

## Sperm competition in the sand lizard, *Lacerta agilis*

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**Abstract.** Sperm competition between males will occur when more than one male successfully inseminates a single female and the viable sperm from different males compete for fertilization of that female's egg(s). The fertilization success of males may depend on at least two factors: the viability of sperm over time, and whether males can prevent other males from fertilizing eggs in subsequent copulations. In this study on the sand lizard, sperm did not survive in the female reproductive tract between ovulations, and sterilized males did not lower the probability of paternity for males in a second copulation occurring within 24 h. Using a genetic marker trait and DNA fingerprinting, we confirmed that multiple inseminations of different fully fertile males led to multiple paternity among offspring. The results provide evidence suggesting that neither mating order nor time between copulations has any impact on the reproductive success of first or last males.

Parker (1970) first pointed out that sperm competition in the uterus and, or, oviduct may be an important form of competition between males. In recent years the significance of this phenomenon has been widely recognized in evolutionary biology over an array of taxa (reviews in Smith 1984; Birkhead & Hunter 1990; Birkhead & Møller 1992). Two factors that set the scene for sperm competition are multiple female copulations within reproductive cycles, and sperm storage within and, or, between reproductive cycles. In species where females copulate with more than one male, several fathers may sire offspring within the same litter; this is not uncommon in reptiles (reviews in Smith 1984; Birkhead & Møller 1992; reptile case studies: Gibson & Falls 1975; Nilson & Andrén 1982; Zweifel & Dessauer 1983; Andrén & Nilson 1987, but see Stille et al. 1986, 1987; Schwartz et al. 1989). Furthermore, squamate reptiles were early described as being capable of sperm storage (Rahn 1940; Gist & Jones 1987), facilitated by sperm tubules or similar structures in the oviducts. This enables females to produce fertile eggs in the absence of males (Cuellar 1966; Adams & Cooper 1988), but may also reinforce

competition between sperm transferred during different ovarian cycles. Thus, reptiles seem to make good model organisms for studies of sperm competition.

A number of adaptations, sometimes quite bizarre, have been acknowledged where sperm competition is believed to be the major selective agent: in a dragonfly, *Calopteryx maculata*, the male's penis is morphologically modified for scooping out sperm of previous males (Waage 1979), while tree cricket males, *Trujana hibinonis*, may lick their own penis clean of a rival's semen before transferring their own sperm (Ono et al. 1989).

Rather than being used for removing sperm transferred by other males, some traits have been suggested to block or hinder insemination in subsequent copulations. In adders, *Vipera berus*, secretions from hypertrophied kidney segments are transferred with the ejaculate (Nilson & Andrén 1982). These secretions make the smooth muscles contract in the female reproductive tract (Nilson & Andrén 1982), and thereby have been argued to lower a male's chances of a subsequent insemination (but see Stille et al. 1986, 1987). In a garter snake, *Thamnophis sirtalis* (Devine 1975, 1977) and rodents (Voss 1979), substances from different accessory glands coagulate into a plug after copulation. In the garter snake, artificial

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administration of the substances that make up the copulatory plug also decreases female receptivity (Ross & Crews 1977). In keeping with this, Devine (1975), Ross & Crews (1977) and Voss (1979) have argued that copulatory plugs have evolved as a means by which a male can prevent other males from inseminating his already mated female. Schwartz et al. (1989) tested the effectiveness of copulatory plugs in a natural population of garter snakes and found that it was surprisingly low: more than 50% of the clutches showed multiple paternity.

Leuckart (1847, cited in Voss 1979) suggested that the function of sperm plugs is rather to prevent sperm from leaking out of the female cloaca. However, this would also be in the interest of the female and cannot explain why female tree squirrels, *Sciurus*, actively remove the plug in between matings with different males (Koprowski 1992). Thus, the function(s) of copulatory plugs is still unclear, at least in some species.

