

MATE GUARDING IN MALE SAND LIZARDS (*LACERTA AGILIS*)

by

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Summary

We investigated factors that may determine mate guarding tactics in male sand lizards. In a sample of lizards from a museum collection, larger males had larger testis, but in laboratory experiments and in a natural population larger males did not sire more offspring. Males with long inter-copulatory intervals were more successful in sperm competition than males with short inter-copulatory intervals. In the wild, the operational sex ratio (OSR, No of receptive females/No of sexually active males) declined throughout the mating season. Mean duration of mate guardings was unaffected by OSR, time to ovulation, female age and mass, and clutch size. Larger males guarded females longer and were more likely to mate guard a female of similar age. Larger males had more partners but there was no correlation between male size or guarding time and the proportion of young that males sired in clutches from females mated with several partners. Males with more partners were more successful at siring offspring in clutches from females that mated with more than one partner. We suggest that fitter males are better at both mate acquisition and have more competitive sperm.

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Introduction

When a female mates more than once, and with different males, the scene is set for competition between sperm in the female's reproductive tract (*e.g.* Parker, 1970; Smith, 1984; Birkhead & Møller, 1992). Such female 'promiscuity' exerts selection on males to out-compete rivals not only in male-male combats, when there is intra-sexual selection, but also at the gametic level. Two principally different, but not mutually exclusive, evolutionary pathways to increased success in sperm competition are (i) to maximize the number and/or the quality of sperm transferred to the female, or (ii) to hinder competing rivals from sperm transfer. Selection for transfer of more sperm may, for example, lead to the evolution of enlarged testis (Møller, 1989, 1991; Birkhead *et al.*, 1993). In some species of lizards and snakes, alternate use of hemipenis in subsequent copulations may have evolved because it enables males to deliver more sperm in each ejaculate, and possibly also to copulate more frequently; each hemipenis is supplied by its own testis (Tokarz, 1988, 1989; Tokarz & Slowinski, 1990; Tokarz & Kirkpatrick, 1991). We do not review here the large number of adaptations that have been described for hindering rival sperm transfer and for removal of rivals' ejaculates in the female reproductive tract (see references in *e.g.* Smith, 1984, and Birkhead & Møller, 1992). Instead we focus on post-copulatory mate guarding, a phylogenetically wide-spread sperm competition tactic (*insects*: *e.g.* Parker, 1970; Convey, 1989; Sakaluk, 1991; *lizards*: Olsson, 1993a, b; *birds*: Birkhead & Møller, 1992 and references therein; *mammals*: Ginsberg & Huck, 1989, and references therein).

Mate guarding is time-consuming and, hence, is likely to be costly in the sense that the guarding male has to give up mating opportunities (*e.g.* Parker, 1978a, b). Therefore, we expect selection to adjust the duration and intensity of mate guarding in relation to factors determining its costs and benefits. In some species other selection pressures than those arising from paternity assurance may select for mate guarding (Grafen & Ridley, 1983; Thornhill, 1984). In pheasants (*Phasianus colchicus*) mate guarding decreases harassment from conspecifics and increases the feeding rate of the guarded female (Ridley & Hill, 1987), and may have formed a transitional stage in the evolution of paternal care in birds (van Rhijn, 1991). Thus, when the aim is to study mate guarding from a viewpoint of sperm competition and paternity assurance, it is desirable to choose a model or-

ganism in which non-genetic benefits to the female from mate guarding are minimal. For those reasons we choose the sand lizard (*Lacerta agilis*) as model organism; it is non-territorial, does not exhibit paternal care, and eggs are predominantly formed from fat reserves accumulated several months before the mate guarding event (Olsson, 1993a; Olsson & Shine, unpubl. data). Swedish sand lizard males emerge from hibernation on average 17 days before females and mate throughout the mating season, *i.e. ca.* May-June (Olsson, 1992b). Thus, during the period when females are receptive, the number of sexually active males can be regarded as approximately constant. Males are polygynous, have widely overlapping home ranges, and guard females but not ecological resources against rivals after a copulation and before resuming mate search (Olsson, 1992a, b, 1993a, b, 1994). Recently mated males have an increased probability of winning staged contests over un-mated males (Olsson, 1993b). Females are not choosy and mate multiply with any male that courts them and do not store sperm between ovulations (Olsson *et al.*, 1994a-c; Olsson & Madsen, 1995).

