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ORIGINAL PAPER

Influence of alternate reproductive tactics and pre- and postcopulatory sexual selection on paternity and offspring performance in a lizard

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Abstract Sexual selection theory predicts different optima for multiple mating in males and females. We used mating experiments and genetic paternity testing to disentangle pre- and postcopulatory mechanisms of sexual selection and alternate reproductive tactics in the highly promiscuous lizard *Eulamprus heatwolei*. Both sexes mated multiply: 30–60 % of clutches were sired by two to four fathers, depending on the experiment. Larger males sired more offspring when we allowed male contest competition: 52 % of large males but only 14 % of small males sired at least one offspring. In the absence of male contest competition, females mated promiscuously and there was no large male advantage: 80 % of large males and 90 % of small males sired at least one offspring, and there was no evidence for last-male precedence. Multiple mating did not yield obvious direct or indirect benefits to females. *E. heatwolei* represents a complex system in which males attempt to improve their fertility success by limiting rivals from access to females and through adopting alternate reproductive tactics. Conversely, females exhibit no obvious precopulatory mate choice but may influence fitness through postcopulatory means by either promoting sperm competition or through cryptic female choice. Our results support the hypothesis that female multiple mating in nonavian reptiles is best explained by the combined effect of mate encounter frequency and high benefits to males but low costs to females.

Keywords Mating system · Reproductive success · Sexual selection · Polygynandry · Promiscuity · Behavior · Lizard

Introduction

The strategies males and females use to influence the paternity of their offspring place them in direct conflict both before and after copulation (Andersson 1994; Eberhard 1996; Simmons 2001; Arnqvist and Rowe 2005). Males can enhance their reproductive success by gaining more mates and by having highly competitive sperm in order to increase the number of offspring they sire (Jennions and Petrie 2000). Because quality varies among males, however, each potential sire offers different benefits to females and their offspring. Therefore, females should only mate with, or use the sperm from, the best male to fertilize their eggs and maximize their reproductive success (Jennions and Petrie 2000). This higher number of females mated with males is a widely recognized source of sexual conflict. Disentangling the relative importance of precopulatory and postcopulatory processes is vital to understanding how sexual conflict affects males and females as they attempt to control fertilization events (Uller and Olsson 2008).

Males in many species may compete for mates directly through contest competition or, more subtly, by adopting alternate reproductive tactics (ARTs) such as female mimicry (Shine et al. 2000; Whiting et al. 2009) or sneaking (Hanlon et al. 2005; Emlen 2008). Males that display dominance behavior may limit the opportunity of other males to mate with females by either controlling space in the form of

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a territory or by guarding one or more females directly. In many species, large males display dominance social behavior, thereby securing more matings, and this is a common phenomenon among lizards (Olsson and Madsen 1998; Gullberg et al. 2003; Tokarz 2008). Under such circumstances, smaller males may adopt condition-dependent ARTs. Alternate reproductive tactics also may evolve through frequency-dependent selection and become fixed if they are coupled with different behavioral strategies and phenotypes, such as in the case of the "rock–paper–scissors" game in the lizard *Uta stansburiana* (Sinervo and Lively 1996).

While precopulatory behavior is often influential in male mating success, males may continue to compete after copulation through sperm competition (Eberhard 1996; Simmons 2001; Stapley and Keogh 2005; Parker and Pizzari 2010; Simmons 2011) or cryptic female choice (Eberhard 1996; Olsson et al. 1996; Birkhead 2000; Tregenza and Wedell 2000). In the case of sperm competition, females store sperm after copulation but before ovulation, and sperm compete for fertilizations either through a lottery system (Parker 1998) or through direct competition as a result of performance (Birkhead 2000). If females store sperm, fertilization also can be affected by cryptic female choice (Olsson et al. 1996; Olsson and Madsen 1998). Cryptic female choice allows females to choose the fittest sperm for fertilization and, although distinct, it is difficult to experimentally distinguish from sperm competition (Eberhard 1996; Birkhead and Møller 1998; Birkhead 2000).

In species that mate multiply, there may be varying costs and benefits for both sexes. In lizards, for example, multiple mating is hypothesized to occur as a consequence of mate-encounter frequency in which there are larger benefits to multiple mating in males but relatively low costs in females (Uller and Olsson 2008). The presence of multiply sired clutches often is attributed to either genetic benefits (Uller and Olsson 2008) or enhanced offspring performance (Evans and Magurran 2000; Ivy and Sakaluk 2005). Despite multiple paternity being extremely common in lizards (Uller and Olsson 2008), the hypothesis that offspring from clutches sired by multiple males perform better has rarely been tested; most studies to date have focused on indirect benefits rather than offspring performance per se (Olsson and Shine 1997; Ivy and Sakaluk 2005; Simmons 2005; Byrne and Keogh 2009).