In the sand lizard, *Lacerta agilis*, both sexes are promiscuous; the males temporarily defend females after copulation for on average 1.3 days (Olsson 1992, 1993a, b), but not ecological resources (Olsson 1993b). Distal kidney segments are sexually dimorphic and become hypertrophied in males during the mating season (Regamy 1935). Mucous secretions from these segments are ejected through the ureters which run parallel to the vas deferens to the cloaca, so one can manipulate the vas deferens and the ejection of semen without interfering with the transfer of renal secretions. After copulation, females sometimes have dried ejaculate in the cloaca, although it may not be formed into a well-defined plug (M. Olsson, personal observation). We therefore investigated whether (1) females store sperm between ovarian cycles, (2) multiple matings with different males within or between ovarian cycles lead to multiple paternity within a clutch, and (3) renal secretions may hinder fertilization in subsequent copulations.

## METHODS

### Sperm Storage

We first investigated whether females can store sperm between ovulations; if they do, only virgin females can be used to analyse multiple paternity and sperm competition between two given males.

Two categories of females were used: virgins ( $N=5$ ) and females that reproduced successfully the year before this study ('non-virgins',  $N=4$ ). All females were hibernated under seminatural conditions, buried in a mixture of sand and peat at  $+5-8^{\circ}\text{C}$  for 10 weeks. After hibernation both groups of females were fed commercially bred crickets ad libitum to induce ovulation.

The virgin females were mated and, after they had laid their first clutch, crickets were again offered ad libitum to induce yolking and a second ovulation. A female's reproductive frequency is determined by her nutritional status and, hence, more than one clutch is produced between two hibernation periods, when food intake is not constrained. No matings were allowed between the first and the second egg laying. Three of these five females ovulated a third time, and these females were mated between the second and the third ovulation.

The non-virgin females were simply kept after hibernation until a first clutch was laid (female sand lizards ovulate spontaneously without a prior copulation), to reveal if any surviving sperm from matings before egg laying the previous season had fertilized the eggs. To control for the possibility that some non-virgin females were sterile during the experiment, each non-virgin female was mated after laying her first clutch and before the second ovulation.

### Multiple Paternity

Multiple matings of females were performed in 1988–1989 using morphological marker traits as cues for paternity. For mating experiments performed in 1990–1991 paternity was established by DNA fingerprinting. The second mating of the female was allowed within either 1 or approximately 24 h. The two periods between copulations were used to detect mechanisms invoked by the first male that may lower the probability of paternity for the second male. Mate guarding (Olsson 1993a, b) suggests that, if such a mechanism is present, it may need time to become influential.

Males of two subspecies, *L. a. chersonensis* and *L. a. agilis*, show morphologically distinct dorsal patterns which were used to identify paternity after copulations between virgin *L. a. agilis* females and males of both subspecies. *Lacerta a. chersonensis* (of Romanian origin, G. Nilson, personal communication) have two stripes running

along the back; this colour morph is unknown from Swedish populations of the *L. a. agilis* subspecies, which have a non-uniform pattern of dots and short lines of varying complexity (Yablakov et al. 1980; M. Olsson, personal observation).

To ensure consistency in dorsal patterns between juveniles with the same father, males were first mated singly with two different females. The *L. a. chersonensis* male produced in all 18 young of both sexes, striped like the father, but with a less stringent underlying pattern. All eggs were fertilized. Brother-sister matings between these offspring yielded 56 young (from 10 clutches), which were used to evaluate the inheritance of the stripes.

The frequency of striped young was tested against the frequencies predicted for single-locus heterozygous crossings (i.e. 3:1 ratio), with the striped pattern as the dominant character. The empirical result was 15 'normal' and 41 striped young, and a chi-squared test for goodness-of-fit did not reject the null hypothesis of heterozygote crossings ( $\chi^2=0.10$ ,  $df=1$ ,  $P>0.8$ ). The Swedish males all produced young with a complex dorsal pattern like their fathers.

To establish paternity using DNA fingerprinting, we sampled blood from the corner of the mouth (v. *angularis*). The blood was transferred to Eppendorf tubes with SSC buffer (0.15 M NaCl, 0.15 mM trisodium citrate, 0.5 mM EDTA pH 7.0) and stored at  $-70^\circ\text{C}$ . Genomic DNA was extracted according to Mullenbach et al. (1989), precipitated with ethanol and dissolved in sterile 0.4–1.5 ml 0.01 M Tris-HCl, pH 8.0 for at least 24 h. DNA was digested with 30 units of Alu I for 4 h at  $37^\circ\text{C}$ , extracted once with phenol/chloroform, once with chloroform and precipitated with ethanol. The digested DNA was dissolved in 25  $\mu\text{l}$  0.01 M Tris-HCl, pH 8.0. DNA fragments (6  $\mu\text{g}$ ) were separated in 0.8% agarose gels for 1680 volt-hours and transferred to Pall Biodyne A transfer membranes by Southern Blotting.

