

FECUNDITY AND MHC AFFECTS EJACULATION TACTICS AND PATERNITY BIAS IN SAND LIZARDS

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Abstract.—We demonstrate that extending copulation enhances probability of paternity in sand lizards and that determinants of copulation duration depend on a males' mating order (first or second). First males, with no information on presence of rivals, extend copulation when mating with a more fecund female. Second males, however, adjust copula duration in relation to a first male's relatedness with his female, which there is reason to believe can be deduced from the MHC-related odor of the copulatory plug. Male-female relatedness negatively influences a male's probability of paternity, and when second males are in a favored role (i.e., the first male is the one more closely related to the female), second males transfer larger ejaculates, resulting in higher probability of paternity. This result corroborates predictions from recent theoretical models on sperm expenditure theory incorporating cryptic female choice and sexual conflict. More specifically, the results conform to a "random roles" model, which depicts males as being favored by some females and disfavored by others, but not to a "constant-type" model, in which a male is either favored or disfavored uniformly by all females in a population.

Key words.—Fecundity, lizards, major histocompatibility complex, paternity bias, sperm competition.

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Sperm expenditure theory predicts that males should manage their ejaculate resources in relation to copulation history of partners and rivals, and in relation to present and future risks of sperm competition. Thus, when information on copulation history of partners and rivals differs between two competing males, theory predicts male-specific ejaculation tactics (Parker 1998). One such example would be when a female exercises preference for one of two males in a situation of sperm competition, because the preferred male is more genetically compatible with the female and would sire more viable offspring. A recent theoretical model depicts this scenario and predicts that when females vary in mate preference with respect to male identity (e.g., due to genetic compatibility, the so-called random roles model), a male in a favored role with respect to probability of paternity should invest more heavily in ejaculate expenditure (Ball and Parker 2003). However, at the planning of this study, we were only aware of parts of Ball and Parker's (2003) results from seminars, and testable predictions were therefore made based on general sperm expenditure theory (summarized in Parker 1998), and then discussed in the light of Ball and Parker's recent discoveries.

Sand lizards (*Lacerta agilis*) are small (up to 20 g, up to 90 mm snout-vent length), ground-dwelling lizards with a wide distribution in Europe and western Asia (Olsson and Madsen 2001). About two weeks after emerging from hibernation, the mating season commences. Males then copulate with the polyandrous females using one of their two ipsilateral hemipenises. These copulatory organs only drain the epididymis on the side of the body corresponding to the hemipenis used, so that expenditure of ejaculate products on the right side is independent of those on the left side. Furthermore, throughout the copulation, sand lizards transfer spermatozoa and kidney secretions that together form a copulatory plug in the female cloaca, and ejaculation is not in-

stantaneous as in most birds and mammals (Olsson and Madsen 1998, 2001; Shine et al. 2000). Although we never collected quantitative information on the relationship between copula duration and ejaculate volume in the current study (because it would have meant destructive sampling of the trials from which the information was gathered), positive copula duration-ejaculate volume relationships have been confirmed in a previous trial series on sand lizards (M. Olsson, pers. obs. 1992), an agamid lizard (*Ctenophorus fordi*; Olsson 2001), and a snake (*Thamnophis sirtalis parietalis*, Shine et al. 2000).

We have shown elsewhere that probability of paternity is biased toward the least related male in sand lizard sperm competition (Olsson et al. 1996). Furthermore, we recently demonstrated that females in staged mate choice trials prefer to associate with males less related at the major histocompatibility complex (MHC) class I loci, and that odor-based mechanisms are likely to explain assortative mating for MHC in a natural population (less partner relatedness than expected by chance; Olsson et al. 2003). This is in agreement with similar findings in mammals (Penns and Potts 1999). In the experiment reported here, we therefore used a sand lizard-specific MHC class I probe to estimate relatedness, indexed as the proportion of shared restriction fragment length polymorphism fragments (Wetton et al. 1987), at confirmed non-neutral loci (Madsen et al. 1999), to look for relatedness effects on sperm expenditure tactics.