In lizards, few studies have focused on sexual conflict and what determines the outcome of mating and fertilization success (reviewed in Uller and Olsson 2008). However, from the few studies that exist, some patterns have emerged. Males often display precopulatory dominance behavior and limit the opportunity of other males to mate with females (Whiting et al. 2003). At the same time, females may exert precopulatory mate choice if they are able to choose among males of varying quality, although female preference in lizards remains poorly understood (Cox and Le Boeuf 1977; Olsson and Madsen 1995; Wikelski et al. 2001). Furthermore, sexual conflict may manifest in male harassment of females (e.g., marine iguanas; Wikelski et al. 1996), making it costly for females to reject mates (Le Galliard et al. 2005). After copulation, however, female lizards may facilitate sperm competition and/or cryptically choose among males by storing sperm prior to ovulation (Olsson et al. 1996; Olsson and Madsen 1998). For example, female sand lizards (*Lacerta agilis*) mate multiply perhaps to gain genetic benefits (Olsson and Madsen 1995) and at the same time cryptically choose the sperm of more distantly related males to fertilize their eggs (Olsson et al. 1996). Another factor that may limit the opportunity for multiple paternity, and thus enhance sexual conflict, includes physiological constraints such as small clutch size (Uller and Olsson 2008). Studies focusing on how sexual conflict is resolved in lizards are limited, as are experiments designed to disentangle multiple mechanisms of sexual selection.

The Australian southern water skink, *Eulamprus heatwolei*, is an excellent system for testing hypotheses concerning sexual conflict. *E. heatwolei* is a viviparous lizard common in the highland regions of southeastern Australia (Morrison et al. 2002; Stapley and Keogh 2005). Females which rely on fat reserves built up in the year before they reproduce and in the years when they do reproduce have few offspring at a time (mean: ca. three offspring per litter), each of which may be sired by a different male (Morrison et al. 2002). Females are generally promiscuous and show high levels of multiple paternity in their clutches (Morrison et al. 2002; Stapley and Keogh 2005; Keogh et al. 2012), but can easily reject mates if desired (JSK, personal observation). Females also may store sperm from several males within a mating season but not across seasons (Morrison et al. 2002), but how it is stored is unknown. Approximately 25 % of adult male and 75 % of female *E. heatwolei* occupy small home ranges on suitable logs. The remaining adults of both sexes employ an ART in which they range widely across the landscape. Although adult body size varies considerably within this population, it is not tightly correlated with ART (Morrison et al. 2002; Stapley and Keogh 2004, 2005). Further, neither male body size nor ART (floater vs. resident) seems to influence mating success, although many males sire no offspring in the wild in a given season (Stapley and Keogh 2005).

We used a series of manipulative experiments, combined with genetic paternity analyses, to disentangle the relative roles of pre- and postcopulatory mechanisms of sexual selection in this species. We experimentally staged male–male contests to test the extent to which male phenotypic traits that are hypothesized to promote higher reproductive success (body size, behaviors, alternative reproductive tactics) influenced male fitness. In a second experiment in which males were not allowed to compete, we examined postcopulatory sperm competition and cryptic female choice and tested if females choose larger (possibly higher quality) males. Given that levels of multiple paternity are so high in this species, these experiments allowed us to test a direct benefits model: the hypothesis that multiple mating increases fertilization success by determining whether or not females are more likely to mate multiply when they have opportunities to mate with higher quality males (Uller and Olsson 2008). In a third experiment, we examined possible indirect benefits by determining whether performance of offspring will be higher when females mate multiply. We examined whether multiple mating positively impacts offspring performance by measuring short-term growth and survival in hatchlings from singly and multiply mated females.

Materials and methods

Study animal

We captured adult *E. heatwolei* by noosing, in mid-September, soon after emergence from hibernation, from a large population in the Tidbinbilla Nature Reserve, 25 km southwest of Canberra in the Australian Capital Territory (elevation 800 m). We measured snout-vent length (SVL) and tail length to the nearest millimeter, head length and head width to the nearest 0.1 mm, and weight to the nearest 0.1 g. Individuals were sexed by checking for hemipenes. Lizards were marked individually with toe clips, and approximately 5 mm of tail tip was sampled for genotyping. All individuals used in this study which had complete or fully regrown tails were free of visible parasites and appeared to be in good health.