We (A) dissected museum specimens, (B) staged laboratory experiments, and performed a long-term field study in which we (C) observed sand lizard guarding behaviours in the field, and (D) looked for trade-offs between a male's number of sexual partners and his probability of paternity (using DNA-fingerprinting). In this way we aimed to establish morphological relationships between testis size and male body size and age, identify key determinants of paternity in staged situations of sperm competition, and relate this information to the behaviours of male sand lizards in a natural population. In each part of the study we primarily considered the following variables and hypotheses.

(A) *Dissection of museum specimens*

(i) Allometric relationships between testis size and male size

Hypotheses: Large, slow-growing males may allocate more resources to reproduction than smaller, fast-growing males. This may affect body size — testis size allometries, ejaculate output and size-specific mate guarding strategies.

(B) *Laboratory experiment*

(i) Male size

Hypothesis: Assuming that larger males have larger testis, larger males could transfer more sperm, which may increase the probability of paternity.

(ii) Time for sperm production and replenishment

Hypothesis: Additional time for sperm production could increase a male's competitive ability in a situation of sperm competition (Tokarz & Slovinski, 1991).

(C) *Field study — Ecology and behaviour*

(i) Risk of subsequent rival matings

Hypothesis: This variable can be approximated by the operational sex ratio and a male would be expected to guard or continue his search for females depending on the risk that a female, with which he has mated, subsequently copulates with other males (Parker, 1978a, b; Møller, 1987; Clark, 1988).

(ii) Timing of the mate guarding in relation to oviposition and, hence, ovulation

Hypothesis: A female which is closer to ovulation could be more attractive to guard since sperm mortality until ovulation is likely to be low and there is less risk that she will mate with a rival (Birkhead, 1982; Møller, 1987; Lessels & Birkhead, 1990; Parker, 1990).

(iii) Male size

Hypothesis: A large male is likely to be more successful at defending a mated female than a small male is (Stockley & Purvis, 1993). We therefore looked for a relationship between male size and number of partners. If there is such a relationship, this predicts that large males are under stronger selection to optimally trade off probability of paternity and mate acquisition.

(iv) Guarding time

Hypotheses: Males that guard females longer should have fewer mating opportunities with other females and, hence, fewer partners. Females that are guarded longer should also have fewer partners.

(D) Field study — *Determinants of probability of paternity*

(i) Probability of paternity

Hypotheses: If paternity assurance is the primary target of selection for post-copulatory mate guarding, then a male that invests more time in mate guarding should increase his reproductive success. Furthermore, according to classic trade off models (Parker, 1978a, b), a male that invests more time in mate acquisition should do so at a cost of reduced probability of paternity per clutch.

Because this study is essentially made up of four different parts, we describe the methods and the results for each part in conjunction. DNA-fingerprinting was used both in the laboratory experiment and in the field study and is described in part (D).

Methods and results

(A) *Dissection of museum specimens*

Material and methods

We analyzed the relationship between testis volume (mm^3) and snout-vent length (mm) in a sample of males from a museum collection (Museum of Natural History, Gothenburg); testis volume was calculated as the sum of the left and right testis (volume = length \times width²). Only specimens collected during breeding seasons were used. Testis volume was cube root transformed, to make the dimensions of the variables equal in the statistical analysis.

Results

Testis volume increased with snout-vent length (SVL) following the regression equation, Testis volume (mm^3) = $556 \times \text{SVL (mm)} - 24\,053$ ($R^2 = 0.56$, $p = 0.008$, $N = 11$). After log-transformation, R^2 dropped from 0.56 to 0.51, and the F -value from 11.5 to 9.4. The data appeared to fit a linear model better than a power function. When a 95% confidence limit was fitted around the regression coefficient in a power function, it

did not deviate significantly from one (2.6 ± 0.84 , SE, $0.84 \times 1.96 = 1.65$, $2.6 - 1.65 = 0.95$). Thus, testis volume increased with SVL according to a linear model and, hence, larger males did not develop larger testis in proportion to SVL than did smaller males.