The insert of human satellite clone 33.15 (Jeffreys et al. 1985) was isolated by preparative restriction enzyme digestion and electrophoresis in low melting temperature agarose. We labelled 50 ng of probe DNA with  $^{32}\text{P}$ -dCTP by the random primer method (Feinberg & Vogelstein 1983) and 250 ng of the synthetic (TC)<sub>n</sub> polydinucleotide (Pharmacia LKB Biotechnology, S-751 82, Uppsala, Sweden) by standard nick trans-

lation (Promega). Prehybridization and hybridization with 33.15 was performed according to Georges et al. (1988) and hybridization with (TC)<sub>n</sub> was performed according to Ellegren (1991). Membranes hybridized with 33.15 were washed  $2 \times 15$  min in  $2 \times \text{SSC}$ , 0.1% SDS at room temperature,  $2 \times 15$  min in  $2 \times \text{SSC}$ , 0.1% SDS at  $58^\circ\text{C}$  and autoradiographed at  $-70^\circ\text{C}$  for 1–6 days using Kodak X-omat AR and intensifying screens. For the (TC)<sub>n</sub> probe the stringency wash was performed  $2 \times 15$  min in 0.1% SSC, 0.1% SDS at  $55^\circ\text{C}$ . Most membranes were subjected to different exposure times to visualize bands of different intensities. The DNA probes were removed from membranes by washing in 0.4 M NaOH and 0.2 M Tris-HCl, pH 7.5 and checked for remaining radioactivity.

To investigate paternity bands >3–4 kb were used. An offspring was considered sired by a certain male if the offspring had at least one male-specific band (a band not shared by the female or the other male). The probability of occurrence of a mutant band with the same length as the male-specific band is negligible. No offspring showed bands specific for both males. In the statistical analysis of paternity experiments performed in 1988–1989 (morphological traits as marker) and 1990–1991 (using DNA fingerprinting) data are analysed and presented (Table I) in a compiled form, as we regard the two methods as equally reliable in determining paternity.

### Renal Secretions and Sperm Competition

We next performed experiments to evaluate whether males transfer substances during copulation that lower the probability of paternity for males in subsequent copulations. Five males were vasectomized after being anaesthetized by injecting 2.7 ml Brietal/kg body weight in the thigh. The lizards became anaesthetized within 5–10 min after the injection and remained unconscious for 30–45 min. The dermis was sterilized with 70% alcohol and then a laparoscopy was performed under a stereoscope. The vas deferens were ligated with catgut and surgically cut. The ventral incision was 10–15 mm long and after the laparoscopy was closed with 'butterflies'. Two males were sham-operated to see whether the operation per se would affect the ability to fertilize eggs. After the operation the lizards were kept in sterile cages measuring  $80 \times 50 \times 60$  cm

Table I. Multiple copulations of females with one Romanian male (H) and different Swedish males (S1–S22)

Male 1	Male 2	Time between copulations (h and min)	Young sired by		Undetermined paternity
			M1	M2	
<b>Short inter-copulation interval</b>					
S11	S12	18 min	1 (11)	8 (89)	2
S5	H	19 min	2 (50)	2 (50)	0
H	S4	21 min	0 (0)	7 (100)	0
H	S6	25 min	2 (40)	3 (60)	0
H	S7	25 min	7 (78)	2 (22)	1
S9	S10	25 min	5 (56)	4 (44)	0
S15	S16	33 min	4 (80)	1 (20)	1
S1	H	36 min	9 (100)	0 (0)	0
S17	S2	1 h 25 min	0 (0)	11 (100)	0
S18	S19	1 h 25 min	4 (50)	4 (50)	0
S1	S22	1 h 30 min	4 (100)	0 (0)	2
S13	S6	2 h	3 (60)	2 (40)	5
Total			41 (43)	44 (46)	11 (12)
<b>Long inter-copulation interval</b>					
S20	S12	18 h 28 min	2 (29)	5 (71)	1
S12	S2	20 h 20 min	7 (100)	0 (0)	2
H	S3	21 h 25 min	0 (0)	7 (100)	3
S1	H	21 h 45 min	0 (0)	9 (100)	3
S5	H	22 h 0 min	8 (100)	0 (0)	0
S2	H	23 h 24 min	3 (60)	2 (40)	0
S2	S21	23 h 58 min	9 (82)	2 (18)	0
S1	H	24 h 24 min	0 (0)	12 (100)	0
Total			29 (40)	37 (50)	7 (10)