Unlike a first male, a second male can smell his rival's ejaculate, which remains lodged in the female's cloaca and is thoroughly investigated by tongue flicking before copulation (Kitzler 1941; Olsson and Madsen 1998). Therefore, it is plausible that a second male could tactically respond to the odor-mediated MHC genotype of his rival and adjust sperm expenditure accordingly, whereas a first male lacks such information on rivals, or may even interpret the absence

of a copulatory plug in the virgin female as a confirmation of absence of competition.

Given this background, and assuming that facultative adjustment of sperm expenditure is made in relation to perceived cues of partners and rivals (not only adjusted by selection to a population average expenditure), we made the following testable predictions: (1) first males should adjust copula duration in relation to female fecundity, since a male in this role is under selection to adjust his investments in relation to risk of sperm depletion (greater risk in more fecund females); (2) under the assumption that second males' reproductive success is much more influenced by rival ejaculates than by his own sperm depletion, second males should primarily consider their role in relation to their rival's competitive ability. In this system we know that the male less related to a female has an advantage in sperm competition. Therefore, we predicted that a second male should primarily adjust ejaculate expenditure in relation to a rival's female relatedness, since this determines his probability of paternity.

METHODS

Males were brought out of an artificial hibernation in January, which induces reproductive behavior and full fertility. After hibernation, all males were allowed to mate with a female not part of the current experiment to clear the sulcus of the hemipenis, since any dried ejaculate remains from copulations in the previous year might interfere with sperm transfer through this narrow canal and inflate the variance in probability of paternity (Olsson and Madsen 1998). A male was then allowed to mate with experimental females until he used the same hemipenis as in his first copulation; that is, we ignored a copulation on the opposite side. In contrast to some other reptile species that appear to tactically alternate between the two hemipeneses in order to maximize sperm transfer, sand lizards are left- or right-biased, resulting in series of copulations using the same hemipenis (M. Olsson, T. Madsen, B. Ujvari, and E. Wapstra, unpubl. ms.). Therefore, more than 70% of the 26 different males allowed to copulate the 13 females in the experiment (two males per female) never used their alternative hemipenis before a second copulation with their "favorite" hemipenis (Fisher's exact test, $P = 0.041$). Before the experiments, males were weighed and their body mass used to size-rank males to minimize any effect of body size-related ejaculation tactics (since small males show sneaker tactics in the wild [Olsson and Madsen 2001] which may perhaps select for size-dependent ejaculation expenditure).

Because of the continuous ejaculate transfer through copulation, we timed copulations using a stopwatch and used copula duration as a proxy of ejaculate investments. Following assignment of paternity using the microsatellites Lv-4-72, Lv-4-x (Boudjemadi et al. 1999), and La-3 (Gullberg et al. 1998), using Cervus Software, (available at <http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.htm>; results reconfirmed manually), we let the proportion of a clutch a male sired estimate his reproductive success. More extensive laboratory, husbandry, and molecular genetics protocols have been published elsewhere (Olsson and Madsen 2001 and references therein).

Statistical analyses were designed to identify (1) relationships between differences in first and second male trait values, and corresponding differences in their probability of paternity (all differences between male attributes were calculated as first-second male trait value), and (2) role-specific male tactics, aiming to identify patterns in sperm expenditure specific for first and second males, respectively.

To compensate for variation in sperm loss in males' first copulations (used to clear the sulcus), we first looked for a negative correlation between first and second copula duration, suggesting sperm depletion. Indeed, such a correlation was evident, which may indicate that first copula durations may constrain optimal male tactics in subsequent copulations ($r = -0.40$, $P = 0.040$, $n = 26$). Therefore, we regressed duration of second copulations on duration of first copulations, and let the residuals index ejaculate investment in relation to expended sperm supplies (hereafter male "effort"; one trial resulted in an outlier mating effort deviating more than 10 standard deviations and was therefore excluded from further analysis). These residual scores are highly correlated with absolute copula duration for both first and second males ($r_s > 0.85$, $P < 0.0004$). In one case, we used residuals from a regression of proportional paternity on effort (itself a residual) to index effort-dependent reproductive success. Although a more direct approach would have been preferable, our relatively small dataset did not allow for techniques requiring large degrees of freedoms (e.g., multivariate analysis). Furthermore, since most of our trait distributions were nonnormal (as is characteristic of most estimates of reproductive success; Arnold and Wade 1984), we used nonparametric Spearman's partial rank-order correlation coefficients (r_s ; available in Proc Corr, SAS 1990), which allowed us to control for confounding variables in a manner similar to multivariate parametric approaches.