(B) *Laboratory experiment*

Material and methods

During 1988-1993 we performed sperm competition experiments by using first generation offspring from wild sand lizards, reared under semi-natural conditions. In mid October the lizards were put into hibernation in plastic boxes with a mixture of peat and sand, and kept in a refrigerator at 4-6°C. In mid December hibernation was interrupted for all males at the same time. Males were allowed to thermoregulate in separate cages for about one week before female hibernation was interrupted (see Olsson *et al.*, 1994c, for details).

When a female became receptive (evident by lifting her tail and by not rejecting male copulation attempts, Kitzler, 1941), she was mated twice with two different males, one hour apart or 24 h apart. The time elapsing between a male's subsequent matings ('time for sperm replenishment/production') was measured in minutes, and the choice of hemipenis in each mating was monitored (left or right). In addition to the data on time-dependent shift in hemipenis use that we collected during this laboratory experiment, we also incorporated equivalent data for individuals from the same laboratory-kept population used in a breeding experiment (Olsson, 1992b).

Paternity was determined by using a morphological marker trait (dorsal stripes), confirmed to be Mendelian inherited and determined by a dominant allele (Olsson *et al.*, 1994c), with DNA-fingerprinting and with a (TC)_n single locus probe (see '(D) Field study — Determinants of probability of paternity' and Olsson *et al.*, 1994c).

Results

Leger & Didrichson (1994) demonstrated that if the within-male variance in reproductive success is high the correct approach is to enter all data in a statistical analysis rather than to delete data at random to achieve single observations per individual. The within-class variance in our study was very high, four of the males had a reproductive success ranging from 0 to 100% (Olsson *et al.*, 1994c), and we therefore entered all data into our statistical model. Before doing so we also tested if the males used more than once in our analyses differed in reproductive success in sperm competition with another male; in an unbalanced ANOVA, the proportion of the clutch sired by a male was used as the dependent variable, and male identification number as the class variable. The ANOVA was not significant, suggesting that these males did not differ in ability to sire

offspring in the experiment ($p = 0.71$, $N = 8$). All proportions were arcsine transformed before statistical analyses (Sokal & Rohlf, 1981).

We aimed to investigate if males that 'rest' longer between copulations gain in reproductive success in situations of sperm competition with another male. To achieve this we performed a multiple analysis of covariance (MANCOVA, PROC GLM, SAS, 1987) comprising the class variables 'interval between a female's matings' (24 h or 1 h), and 'male mating order' (first or last), and the covariates 'time since a male's previous mating', number of a male's subsequent matings ('male copulation number'), and 'male snout-vent length'. The response variable was the proportion of the clutch sired by a male.

The model was statistically significant ($F = 3.2$, $p = 0.02$, $R^2 = 0.38$, Table 1), but the only significant variable *per se* was time since a male's previous copulation ($p = 0.001$). This time was on average 4409 min (73 h 29 min), (± 3816 , SD, median = 2360, range 1130 min (18 h 50 min) – 10 080 min (168 h)). Mating order was the variable with the second highest F -value in the model ($F = 1.88$, $p = 0.18$); first males sired on average 40.9% of the offspring (± 39.8 , SD), while the equivalent figure for second males was 59.1% on average (± 40.2 , SD).

When the time between a male's two subsequent matings was less than 24 h, males altered between the right and the left hemipenis in 17 of 25 copulations (68%). When the time between matings was more than 24 h, males altered between using the different hemipenis in 9 of 18 cases (50%).