The absolute number of young sired by male 1 (M1) and male 2 (M2) are given as well as the percentage of sired young (within parentheses). The sum of unhatched eggs, and dead young for which paternity could not be determined, are given under 'Undetermined paternity'.

(width × depth × height) with filter paper as floor cover. The filter paper was changed daily.

No lizard showed any ill-effects of the anaesthesia, some of them fed only a few hours after the operation and they all did so within 24 h. All operated males recovered completely, no infections occurred and the incisions were healed after approximately 2 weeks.

Once males had fully recovered from the laparoscopy, we confirmed that they were sterile by mating them with receptive (and yolking) females, and by monitoring fertilization of the eggs. Control matings of the sham-operated males revealed that they were able to copulate and were fertile. The sterilized males successfully mated with 10 females, which were subsequently remated with fully fertile males (confirmed by single matings with other females). Eight of the second copulations took place after 22 h while two were within half an hour of the first copulation (to avoid the

possible loss of female receptivity prior to a second copulation).

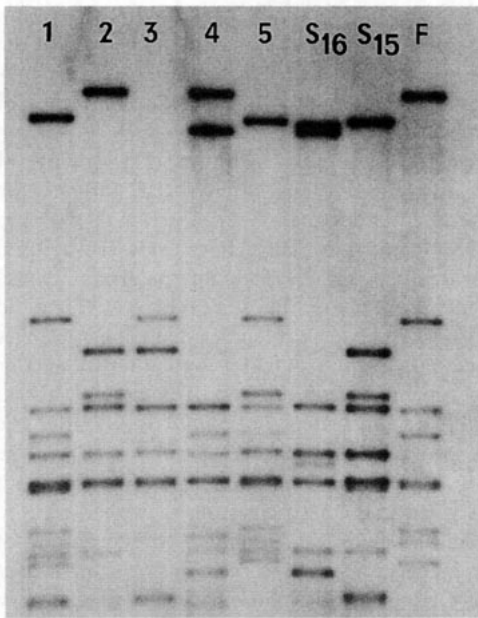
## RESULTS

### Sperm Storage

The virgin females all produced fertilized first clutches; their second clutch, with no additional mating before the second ovulation, were all unfertilized. The three females that were mated again before a third ovulation produced fertile eggs and young sired only by the last male.

The non-virgin females ( $N=4$ ) used to test for sperm storage between reproductive seasons all laid a non-fertilized first clutch, but produced a second clutch of fertilized eggs when mated before the second ovulation; thus, they were all fertile.

Thus, among the nine females that were used in these experiments none could be demonstrated to



**Figure 1.** DNA fingerprint of a female sand lizard (F), her five offspring (1–5) and two potential fathers (S15 and S16) that were used in the experiment with multiple matings. Offspring 4 was sired by male S16 and the other four offspring were sired by male S15.

store functional sperm between ovary cycles, either between years or between cycles within the same year.

### Multiple Paternity

Table I summarizes the results from experiments with multiple matings of females where the second mating of the female was allowed within approximately either 1 or 24 h. In 11 of the 20 clutches examined, there was mixed paternity, unambiguously showing that multiple paternity was possible after copulations with different males (Fig. 1). There was no significant difference in the probability of mixed paternity between the two time intervals separating copulations (Fisher's exact test,  $P > 0.36$ ); however, 75% of the litters in the 1-h group had multiple paternity and only 38% in the 24-h group, which may suggest an interval effect although the sample size is low.