RESULTS

When we controlled for male time in copula and rival relatedness (which lowers probability of paternity when rivals are closely related; Olsson et al. 1997), male probability of paternity was negatively correlated with partner MHC relatedness in both first and second males ($r_s = -0.84$, $P = 0.035$; $r_s = -0.82$, $P = 0.046$, respectively). These results thus confirm that male probability of paternity is negatively influenced by partner relatedness (Olsson et al. 1996).

We also confirmed that our results were not due to differences between first and second males in the mean values of a number of traits potentially linked to probability of paternity, such as mean first copula duration (pairwise t -tests; -127.8 ± 93.9 , $t = -1.4$, $P = 0.20$), second copula duration (25.3 ± 34.9 , $t = 0.72$, $P = 0.48$), body mass (-0.02 ± 1.19 , $t = -0.01$, $P = 0.990$), and MHC relatedness with the female (-0.05 ± 0.07 , $t = -0.78$, $P = 0.45$). Furthermore, there was no significant difference in mean probability of paternity between first and second males (-14.8 ± 23.6 , $t = 0.63$, $P = 0.54$), nor was there any relationship between the time separating a first and a second male's copulation (mean $131 \text{ min} \pm 271$, SE) and the time in copula, or between copula separation time and mean proportional paternity for either male ($-0.003 < r_s < 0.12$; $0.685 < P < 0.993$, respectively).

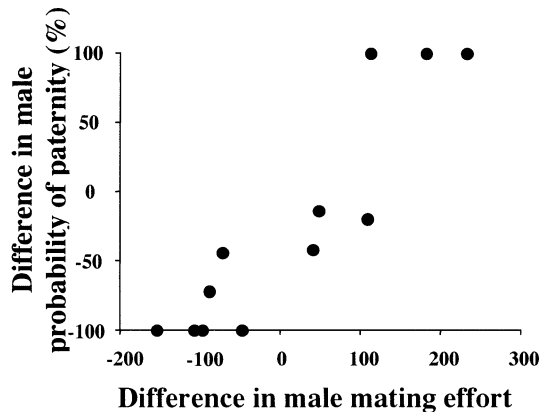


FIG. 1. Large differences in mating effort (residual scores) between males in sperm competition result in large differences in reproductive success (Spearman's partial rank-order correlation analysis, $r_s = 0.92$, $P = 0.0001$).

Once these confounders were ruled out, we first confirmed that male decisions regarding ejaculation duration should be under strong selection in sand lizards. This is strongly supported by the correlation between difference in mating effort between males, and their corresponding difference in probability of paternity (Fig. 1; $r_s = 0.92$, $P = 0.0001$). We then looked for specific role-dependent effects.

There was no relationship between a first male's time in copula and his body mass ($r_s = -0.31$, $P = 0.331$), or with his relatedness with the female ($r_s = 0.13$, $P = 0.596$). However, in accordance with predictions, first males prolonged copulations relative to second males when females were carrying larger clutches (clutch size, range 3–10, mean 6.9 ± 1.85 SD; mean time in copula, $226.6 \text{ sec} \pm 63.2$ SD), resulting in an increasing difference in copula duration between males with increasing clutch size ($r_s = 0.55$, $P = 0.05$), and a corresponding increasing difference in proportional paternity between males with female fecundity ($r_s = 0.68$, $P = 0.011$). A very strong relationship between clutch size and a first male's effort-dependent probability of paternity is likely to further contribute to this result (Fig 2; $r_s = 0.91$, $P = 0.0001$). Thus, this may suggest that first males not only copulate for longer, but also transfer sperm at a higher rate, when females are more fecund. Unfortunately, we have no means to control for this in our analysis, but the relatively longer copulations with more fecund females strongly suggest that at least part of this result is explained by mere prolonged copula duration.