Experiment 1: precopulatory male–male competition: size vs. alternate reproductive tactic

We performed an experiment to simultaneously examine male reproductive success based on both male body size and male mating strategy (resident vs. floater males). Thirty-three adult females (SVL 86–103 mm) were assigned randomly to 11 outdoor circular enclosures, each is 2 m in diameter, with three females per enclosure. Each enclosure contained a bark-chip substrate, three large tussock grass clumps, five wooden boards, and three roof tiles to serve as retreat and basking sites. The enclosures were located in the campus of the Australian National University and experienced similar temperatures to the lizard's natural environment. We assigned 44 males to small- (SVL 80–89 mm) and large-size (SVL 92–96 mm) classes in equal proportions. It is not possible to know the actual reproductive strategy of each male without an entirely different long-term study of individual males over multiple seasons, so we simulated male reproductive strategy experimentally by manipulating encounter rates. To do this, we randomly designated half of the males as "floaters" or "residents" in each size group and then adjusted the time spent with females, with residents spending 22 days with the same females and floaters spending only two days with a group of females. There were 11 males each in the four size/strategy categories: "small floater," "large floater," "small resident," and "large resident," and females had mating opportunities with each of the four categories of male.

The mating experiment ran over 44 days, during which each trio of females spent 22 days with a single pair of resident males in 11 nonconsecutive 2-day blocks and 22 days with 11 pairs of floater males, one pair of males for each of 11 nonconsecutive 2-day blocks in alternation with the resident pair. Thus, each trio of females spent only 2 days each with 11 different pairs of floater males (Fig. 1). The experiment was set up in the following way: one pair of resident males was released into each of the 11 enclosures containing three females on the first day of the experiment (16 October). These males remained in the enclosures for 2 days and then were removed and replaced with pairs of floater males. After further 2 days has passed, the floater males were removed and the same resident pairs of males were again placed with the same females. Every 2 days thereafter, the same resident males were removed and replaced with a new pair of floater males, or vice versa. Importantly, the males used as floaters were allocated to different pairs and different enclosures for each replacement, so no two floater males were ever paired together more than once and no female encountered any floater male for more than one 2-day period. On the days residents or floaters were not in the enclosure, they were kept in the shade in individually ventilated snap-lock containers (30 L×21 W×9 H, in centimeter), containing water, bark-chip bedding, and a small cardboard retreat site. Thus, all males experienced the same conditions when not in the enclosures. One potential confounding factor in male reproductive success in this experiment is that resident pairs of males would have an established dominance hierarchy early on whereas the pairs of floater males had to reestablish that hierarchy each time. We dealt with this issue by removing male competition all together in the next experiment (experiment 2 below).

In analyzing the paternity data for this experiment (see below), we used observed and expected numbers of offspring across the entire experiment. Individually, resident males had access to only three females over 22 days while floater males were rotated through all 11 enclosures and therefore had access to all 33 females over 22 days. However, at the level of the entire experiment, resident

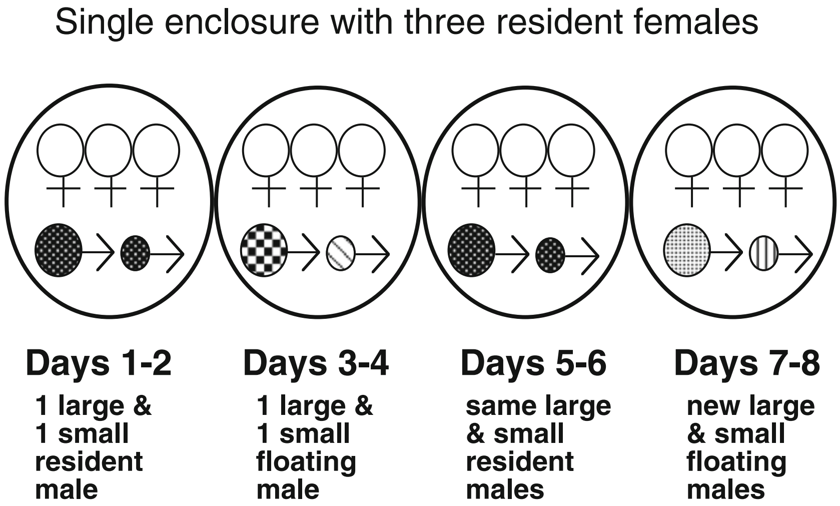

Fig. 1 Depiction of the experimental design for experiment 1 showing the categories of males by size and ART that were presented to females. Each enclosure contained three females that remained in the enclosure for the duration of the experiment while males were rotated among different females (floaters) or with the same female (residents) but, alternately, with rotating groups of floaters (see text for details)

males also had access to 33 females; therefore, when the number of sired offspring is pooled within each of the four classes of male category, then the total number of expected offspring is the same across each of the four classes. A total of 62 offspring were produced from this experiment (see “Results”); therefore, our expected number of offspring was 15.5 for each class. All adults and offspring were genotyped and paternity-determined (see below). We then quantified reproductive success of males in each of the four size/strategy categories. Our prediction was that under direct male–male competition, large males of both mating strategies would achieve higher reproductive success.

During the experiment, males were exchanged after the lizards had chosen overnight retreat sites in the early evening. Each time a male was caught, we also recorded the number of females with which he was sharing a retreat site to obtain a behavioral measure of male–female association. The number of females with which an individual male shared a retreat site was averaged over all nights for each male and compared among the four male size and strategy categories for significant differences. Our prediction was that under direct male–male competition, large males of both mating strategies would exclude small males from mating opportunities and share retreat sites with more females.