TABLE 1. Results of the MANCOVA considering variables potentially important for siring offspring in competition with another male

Variables	df	Type III SS	F	p
Snout-vent length	1	0.04	0.19	0.67
Interval	1	0.18	0.93	0.34
Male mating order	1	0.36	1.88	0.18
Male mating number	1	0.16	0.86	0.36
Time	1	2.53	13.13	0.001

Interval = interval between a female's matings (1 h or 24 h), Time = time since a male's previous copulation.

This difference was not statistically significant ($\chi^2 = 1.38$, $p = 0.20$, $df = 1$).

(C) *Field study — Ecology and behaviour*

Material and methods

The data on mate guardings in the present study were gathered during 1987-1991 from a natural population of individually marked sand lizards at Asketunnan, 50 km S Gothenburg on the Swedish west coast. The lizards were caught by hand or by noosing, measured snout to vent to the nearest mm, weighed to the nearest 0.1 g, marked by toe-clipping and an individual number painted on an adhesive cloth tape placed on the lizard's back. Toe-clipping does not lower the survival rate of wild sand lizards (Olsson, 1994), and the removed phalanges were used for aging by skeletochronology. Each toe was sectioned and stained with Ehrlich-Haematoxyline whereafter visualized growth rings were counted according to Hemelaar (1985). For a more detailed description of field methods see Olsson (1992b, 1993a, 1994).

From the first day after hibernation that sand lizards were observed (usually around 15 April) we numbered the days and assigned a 'day number' for the onset of each mate guarding. Mate guarding investments were estimated by recording duration (days) of individual mate guardings, achieved by visiting 'pairs' mornings and evenings and monitoring when the male left the female.

About one week before oviposition, which was evident from egg contours on the females' body sides (Olsson, 1992b), they were brought to the laboratory, and kept separately in cages 40 cm × 50 cm × 60 cm, with a 40 W spotlight arranged to allow thermoregulation. Each female was checked twice daily for recent oviposition, which is evident from lateral skin folds. No female in this study laid more than one clutch per year. Since ovulation and, hence, conception, cannot be determined by exterior cues, we used egg-laying date as an indicator of ovulation day; ovulation occurs *ca.* two weeks earlier than oviposition (Olsson *et al.*, 1994c), which will not affect our analyses providing the time between ovulation and oviposition is approximately constant between females. Females that mated late also oviposited late (correlation between copulation date and laying date, $r_s = 0.41$, $p = 0.003$, $N = 49$), which supports our assumption that the temporal separation of mating, ovulation and oviposition is approximately constant between females. We define the time from the day of copulation and/or onset of mate guarding to the estimated day of ovulation as 'time to ovulation'.

Operational sex ratio is defined as "the average ratio of fertilizable females to sexually active males at any given time" (Emlen & Oring, 1977). When males become sexually active before females and female receptivity is a-synchronous, the ratio between breeding females and males is likely to be an inflated estimate of OSR. The number of receptive females in any given day depends on to what extent the reproductive cycles of females are synchronized and, hence, to what extent their receptive periods overlap. We calculated the duration of receptive periods in the natural population by counting the number of days separating a female's first and last copulation and/or first and last day of mate guarding. This estimate is dependent on the availability of sexually active males, which however is unlikely to affect our estimate of OSR; OSR is heavily skewed towards males (Table 2), the encounter

rate of females is relatively high (Olsson, 1993a), males are polygynous, mate throughout the mating season, and do not hold site-related territories (Olsson, 1992b). Thus, there is little risk that receptive females will be unattended by sexually active males for significant time, which would make us underestimate the receptivity period. The female receptive period in our sample was on average about 9 days (8.7 ± 5.0 , SD, $N = 50$, 1987-1991), and did not vary significantly between years for females for which we had observations in more than one year (ANOVA, identification number as class variable, $N = 17_{\text{females}}$, $R^2 = 0.002$, $F = 0.03$, $p = 0.86$). This also supports the assumption that individual females do not vary significantly in temporal separation of mating, ovulation and oviposition.