In contrast to first males, second males mated for longer when larger (correlation between body mass and effort, $r_s = 0.81$, $P = 0.001$), but did not prolong copulations when mating with more fecund females ($r_s = -0.49$, $P = 0.10$). Furthermore, males in second copulations seemed to be negatively influenced by long copulations by the first male, that is, when copulation plugs were larger ($r_s = -0.62$, $P = 0.03$). However, even after we controlled for these confounders in the partial correlation analyses, second males showed strong rival relatedness effects. A second male copulated for longer when a first male was more closely related with the female (correlation between first male-female relatedness and second male copula duration, $r_s = 0.65$, $P = 0.043$), which resulted

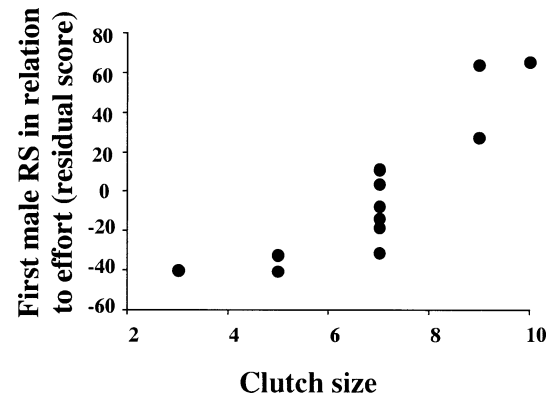


FIG. 2. First male effort-dependent reproductive success (RS) increases significantly with female fecundity (Spearman's partial rank-order correlation analysis, $r_s = 0.91$, $P = 0.0001$).

in a significant increase in probability of paternity for second males in longer copulations (Fig. 3; $r_s = 0.94$, $P = 0.0001$).

DISCUSSION

Our results largely agree with our previous finding that female genotype significantly influences male paternity (Olsson et al. 1996; Olsson and Madsen 2001). An important novel finding, however, is that males in different mating roles (first or last) respond to different cues when making tactical ejaculation decisions. In first males, with no information on the presence of rivals or their genotypes, higher female fecundity elicits longer copulations, which is in agreement with recent work confirming the influence of sperm depletion on both male and female mating tactics (Wedell et al. 2002). Second males, however, with information on prior male presence, increase their probability of paternity by prolonging copula duration when a first male is more closely related with his female and thus his fitness returns on ejaculate investments are higher. This seems to suggest that males not only invest in ejaculates in relation to potential costs of sperm depletion, but also in relation to an immediate, facultative gain in probability of paternity when genetic stakes are in favor. This result is in accordance with a recent mathematical

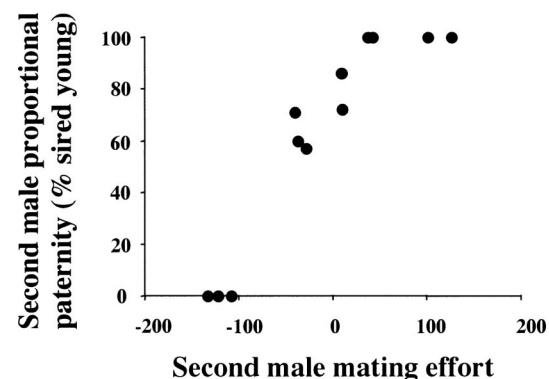


FIG. 3. In response to the prolonged matings by second males when females were more closely related with their rivals, second males increased their reproductive success significantly (Spearman's partial rank-order correlation analysis, $r_s = 0.94$, $P = 0.0001$).

model (published subsequently to this empirical study), in which males are depicted as being in random roles (i.e., preferred by some females but not others, e.g., due to genetic compatibility), or being in constant roles (i.e., preferred or rejected by all females, as in a “good genes” situation). Our results specifically agree with the predictions from the random roles model, in which males allocate more ejaculate when in a favored rather than disfavored role.

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