Experiment 2. Postcopulatory processes: sperm competition, cryptic female choice, and a test of female preference for large males

Using identical outdoor enclosures to those described above, we randomly assigned 27 adult females to 10 enclosures (two to three females per enclosure). Females were offered sequential mate choice between small and large males but without any male–male competition or female experience of male mating strategy. Ten small (SVL 82–90 mm) and 10 large (SVL 95–96 mm) adult males were rotated through the 10 enclosures such that on any given day, each enclosure had one adult male and the other 10 adult males were housed in small containers as described in experiment 1. After 2 days, the males in the enclosures were captured and all of the males rotated such that the 10 males from the enclosures were put into the boxes and the 10 males from the boxes were put into enclosures. Therefore, over 40 days of their mating season, every group of females was exposed to all 20 males for 2 days each, but one at a time and alternating between small and large males. In the absence of male–male competition, we predicted that large males should sire more offspring in more litters if females prefer larger males or are trading up to larger males. All adults and offspring were genotyped and paternity-determined (see below). We then quantified the number of litters with multiple paternity, the number of fathers that sired each litter, the total number of offspring each male produced and in how many litters.

Experiment 3. Offspring performance in singly and multiply sired litters: a test of the indirect benefit hypothesis

We tested whether or not multiple mating positively impacts several aspects of offspring performance by comparing performance of hatchlings from singly and multiply mated females reared in common enclosures. Using identical outdoor enclosures to those described above, we randomly assigned 26 adult females to 10 enclosures (two to three females per enclosure). These females were offered no mate choice over a period of 40 days. We introduced a single adult male of intermediate size (90–91 mm) to each enclosure, and he stayed with the females for the duration of the experiment. We then compared survival and short-term growth rates in hatchlings produced from these single-father litters and the offspring produced when females were able to choose among multiple males (experiment 2). The first few months of life are critical for young lizards in cold environments because they need to attain the resources necessary for surviving their first hibernation (Civantos and Forsman 2000), and initial body size and growth rate are key predictors of long-term survival and fitness in reptiles (Tinkle et al. 1993).

On their birthday, all offspring were weighed and then randomly assigned to one of two large (diameter 4 m), seminatural outdoor enclosures in equal proportions. Each enclosure had dense vegetation and numerous retreat sites. Offspring from both singly mated and multiply mated females were housed together, but at sufficiently low density to prevent competition for food. Offspring fed on naturally available insects and we did not provide supplementary food. After approximately 100 days, and just before entering hibernation, we captured all offspring on a single day in order to score survival and record their body mass. Our prediction was that offspring produced by females with extreme levels of mate choice (and multiple paternity) would have higher survival and growth rates than those from singly mated females. We also tested for direct benefits of multiple mating, specifically, whether multiple mating increased fertilization success by gauging and whether multiply mated females had larger litters when female body size was controlled for.

Paternity assignment

At the conclusion of all the three experiments, the males were relocated to separate enclosures and the females were kept in their original enclosures until they were noticeably gravid. Females were brought into the laboratory in early January and housed in individual snap-lock containers (30 L×21 W×9 H, in centimeter), in a temperature-controlled (18 °C) environment with a natural light cycle (12-h light and 12-h dark). We provided lizards with a bark-chip substrate, a cardboard retreat site, and heat tape (30 °C) for basking 8 h a day to allow natural thermoregulation. Lizards were provided with fresh water ad libitum and wet dog food and mealworms every second day. The enclosures were checked for offspring twice daily until females gave birth. Neonates were removed upon discovery and housed separately. We toe-clipped neonates individually and removed approximately 5 mm of tail tip for genotyping. All offspring, mothers, and potential sires from experiments 1 and 2 were genotyped for three highly polymorphic microsatellite loci: Ek37, Ek100, and Ek107, as described in Scott et al. (2001) and applied in our earlier studies (Morrison et al. 2002; Stapley and Keogh 2004). To assign paternity, we first matched maternal alleles in the offspring and then went through the alleles of all the potential fathers until there was a match at all three paternal alleles. The three microsatellite loci were sufficiently variable (25, 22, and 21 alleles, respectively) for us to assign paternity with 100 % certainty in this closed population. The design of our experiments allowed us to date the mating events that resulted in offspring to within 2 days for floaters in the precopulatory experiment (experiment 1) and for all males used in the postcopulatory experiment (experiment 2).

