspring, n = 50 females)				
SVL ~ intercept	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
SVL ~ Sex + FCS + Fcond + FSVL	683.33	0.00	0.51	1.00
Offspring mass (n = 204 offspring, n = 50 females)				
Mass ~ intercept	−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
Mass ~ Sex + FCS + Fcond + FSVL + OffSVL	−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
Offspring sprint speed (n = 175 offspring, n = 50 females)				
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
SS ~ Sex + OffSVL	−29.53	0	0.35	1.00
SS ~ Sex + OffSVL + TimeCap	−27.44	2.09	0.12	2.84
Offspring endurance (n = 175 offspring, n = 50 females)				
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
Endur ~ TimeCap	−93.44	0	0.39	0.20
Offspring growth (captive) (n = 181 offspring, n = 53 females)				
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
Growth_cap ~ Sex	−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 offspring, n = 42 females)				
Growth_wild ~ intercept	−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

We report the number of females and offspring born to these females in brackets for each analysis because of missing data. 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. NL means that the ER is greater than 1200 and thus the model is not likely (NL). Best-supported models are represented in boldface.

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 toward males, suggesting that some form of cryptic female choice may be operating. Sex ratio adjustment has been shown in

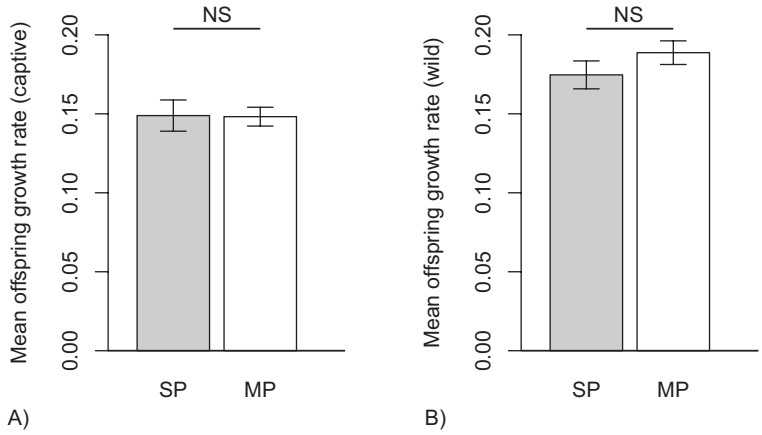

Figure 4
Mean (±SE) offspring growth rates in captivity (A) and in seminatural enclosures (B) for SP and MP clutches. NS indicates that differences are not statistically significant at $\alpha = 0.05$.

side-blotched lizards (*Uta stansburiana*) in response to sperm from large and small males (Calsbeek and Sinervo 2004). For example, SP females may mate multiply but more strongly bias paternity toward 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 these hypotheses 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 *L. 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 has 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 data set 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 D, personal observations). Furthermore, behavioral observations of individuals during the breeding season suggest that male harassment is minimal in this species (Noble D, Keogh JS, 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 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 MP 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, overlooked hypothesis explaining the evolution of polyandry (Sheldon 1994; Uller and Olsson 2008; Slatyer et al. 2012), yet in many systems, it remains untested (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 suggest 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, MP 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, 2011; Lancaster et al. 2009). 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 variation exists in sperm quality among males and this is worth investigating further in a diverse range of systems.

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