When all females had laid their eggs, we stacked their mean receptive periods in a diagram from the date of ovipositions retrospectively (Fig. 1). We then counted the number of overlapping receptive periods each day and divided this figure by the total number of females in the sample. This proportion was then multiplied by the total number of reproductive females in the natural population, yielding the absolute number of receptive females in any given day in the wild. OSR was obtained by calculating the ratio between the number of receptive females and the number of sexually active males per day, thus, estimating the number of receptive females available to the sexually active males in any given day and year.

Field data were pooled for all years and were therefore standardized by year (PROC STANDARD, SAS) to remove potential between-year effects (Lande & Arnold, 1983; SAS, 1987). When a male had been observed more than once, we used the means of the standardized scores in all statistical analyses. Several variables could not be normalized by transformation, and therefore we used non-parametric statistical techniques. All non-parametric partial correlations were performed in SAS (Spearman's partial rank order correlation analysis, PROC CORR, SAS, 1987).

Results

The descriptive data, before it was standardized and before we calculated mean values of the standardized scores, are presented in Table 2.

TABLE 2. *Descriptive data*

Variable	Mean	SD	Median	Range	<i>N</i>
F Mass (g)	3.6	2.31	13.6	9.0-19.5	146
F Age (calendar years)	6.5	1.6	6.0	4-11	143
Clutch size	8.9	2.2	9.0	5-15	105
M Mass (g)	12.3	2.1	12.5	6.8-19.2	143
M Age (calendar years)	6.3	1.9	6.0	3-11	124
Guarding duration	1.4	1.2	1	1-9	148
Time to ovulation	29.3	9.3	29.0	0-56	117
OSR (No F/No M)	0.44	0.24	0.46	0.03-1.0	106

F = Female, M = Male.

Female reproduction: timing and reproductive output

Heavier females laid larger clutches ($r_s = 0.63$, $p = 0.0001$, $N = 47$), independent of age ($r_s = 0.57$, $p = 0.0001$, $N = 45$, factoring out the effect of age in a partial correlation). Age was unrelated to clutch size when body mass was factored out ($r_s = 0.08$, $p = 0.064$, $N = 46$). Older females mated earlier than younger females ($r_s = -0.32$, $p = 0.02$, $N = 54$, correlation between copulation day and age). Since OSR declined throughout the mating season ($r_s = -0.41$, $p = 0.004$, $N = 46$, correlation between OSR and copulation day), older females also mated when there were more receptive females available to males. Females that mated later also oviposited later ($r_s = 0.41$, $p = 0.003$, $N = 49$).

Mate guarding: male investments and timing in relation to ovulation

We first performed a non-parametric analysis of variance on males observed to mate guard more than once, using male identification number as class variable and guarding time as continuous variable. If males could be assigned to categories of guarding strategies like 'guarders' and 'sneakers' we predicted a significant result in such an analysis. The analysis was not statistically significant (Kruskal-Wallis analysis of variance, χ^2 approximation = 38.2, $p = 0.37$, $df = 36$).

Male age and body mass were not correlated with copulation day ($r_s = -0.08$, $p = 0.57$, $N = 54$; $r_s = 0.04$, $p = 0.78$, $N = 50$, respectively). Older males were more likely to guard older females ($r_s = 0.42$, $p = 0.003$, $N = 48$), a result which was independent of male body mass ($r_s = 0.29$, $p = 0.05$, $N = 48$, body mass factored out). In the corresponding partial correlations, holding age constant, heavier males did not guard heavier or older females, or females with larger clutch size (Table 3).

Mean guarding time in our sample was 1.4 days (Table 2). Larger and older males guarded females longer than smaller and younger males (Table 3). When the effect of body size was factored out, male age was no longer correlated with guarding duration ($r_s = -0.01$, $p = 0.95$, $N = 49$). However, the correlation between male body mass and guarding duration was robust and remained significant when male age, OSR, and copulation day were factored out in partial correlations (Table 3). Mating day, OSR and the time to oviposition from mating were not significantly correlated with guarding duration (Table 3).