Results

Experiment 1. Precopulatory male–male competition: size and alternate reproductive tactic

Of the 33 females, 20 (60 %) gave birth to a total of 62 offspring, and litter size ranged from one to five offspring (mean ± SE=3.2±0.22). It is not surprising that 13 females did not give birth as female *Eulamprus* often skip years in reproduction (Morrison et al. 2002; Schwarzkopf 1993). Multiple paternity was identified in 6 of the 20 litters (30 %), five of which had two fathers and one had three fathers.

Comparing all four size and strategy categories of males (large floater, small floater, large resident, small resident) large floater males sired the most offspring (52 %) (Fig. 2). The distribution of male fertilization success was significantly different from the null hypothesis of equal numbers of offspring across all four classes of male (Fig. 1a: X^2=26.13, *df*=3, *P*<0.0001), with large males of both strategies siring more offspring than either class of small males. Nearly three quarters (72.7 %) of the large floater males sired at least one offspring and 45.5 % of the large resident males sired at least one offspring. In contrast, only a single small floater male (9.1 %) and two small resident males (18.2 %) sired at least one offspring. Considering size class alone, 52.1 % of the large males sired at least one offspring, while only 13.6 % of the small males sired at least one

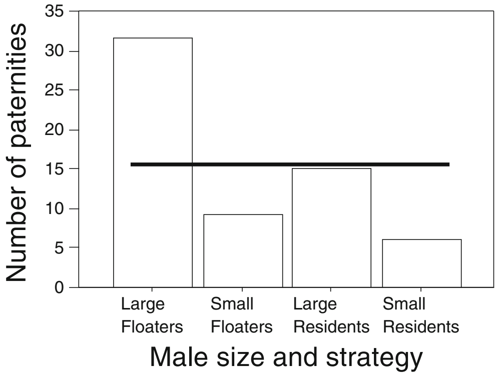

Fig. 2 Male fertilization success in the presence of precopulatory male–male competition showing that large males sired significantly more offspring than small males, regardless of reproductive tactic (experiment 1). The expected number of offspring is indicated with a *horizontal line*, taking mating opportunity into account (see text for details)

offspring. Three males, all floaters, sired offspring in three or more litters. In summary, under direct male–male competition, large males, and large floater males in particular, sired far more offspring than other classes of males.

The male–female associations (refuge sharing) revealed a similar pattern to the data on male fertilization success. The mean number of females with which a male shared a retreat site over the course of the experiment was significantly different among the four size and strategy categories (Fig. 3; ANOVA: $F_{1,3}=22.65$, $P<0.0001$), with large males of both strategies sharing more retreat sites with females than either class of small males.

Experiment 2. Postcopulatory processes: sperm competition, cryptic female choice, and a test of female preference for large males

A total of 22 of the 27 females (81.5 %) which were given sequential mate choice (no male–male competition) gave birth to a total of 69 offspring, and litter size ranged from one to six offspring (mean ± SE=3.1±0.27). Seven of the 23 litters had one father, and multiple paternity was identified in 15 litters (65.2 %), six of which had two fathers, six had three fathers, and three litters had four fathers. There were seven litters, ranging in size from two to four offspring, where each offspring had a different father (including two litters of four offspring). The number of fathers in a litter was correlated with litter size ($r^2=0.303$, $F_{1,19}=7.159$, $P=0.0145$). Three of the 20 males, two large and one small, did not father any offspring. Of the 17 males that did father offspring, the number of offspring per male ranged from 1 to 9 (mean ± SE=4.1±0.72). These males fathered offspring in one to eight litters (mean ± SE=2.9±0.52). As expected, the number of offspring sired was highly correlated with the number of litters in which males fathered offspring (Fig. 4; $r^2=0.84$, $F_{1,19}=94.78$, $P<0.0001$) but male body size (small vs. large groups) was not related to the number of litters in which a male sired offspring (ANOVA: $F_{1,18}=0.009$, $P=0.92$) or the number of offspring produced (ANOVA: $F_{1,18}=0.50$, $P=0.26$). The number of offspring produced from successful matings was not related to the order of those matings within multiple paternity clutches (ANOVA: $F_{3,37}=0.380$, $P=0.7678$). In summary, there is no evidence that females preferentially mated with large males or were “trading up” for large males in the absence of male–male competition, but instead were highly promiscuous.

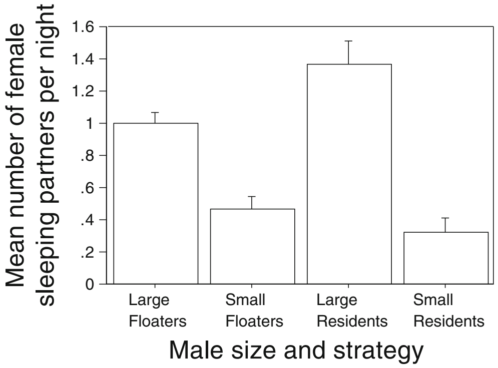

Fig. 3 Mean number of female sleeping partners per night in the presence of precopulatory male–male competition showing that large males shared overnight retreat sites with significantly more females than small males, regardless of strategy (experiment 1)