The litters with single male paternity were not significantly biased against either male: four clutches were sired by the first male and five by the second. More interesting was that the probability

of paternity of the second male did not decrease with a longer time between copulations (chi-squared test,  $\chi^2 = 0.007$ ,  $df = 1$ ,  $P > 0.98$ , Table I). We conclude that, when more than one male inseminates a female, mixed paternity is possible and sperm competition may still operate when copulations are 24 h apart.

### Renal Secretions and Sperm Competition

The five vasectomized males successfully mated with 10 females, which were subsequently remated with fully fertile males. One of these laid an unfertilized clutch and one laid 10 fertilized eggs but also one unfertilized egg. The remaining females laid only fertilized eggs. The total number of eggs was 87, which yield a hatching success of 87%, even higher than the average for captive females (77%, M. Olsson, personal observation). Thus, nothing seems to be transferred from the ureter during a copulation that prevents fertilization in a second copulation within 24 h.

## DISCUSSION

Sand lizard females did not store functional sperm between ovulations, either between or within seasons. We also showed that the mucous secretions produced during the mating season from the sexually dimorphic male kidneys (Regamy 1935) do not interfere with sperm transfer in subsequent copulations. An alternative hypothesis to the function of renal secretions in male competition is that the ejaculate is a resource for developing young; however, there is no relationship between number of repeat matings with the same male and hatching success, clutch size or offspring mass in sand lizards (M. Olsson, personal observations). A third hypothesis that may explain the evolution of renal secretions is that they act as a medium for successful transfer of sperm; however, this would be difficult to test experimentally as the ureter cannot be surgically cut like the vas deferens. In mammals, accessory glands, like the prostate, produce plasma that constitutes a significant part of the seminal fluid (Price & Williams-Ashman 1961), which possibly acts as a vector for sperm.

With our experimental design we cannot establish for how long functional sperm can be stored within an ovary cycle, as we do not know the exact time of the ovulation and fertilization.

However, Rudeberg (1955) gave a detailed description of mating behaviour and embryological development in a small population of sand lizards kept in an outdoor terrarium in south Sweden. He dissected eggs from the same clutch (all eggs are laid at the same time in sand lizards), at different stages of development, and described the embryonic development of the siblings. We fitted his data on embryo snout-vent lengths to the corresponding dates of development in a regression model and backcalculated when embryo snout-vent length would have been zero (this relationship can be described by the function  $Y=1.55X-9.64$ ,  $N=6$ ,  $P<0.0001$ ). This estimate, although gross, suggested that the zygotes were formed 6 days before oviposition (11 and 17 June, respectively). The matings in Rudeberg's study took place on 8 and 9 May. This suggests that enough sperm survive for at least a month in the female reproductive tract within an ovary cycle to ensure fertilization of an entire clutch. However, how large a proportion this is of the sperm in the ejaculate is not known.

Mating order appeared to have no influence on the probability of paternity in the sand lizard (although this result should be interpreted with caution as our sample size is low). A similar observation has been made in a rodent, *Peromyscus maniculatus* (Dewsbury & Baumgardner 1981). However, contrasting patterns have been identified even in related species, with paternity biased in favour of the first male, *Mus musculus* (Levine 1967) and the last male, *Microtus ochrogaster* (Dewsbury & Baumgardner 1981). Ginsberg & Huck (1989) reviewed mating order effects in mammals and concluded that such effects are an artefact of when inseminations occur relative to ovulation.

The probability of paternity may be influenced by the 'quality' of the sperm itself (e.g. swimming ability, longevity, meiotic drive) and 'other factors' that make a male competitive in sperm competition (e.g. number of sperm produced, quality of seminal fluid, time between copulations, copulatory plugs, copulation frequency, etc.). Martin et al. (1974), for example, demonstrated differences in the quality of sperm between males of the two races of cocks, *Gallus g. domesticus*, Columbian and Leghorn. Their experiment clearly demonstrated that the proportion of sired offspring between the races was constant (34% and 66%, respectively), irrespective of the total num-

ber of sperms inseminated; when the proportion of sperm inseminated from each male varied, the ratio of sired offspring closely matched that predicted. Unfortunately, in behavioural studies of sperm competition mechanisms, such as mating order, investigations of the sperm quality of individual males has rarely been part of the experimental design.