TABLE 3. *Correlation coefficients between guarding duration (days) and some male and female traits*

Variables	r_s	p	N
Male mass	0.38	0.0005	45
Partial: Age	0.31	0.03	49
Partial: OSR	0.41	0.006	45
Partial: Copulation day	0.38	0.004	45
Female mass	0.18	0.19	53
Female age	-0.008	0.96	48
Clutch size	0.4	0.78	46
Mating day	0.001	0.99	46
OSR	0.09	0.50	55
Time to oviposition (and ovulation)	-0.07	0.64	49

When a third variable was held constant in a partial correlation this is indicated by 'partial'.

We also looked for costs and benefits of mate guarding, depending on when and for how long males guarded their sexual partner(s). A positive correlation between time to ovulation and a female's number of sexual partners was bordering on significance at the 0.05 level ($r_s = 0.30$, $p = 0.06$, $N = 40$; mean number of mates per female = 1.7, ± 0.78 , range 1-5). Thus there was a non-significant trend suggesting that matings early in a female's receptive period were likely to be followed by matings by other males. There was no significant negative relationship between guarding duration and the number of males that a female mated with ($r_s = 0.22$, $p = 0.15$, $N = 45$).

(D) Field study — Determinants of probability of paternity

Material and methods

Male blood samples were collected at first capture with a capillary tube from a minute cut in the corner of the mouth (*v. angularis*). In the statistical analyses of male probability of paternity we incorporated male mass, guarding time, and the day that a male emerged from hibernation (first capture), since this may influence male spermatogenesis and, hence, sperm quality (Saint-Girons, 1985). Males that had been observed within a female's home range during the mating season were included in the paternity analysis of her offspring. Five of the females that were brought back to the laboratory for oviposition were sampled at random for analyses of the paternity of their offspring. The home ranges of these females were geographically well separated. After oviposition a blood sample was taken from the female as described for males. A few days after hatching, the offspring were blood sampled as described for adults. For a detailed description of the laboratory methods see Olsson

et al. (1994c). For the single locus probe, the synthetic (TC)_n, the high stringency wash was performed at 62°C. For assigning paternity with DNA fingerprinting we used male specific bands (bands not shared by the female or other males) >3-4 kb and with the (TC)_n-locus we used the male allele in all offspring. The mutation rate in the minisatellites used for DNA-fingerprinting is probably not higher than 1×10^{-3} because in 92 offspring in a laboratory population with a total of 879 bands in their DNA-fingerprints (Olsson *et al.*, 1994c) we found no mismatch band (a band that could not be traced to either of the parents). The risk that an offspring obtained a band from a mutation event with exactly the same length as the male-specific band is therefore very low. An offspring was considered sired by a male if it had one or more male-specific bands.

Results

Our DNA fingerprinting data on wild sand lizards is on a small subset of males ($N = 12$) and females ($N = 5$) (Table 4). Of these males ten were observed with females and eight mate guarded females for at least one day. We present results of statistical analyses both including and excluding the two males which were never observed with the females. This is because no straightforward interpretation of the relationship between mate acquisition and success in sperm competition can be made for these males. The variance in the mass of the males approximately represents the

TABLE 4. *Data for the analyses of the relationship between success in sperm competition and mate acquisition*

Male number	Female number	Day	Partners	Male mass (g)	Sired young (%)	Guarding time (days)
158	80	1	3	14.1	67 (6)	2
893	80	10	0	8.0	33 (6)	—
228	80	1	2	13.2	0 (6)	—
599	213	13	2	7.0	25 (8)	4
193	213	1	6	15.3	75 (8)	3
362	357	14	4	12.7	100 (6)	1
593	357	9	1	7.9	0 (6)	1
287	396	15	2	10.8	50 (6)	—
899	396	17	2	7.2	33 (6)	1
282	396	2	0	10.2	17 (6)	—
124	398	2	2	13.1	17 (6)	8
898	398	16	1	6.4	0 (6)	1

The number of DNA-fingerprinted young is given within parentheses in the 'Sired young' column. 'Day' denotes male emergence day in relation to the first observed male. 'Partners' denotes a male's number of sexual partners.

sexually active male population (range 7.0-15.3 g, Table 4), thus from a male size perspective our sub-sample is unbiased.