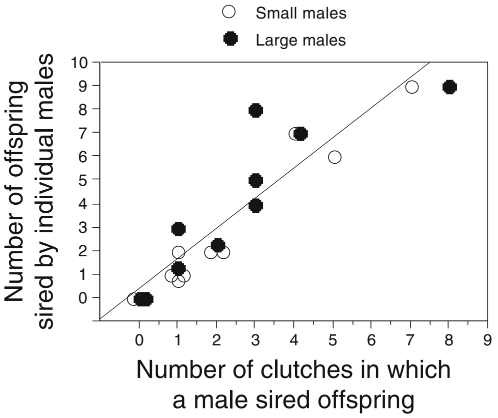

Fig. 4 Total number of offspring produced and total number of clutches in which a male sired offspring for all males in the multiple-mating treatment in experiment 2

Opportunity for sperm competition in experiments 1 and 2

The design of both experiments meant that we could accurately determine mating date of matings that resulted in offspring in mixed-paternity clutches and so test the opportunity for sperm competition and/or cryptic female choice. In experiment 2, 15 litters had more than one father and the number of days between matings with different sires ranged from 4 to 36 (mean=14.6 days; Fig. 5). In experiment 1, two litters comprised offspring sired by two floater males. The

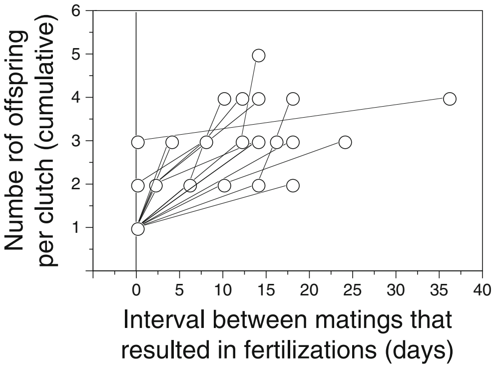

Fig. 5 Opportunity for sperm competition and/or cryptic female choice. Summary of the interval (in days) between the first mating that resulted in an offspring (day 0) and all subsequent matings that resulted in an offspring (experiment 2). Each *line* represents one clutch and the figure shows the number of offspring sired and when

mating dates of the two males that resulted in offspring were 4 days apart in one litter and 16 days apart in the other. Despite the large number of days between matings that produced offspring in both experiments, all offspring within a litter always were delivered on the same day, suggesting that fertilization of all offspring within a litter occurred at the same time. In addition, 90 % of all litters were delivered within a 10-day period, suggesting that females start mating and storing sperm many days before they ovulate. These data demonstrate that this species has at least short-term sperm storage and the opportunity for sperm competition and/or cryptic female choice within a mating season.

Experiment 3. Offspring performance in singly and multiply sired litters: a test of the indirect benefit hypothesis

A total of 19 (73 %) of the 26 females which were given no mate choice gave birth to a total of 62 offspring, and litter size ranged from two to four offspring (mean ± SE=3.1± 0.14). Mean litter size was not significantly different between this experiment and the multiply mated females in experiment 2 (ANOVA: $F_{1,39}$=0.071, P=0.79). We evaluated two aspects of offspring performance (survivorship and growth) over the initial 3.5 months (mean age in days ± SD=103±5.2) of their life. Survivorship was high for both experiments: only 9 % (singly mated) and 13 % (multiply mated) of offspring died over this period and there was no difference among the experiments in mean survivorship (ANOVA: $F_{1,39}$=0.303, P=0.59). Mean litter mass among experiments did not differ at birth (ANOVA: $F_{1,39}$=0.122, P=0.73) or after 3.5 months of growth, corrected for survivorship by excluding offspring that did not survive (ANOVA: $F_{1,39}$=0.162, P=0.69). This result persisted when their mother's SVL (results shown) or mass (results not shown) was included as a covariate (analysis of covariance (ANCOVA) on mother's SVL: birth—slopes homogeneous $F_{1,37}$=0.287, P=0.59 and intercepts $F_{1,38}$=0.126, P=0.72; ANCOVA: 3.5 months—slopes homogeneous $F_{1,37}$=0.612, P=0.44 and intercepts $F_{1,38}$=0.793, P=0.38). The offspring from both experiments grew substantially over this time period with some individuals tripling their weight (Fig. 6). Weight gain (growth), corrected for survivorship, did not differ between experiments (ANOVA: $F_{1,114}$=0.492, P= 0.48), and the result was the same when age in days (ANCOVA: slopes homogeneous $F_{1,112}$=0.030, P=0.86 and intercepts $F_{1,113}$=0.918, P=0.34) or their mother's body size (ANCOVA on mother's SVL: slopes homogeneous $F_{1,112}$=0.019, P=0.89 and intercepts $F_{1,113}$=0.745, P= 0.39) was included as a covariate. Similarly, within-litter growth, adjusted for survivorship and with their mother's body size as a covariate, did not differ between experiments (ANCOVA: slopes homogeneous $F_{1,37}$=0.23, P=0.63 and intercepts $F_{1,38}$=0.095, P=0.76). The number of fathers in a litter was unrelated to growth rate across both experiments (r^2<0.001, P=0.99) and just within the multiply mated experiment (r^2=0.003, P=0.81). In summary, we found no evidence to support our prediction that short-term survival or growth was higher in offspring produced from females with an opportunity to mate multiply.