The Romanian male (H in Table I) was used repeatedly when performing paternity experiments using the morphological marker. To avoid confounding effects of sperm depletion no males were mated within 24 h of a copulation. While our experiments were in progress, Tokarz & Slowinski (1990) demonstrated that *Anolis sagrei* alter the use of the right and left hemipenis when the interval between copulations is 24 h or less, but not when it is 72 h suggesting they need time to replenish sperm. No significant alternation of hemipenis use was observed in the sand lizards (M. Olsson, personal observation).

The time between copulations did not have any predictable influence on a male's reproductive success and a first male advantage could not be confirmed for copulations that were 24 h apart. However, the hypothesis of a first male advantage was formulated assuming that mate guarding is a primary target for sexual selection. Another hypothesis could be that males need time to replenish sperm, for a time period equal to an average mate guarding, and that time is better invested in guarding an already mated female than in searching for a new one. If so, mate guarding would not be the main target for selection but a secondary corollary to selection, and constraints, on the replenishment of gametes. Such trade-offs in time investment between, for example, sperm production, mate search and mate guarding have been modelled with optimality theory (e.g. Parker 1974, 1978), and game theory (Parker 1990) but these models are difficult to test empirically. Our results for the sand lizard, however, provide evidence suggesting that mating order and time between copulations (within 24 h) have no impact on the reproductive success for first or last males.