Larger males emerged earlier after hibernation than did smaller males ($r_s = -0.77$, $p = 0.003$, $N = 12$; $r_s = -0.82$, $p = 0.003$, $N = 10$, correlation between emergence date and body mass), and had higher mating success ($r_s = 0.62$, $p = 0.03$, $N = 12$; $r_s = 0.73$, $p = 0.02$, $N = 10$), also when we controlled for emergence date in a partial correlation ($r_s = 0.72$, $p = 0.01$, $N = 12$; $r_s = 0.74$, $p = 0.02$, $N = 10$). The time difference between the first and the last emerging male (17 days) does not exceed the time difference in mean emergence date between males and females (17 days, Olsson & Madsen, 1995). Thus, the difference in emergence date between males does not violate our assumption of a constant number of sexually active males once females become active.

When males invest more time in guarding a female we expect this to increase that male's probability of paternity; in our study it did not. There was no correlation between guarding time and probability of paternity ($r_s = 0.05$, $p = 0.90$, $N = 8$, Table 4). When males invest time in finding more partners we expect this to carry the cost of a reduced proportion of young sired per clutch. Therefore, the number of partners ought to

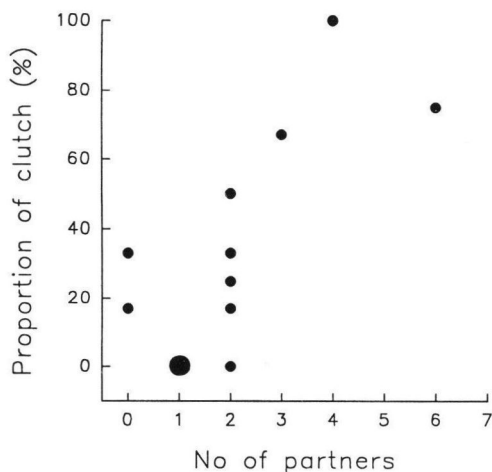


Fig. 2. Correlation between a male's estimated number of partners and the proportion that he sired in a female's clutch ($r_s = 0.70$, $p = 0.01$, $N = 12$). The larger dot represents two readings.

be negatively correlated with the proportion of sired offspring in clutches from females mated with several partners. Larger males did not sire more offspring ($r_s = 0.45$, $p = 0.14$, $N = 12$; $r_s = 0.45$, $p = 0.19$, $N = 10$). However, males with *high* mating success sired *more* offspring in clutches from multiply mated mothers ($r_s = 0.70$, $p = 0.01$, $N = 12$; $r_s = 0.91$, $p = 0.0006$, $N = 10$; Fig. 2). Thus, a trade-off between number of partners and number of sired offspring per clutch could be masked by positive genotypic and/or phenotypic correlations between fitness components in successful males. Our data also demonstrated that two males that were never recorded mating or mate guarding still sired offspring. Both these males were below the average size in the sample (mean = 12.3 g, $N = 143$, Table 2; male 893 and 282, Table 4).

Discussion

Testis size was not allometrically related to body size. Thus, larger males do not seem to invest proportionally more in gamete production than smaller mature males, which may occur in mammals (Stockley & Purvis, 1993). Larger males have larger testis and ought to transfer more sperm in absolute numbers with each ejaculate, but we could not confirm a male size-advantage in our sperm competition experiment; 'Time since a male's previous mating', was the only variable that had a significant effect on a male's probability of paternity. The major results of the field study were: (i) larger males emerged earlier after hibernation and had more partners; (ii) the operational sex ratio (OSR) declined throughout the mating season; (iii) OSR, time to ovulation, clutch size and female mass had no impact on mate guarding duration; (iv) larger males guarded females longer than smaller males did; (v) a longer guarding of a female did not significantly decrease her number of partners; (vi) males with many partners also sired a higher proportion of offspring in clutches from multiply mated females. The results of the field study were mainly arrived at by correlation analyses, several on each of the three subsets of data. This increases the risk of Type I-error. However, in each case when we achieved significant correlations, we also looked for effects of confounding variables in partial correlations. The major results were all robust in such analyses.