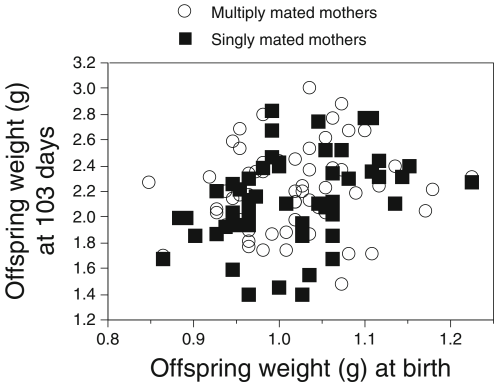

Fig. 6 Growth of all offspring produced from the singly mated females and multiply mated females showing no significant difference between the treatments (experiment 3)

Discussion

A key question in sexual selection theory is the relative contributions of male contest competition and female mate choice in determining fitness (Arnqvist and Rowe 2005). We simultaneously examined the relative importance of pre- and postcopulatory mechanisms of sexual selection for both male and female *E. heatwolei* in determining fitness and offspring performance. When we provided the context for males to interact and compete with one another (male contest competition), large males sired more offspring than small males, irrespective of ART. Large males also were more likely to share overnight retreat sites with females, and males generally did not share retreat sites with each other. In the absence of precopulatory male–male competition, however, male reproductive success was unaffected by male body size or ART and females showed no evidence of preferring large males or trading up. Females in both experiments showed high levels of promiscuity and multiple paternity, with some clutches being sired by as many as four males. By controlling mating interval, we show that females store sperm and that postcopulatory sexual selection is likely important because males that mated as many as 36 days after the first male still sired offspring. We found no evidence that mating order was important. While we detected no differences in body size or short-term survival between offspring from singly vs. multiply mated offspring, there may be less obvious indirect benefits that we were unable to detect.

Male contest competition can be highly intense in the case of species that patrol territories, that signal assertively, or that use armaments to battle rival males (Andersson 1994; Whiting et al. 2003, 2006). Alternatively, males may be devoid of obvious armaments and instead use body size, subtle signals, or occasional aggressive bouts to deter rivals from intruding on their space or approaching females in their immediate proximity. *E. heatwolei* appears to fit this latter category: they do not patrol territories but do defend a relatively small space near a refuge or log, and larger males will dominate smaller ones (Morrison et al. 2002; Stapley and Keogh 2004, 2005; Keogh et al. 2012). Therefore, while contest competition may be less intense, it is still likely sufficient to have a major impact on fitness if smaller males have less access to females. Our data show that large males are more successful at siring offspring than small males when they are allowed to compete prior to copulation, and this is further supported by the observation that larger males are more likely to share shelters with females, perhaps guarding them from further mating events (Olsson 1993; Sinervo et al. 2000).

When females are a limited resource and sexual selection is intense, males of many species adopt alternate reproductive tactics that may be condition dependent or fixed (Gadgil 1972; Andersson 1994; Gross 1996; Zamudio and Sinervo 2000; Baird et al. 2007). For example, instead of engaging in costly physical fights with larger rivals, smaller males may adopt a roaming strategy and seek sneaky copulations (Jenssen et al. 2005; Olsson et al. 2008; Whiting et al. 2009; Keogh et al. 2012). For *E. heatwolei*, we show that when exposed to precopulatory male–male competition in enclosures, small males sired fewer offspring and shared fewer retreat sites than larger males. This does not exactly mimic results of a small-scale field-based study where we show that neither male body size nor reproductive tactic seemed to influence male reproductive success (Morrison et al. 2002), but in patches of good habitat (e.g., log and rocks with refugia and suitable for basking), multiple male and female residents may be found together, and these aggregations would promote precopulatory competition among males. It is in these scenarios that large males will sire more offspring than small males (Stapley and Keogh 2005). Floater males, however, are more widely distributed, likely have higher encounter rates with females, and are perhaps able to access females that may be occupying or moving through a wider range of habitats. As such, male fertilization success may be linked to male reproductive tactics in the context of habitat use.