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## REFERENCES

- Adams, C. S. & Cooper, W. E. 1988. Oviductal morphology and sperm storage in the keeled earless lizard, *Holbrookia propinqua*. *Herpetologica*, **44**, 190–197.
- Andrén, C. & Nilson, G. 1987. The copulatory plug: does it keep sperm in or out. *Oikos*, **49**, 230–239.
- Birkhead, T. R. & Hunter, F. M. 1990. Mechanisms of sperm competition. *Trends Ecol. Evol.*, **5**, 48–52.
- Birkhead, T. R. & Møller, A. P. 1992. *Sperm Competition in Birds: Evolutionary Causes and Consequences*. London: Academic Press.
- Cuellar, O. 1966. Oviductal anatomy and sperm storage structures in lizards. *J. Morphol.*, **119**, 7–20.
- Devine, M. 1975. Copulatory plugs in snakes: enforced chastity. *Science*, **187**, 844–845.
- Devine, M. 1977. Copulatory plugs, restricted mating opportunities and reproductive competition among male garter snakes. *Nature, Lond.*, **267**, 345–346.
- Dewsbury, D. A. & Baumgardner, D. J. 1981. Studies of sperm competition in two species of murid rodents. *Behav. Ecol. Sociobiol.*, **9**, 121–133.
- Ellegren, H. 1991. Fingerprinting birds' DNA with a synthetic polynucleotide probe (TG)<sub>n</sub>. *Auk*, **108**, 956–958.
- Feinberg, A. P. & Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.*, **132**, 6–13.
- Georges, M., Lequarré, A.-S., Castelli, M., Hanset, R. & Vassart, G. 1988. DNA fingerprinting in domestic animals using four different minisatellite probes. *Cytogenet. Cell Genet.*, **47**, 127–131.
- Gibson, A. R. & Falls, J. B. 1975. Evidence for multiple insemination in the garter snake, *Thamnophis sirtalis*. *Can. J. Zool.*, **53**, 1362–1368.
- Ginsberg, J. & Huck, U. 1989. Sperm competition in mammals. *Trends Ecol. Evol.*, **4**, 74–79.
- Gist, D. & Jones, J. M. 1987. Sperm storage in the reptilian oviduct. *Scan. Microscopy*, **1**, 1839–1849.
- Jeffreys, A. J., Wilson, V. & Thein, S. L. 1985. Hyper-variable 'minisatellite' regions in human DNA. *Nature, Lond.*, **314**, 67–73.
- Koprowski, J. L. 1992. Removal of copulatory plugs by female tree squirrels. *J. Mammal.*, **73**, 572–576.
- Levine, L. 1967. Sexual selection in mice. IV. Experimental demonstration of selective fertilization. *Am. Nat.*, **101**, 289–294.
- Martin, P. A., Reimers, T. J., Lodge, R. & Dziuk, P. J. 1974. The effects of ratios and numbers of spermatozoa mixed from two males on proportions of offspring. *J. Reprod. Fert.*, **39**, 251–258.
- Mullenbach, R., Lagoda, P. J. L. & Welter, C. 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Gen.*, **5**, 391.
- Nilson, G. & Andrén, C. 1982. Function of the renal secretion and male hierarchy in the adder, *Vipera berus*, during reproduction. *Horm. Behav.*, **16**, 404–413.
- Olsson, M. 1992. Sexual selection and reproductive strategies in the sand lizard, *Lacerta agilis*. Ph.D. thesis, University of Göteborg, Sweden.
- Olsson, M. 1993a. Male preference for large females and assortative mating for body size in the sand lizard, *Lacerta agilis*. *Behav. Ecol. Sociobiol.*, **32**, 337–341.
- Olsson, M. 1993b. Contest success and mate guarding in male sand lizards, *Lacerta agilis*. *Anim. Behav.*, **46**, 408–409.
- Ono, T., Siva Jothy, M. T. & Kato, A. 1989. Removal and subsequent ingestion of rival's semen during copulation in a tree cricket. *Physiol. Entomol.*, **14**, 195–202.
- Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.*, **45**, 525–567.
- Parker, G. A. 1974. The reproductive behavior and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). IX. Spatial distribution of fertilization rates and the evolution of male search strategy within the reproductive area. *Evolution*, **28**, 93–108.
- Parker, G. A. 1978. Evolution of competitive mate searching. *Ann. Rev. Entomol.*, **23**, 173–196.
- Parker, G. A. 1990. Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. Lond. B.*, **242**, 127–133.
- Price, D. & Williams-Ashman, H. G. 1961. The accessory reproductive glands of mammals. In: *Sex and Internal Secretions, Vol. 1*. 3rd edn (Ed. by W. C. Young), pp. 366–448. Baltimore: Williams and Wilkins.
- Rahn, H. 1940. Sperm viability in the uterus of the garter snake, *Thamnophis*. *Copeia*, **4**, 109–115.
- Regamy, J. 1935. Les caractères sexuels du lézard (*Lacerta agilis*). *Rev. Suisse Zool.*, **42**, 89–117.
- Ross, P. Jr & Crews, D. 1977. Influence of the seminal plug on mating behaviour in the garter snake. *Nature, Lond.*, **267**, 344–345.
- Rudeberg, S. 1955. Studier över sandödlan sommaren 1954 med speciell inriktning på artens parning och embryologi (in Swedish). *Fauna Flora*, **50**, 83–107.
- Schwartz, J. M., McCracken, G. F. & Burghardt, G. M. 1989. Multiple paternity in wild populations of the garter snake, *Thamnophis sirtalis*. *Behav. Ecol. Sociobiol.*, **25**, 269–273.
- Smith, R. L. 1984. *Sperm Competition and the Evolution of Animal Mating Systems*. Orlando: Academic Press.
- Stille, B., Madsen, T. & Niklasson, M. 1986. Multiple paternity in the adder, *Vipera berus*. *Oikos*, **47**, 173–175.

- Stille, B., Niklasson, M. & Madsen, T. 1987. Within season multiple paternity in the adder, *Vipera berus*. *Oikos*, **49**, 232–233.
- Tokarz, R. R. & Slowinski, J. B. 1990. Alternation of hemipenis use as a behavioural means of increasing sperm transfer in the lizard *Anolis sagrei*. *Anim. Behav.*, **40**, 374–379.
- Voss, R. 1979. Male accessory glands and the evolution of copulatory plugs in rodents. *Occas. Papers Museum Zool. Univ. Michigan*, **689**, 1–27.
- Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science*, **203**, 916–918.
- Yablakov, V. A., Baranov, S. A. & Rozanov, S. A. 1980. Population structure, geographic variation, and microphylogenesis of the sand lizard, *Lacerta agilis*. *Evol. Biol.*, **12**, 92–127.
- Zweifel, R. G. & Dessauer, H. C. 1983. Multiple insemination demonstrated in the kingsnake *Lampropeltis getulus*. *Experientia*, **39**, 317–319.