Olsson (1992b) reported that the OSR of the same natural population as in the present study was approximately 1:1. However, this estimate of OSR was based on the ratio of sexually active females and males in a given year and overestimates the availability of females in relation to males per day with a factor 2. The lack of influence of daily OSR on male guarding behaviors could be due to difficulties for non-territorial lizard males to perceive rivals in a heterogenous habitat. Thus, OSR ought to affect guarding tactics more in species in which scanning for rivals is less constrained. This may explain why our results contrast with significant effects of OSR on guarding tactics in birds (Møller, 1987).

Female sand lizards do not choose males (Olsson & Madsen, 1995) so a male's mating success depends primarily on his ability to encounter receptive females and achieve matings in competition with other males. Larger males' higher resource holding power make them more likely to obtain mates and to be better mate guards. Superficially, an increase in reproductive success from mate guarding seems obvious in this species. Even if mating order effects were not statistically significant, there was mixed paternity and males that can prevent sperm competition would clearly be at a selective advantage. What are the costs and benefits of doing so?

Because females are promiscuous, a successful mate guarder is not guaranteed to sire all offspring in a clutch unless he mates as soon as the female becomes receptive and guards her to conception. On average, doing that would leave him 8.9 offspring (Table 2); a mating of 'his' female by a second male would likely reduce his reproductive output to *ca.* 40% of 8.9 young, *i.e.* *ca.* 3.6 young. If he does not mate guard at all but leaves the female after mating, his mating success should primarily depend on the total number of females that he mates with, the number of partners that 'his' female mates with, and the relationship between a female's number of partners and the proportion of the clutch sired by each male (mating order effects). Female sand lizards mate on average 3.7 times with one to five partners (Olsson *et al.*, 1994a). The number of partners is likely to be underestimated as there is a minority of males that sire offspring without being recorded mating or mate guarding. Assume that the proportion of offspring of the mating males are shared equally. In the extreme case a female's clutch may be shared between five males; a male then sires $1/5 = 20\%$ of a clutch, *i.e.* 1.8 young on average. A large male mates with

up to six females (Olsson, 1994) which would then render him 10.8 young, increasing his reproductive success with more than 20%. Thus, also under strong sperm competition a large successful male would be selected to mate with many females. Such a male also appears to be more successful in sperm competition, which reduces his costs for leaving the female. However, it would naturally benefit him even more if he can further increase his probability of success in sperm competition, *e.g.* by transferring more sperm at each mating. We demonstrated that a male may increase his chances in sperm competition by prolonging inter-copulatory intervals. Larger males would, due to their stronger resource holding power, be more likely than smaller males to freely trade off time between mate guarding and mate acquisition. Larger males also mate guard longer, which suggests that prolonged inter-copulatory interval is adaptive. Thus, mate guarding could have evolved as a means by which males prevent rival matings while they allow sperm number to build. The ultimate test of the hypothesis that prolonging the inter-copulation interval increases a male's reproductive success would be to look for effects of mate guarding on the probability of paternity in the clutch of the *next* female that a guarding male mates with. Unfortunately our data does not admit such analyses. Interestingly, in many snake species, males and females remain in coitus for hours up to days (*e.g.* Klauber, 1956). Thus, perhaps the prolonged mating in snakes is functionally comparable to mate guarding in sand lizards. A male snake could prevent other males from mating while replenishing sperm in the testis and epididymis that is not currently being used.

In conclusion, male mate guarding tactics in sand lizards seem to be determined by positive effects of prolonged inter-copulatory interval, and male fighting ability. An important result is that sperm performance may vary between males and correlate with other male fitness components, such as success in mate acquisition. When correlations between male fitness components are positive, some males are 'good at everything', optimal trade-offs of time between for example mate acquisition and mate guarding may be masked. In such cases, optimality models are unlikely to reveal trade-offs. If they exist, they have to be demonstrated experimentally.

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