We found high levels of multiple mating by both males and females and high levels of multiple paternity within individual clutches in experiments 1 and 2. Furthermore, considerable time elapsed between copulations that produced offspring. Males were able to obtain sire offspring up to 36 days after multiple mating trials began, suggesting that the opportunity for postcopulatory sexual selection through either sperm competition or female sperm selection is high, although the order of mating had no bearing on male fertilization success in experiment 2. So why do female *E. heatwolei* mate multiply and why are there such high levels of multiple paternity? This pattern may be explained in a variety of ways (reviewed in Jennions and Petrie 2000). Females may gain direct benefits through increased fertilization (Michalczyk et al. 2011) or indirect benefits by using the sperm of many males to enhance offspring genetic diversity and subsequent offspring performance. Females also may mate multiply to reduce the risk of genetic incompatibility or simply to secure good genes (Byrne and Whiting 2011). One mechanism through which sons may gain an advantage is when their sperm is more competitive or more likely to be selected by a female (Jennions and Petrie 2000; Gage et al. 2004). We designed our experiments to test whether females preferred higher quality (larger) males (experiment 2) and to test the direct and indirect benefits hypotheses by comparing fertilization success (clutch size) and offspring performance between singly vs. multiply mated females. While male contest competition appears to be an important predictor of male fertilization success in this system, we found little evidence of precopulatory female mate choice: when direct male contest competition was removed, females mated with both small and large males and showed no evidence of trading up to larger males. We also found no evidence for direct or indirect benefits arising from multiple mating. There was no difference in fertilization success or clutch size between singly and multiply mated females, and offspring from multiply sired litters did not perform any better than litters sired by a single father, with no effect on offspring birth weight, growth rate, or short-term survival. A recent meta-analysis of 46 studies, in which matings by females assigned to monandry and polyandry treatments were fully controlled, found that polyandry was not significantly beneficial for any single measure of offspring performance, including growth rate and survival, but was marginally beneficial for egg hatching success, clutch production, and fertility (Slatyer et al. 2012). Yet the fact remains that this species displays high levels of multiple mating, multiple paternity, and has at least short-term sperm storage and thus the opportunity for sperm competition of cryptic female choice. One explanation is that females mate multiply to avoid the costs of male harassment rather than gain benefits, but females can easily reject male mating attempts. It seems more likely that multiple mating perhaps offers other indirect genetic benefits resulting in reproductive advantage to offspring later in life, benefits we have not been able to detect by measuring short-term growth rates and survival. One possibility might be generic bet hedging, in which production of a genetically diverse clutch will mean that certain individuals will have a survival advantage should there be a stochastic event resulting in a significant alteration of environmental conditions (Fox and Rauter 2003; Byrne and Keogh 2009). Testing this hypothesis will require much longer experiments that take into account lifetime reproductive success, something that is difficult to do with a long-lived vertebrate.

In an important recent review of multiple paternity in reptiles, Uller and Olsson (2008) found no evidence that high levels of polyandry are explained by direct benefits to females and support for indirect genetic benefits was weak at best. Therefore, while genetic models explaining multiple mating have rightly received much recent attention, female multiple mating can still occur in the absence of either direct or indirect genetic benefits. The conclusion from the extensive review of Uller and Olsson (2008) is that patterns of female promiscuity are most parsimoniously explained by the combined effect of mate encounter rates and the large benefits to males, and small costs to females, of multiple mating. This conclusion has recently been questioned. Madsen (2011) provides indirect evidence for higher predation risk to female adders (*Vipera berus*) during the breeding season as a result of frequent male attention, which likely serves to attract avian predators in comparison to solitary (stationary) snakes that appear to suffer significantly lower levels of predation. Uller and Olsson (2008) call for experimental tests of the assumptions and predictions associated with the evolution of female multiple mating in nonavian reptiles to address the costs and benefits of multiple mating to females. More recently, however, they show that sand lizard (*L. agilis*) offspring from multiply mated females have lower incidences of malformations and improved first year survival (Olsson et al. 2010, 2011). In our present experimental study, we were unable to detect any benefits or costs (although we cannot discount increased predation risk) to polyandry in *E. heatwolei*, and hence our results support the hypothesis suggest by Uller and Olsson (2008).

In summary, *E. heatwolei* has a polygynandrous mating system in which both males and females mate multiply. Larger males have an advantage by excluding smaller males from females, but females are not choosey and do not preferentially mate with larger males. One interpretation of our results is that postcopulatory processes such as sperm competition and cryptic female choice may be important mechanisms driving sexual selection (Slatyer et al. 2012). We found no benefits of multiple mating to female *E. heatwolei* because offspring from single vs. multiply mated females had similar life history traits and were equally likely to survive over the short term. While we cannot exclude the possibility that there may be indirect genetic benefits to offspring later in life, the most likely explanation for high levels of female multiple mating supports the Uller and Olsson (2008) hypothesis of a combined effect of mate encounter rates (high in our experiments) and low costs to females in the face of high benefits to males.

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