Behav Ecol Sociobiol (1993) 32:313–319

Behavioral Ecology and Sociobiology

Females influence sperm storage and use in the yellow dung fly *Scathophaga stercoraria* (L.)

Paul I. Ward

Zoologisches Museum, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received July 27, 1992 / Accepted December 19, 1992

Summary. The influence of the female on the process of sperm storage and use was examined. Copula duration, the condition of the female and whether or not a copula terminated naturally influenced the number of spermathecae (of three) in which once-mated females stored sperm. Females stored more sperm the larger their mate and the sperm from larger males were stored more unevenly amongst the spermathecae than were those from smaller males. Double-mated females had sperm in fewer spermathecae the larger the second of their mates and these spermathecae tended to be the ones which lay together within the female. The P2 values over three successive clutches were constant and sperm precedence was complete when the larger male was second to mate but began low and increased over subsequent clutches when the smaller male mated second. These results suggest females prefer, and are able, to use the sperm of larger males to fertilise their eggs. It is proposed that multiple spermathecae in Diptera have evolved to give females better control over offspring paternity.

Introduction

Sperm competition is common in insects and has influenced the evolution of many mating systems and adaptations (Parker 1970a, 1984; Smith 1984). In many situations there will be a conflict between males and females over mating and subsequent events (Parker 1979). Selection for a male to increase the proportion of a female's eggs his sperm will fertilise after mating is expected to be stronger than selection for a female to resist either mating or the use by males of adaptations, such as sperm plugs, which appear designed to increase the success of the male's sperm. This is because the issue for males is whether they produce offspring or not, whereas for females the issue may be only a matter of offspring quality (Parker 1984). However, as fertilisation usually takes place within a female, she may have control over the detailed movements of sperm and eggs and could possibly influence sperm use after the male has no further opportunity to do so. This makes it unclear how an evolutionary conflict between the sexes will be resolved (Parker 1984; Knowlton and Greenwell 1984).

The mechanisms of sperm competition are poorly known but it is clear that females can influence the movements of sperm within their sperm storage organs (see Birkhead and Hunter 1990; Eberhard 1985, 1990). For example, the distribution of sperm within female *Dryomyza anilis* is influenced by interactions between the male and the female after copula is complete and this could influence sperm precedence, though the precise mechanism is still unclear (Otronen and Siva-Jothy 1991).

Sperm competition in the yellow dung fly *Scathophaga stercoraria* (L.) (often *Scatophaga*, see Kloet and Hincks 1975) has been the subject of detailed investigation by Parker and co-workers since Parker's pioneering work (see Parker 1978 for a summary of the early work; Parker et al. 1990; Ward and Simmons 1991; Parker 1992; Parker and Simmons 1991; Simmons and Parker 1992). This work has concentrated on comparing an observed copula duration in the field of 35 min with the optimal copula duration a male should show with a particular female to maximise his reproductive success. Reproductive success of males which have mated with non-virgin females has usually been measured in the laboratory by estimating the P2 value, which is the proportion of eggs laid by the female fertilised by the second male to mate with her.

The classical method used to double-mate females experimentally is to allow the second male to copulate shortly after the first male has completed mating. This method does not give the female much opportunity to influence the storage of the first ejaculate before the second male can also influence its fate, probably by flushing it out of the female with his own fluid (Parker and Simmons 1991). However, females can store sperm for long periods and later matings do not always follow soon after previous ones. The natural time between matings for a female will normally be the time it takes to

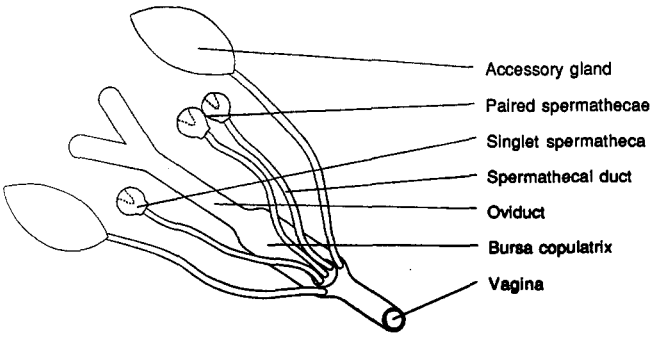

Fig. 1. The female internal genital morphology

develop a clutch, which is around a week in the laboratory. Thus, females have the opportunity to influence the storage and use of sperm and this could go undetected by the classical method.

The aim of this paper is to show that females can influence the process of sperm storage and later use. The methods employed allow the female a greater degree of control over sperm movements than the classical method. The object is to detect non-randomness in sperm storage and use with respect to a female's mates. The main male characters investigated were the mating order and body size, a character known to have a large influence on this species' mating system (Borgia 1979; Sigurjonsdottir and Parker 1981) and to be partially heritable (Simmons and Ward 1991).

The female sperm storage system normally consists of a bursa copulatrix and three spermathecae (Fig. 1), though there is some natural variation in this morphology (unpublished data). Each spermatheca has its own duct with two lying on one side of the oviduct and one "singlet" spermatheca on its own on the other side. The three ducts are attached close together to a fold on the dorsal wall of the bursa. The fold appears to be somewhat extensible. The male aedeagus is too large to enter the ducts and appears to have no extensible parts. Sperm are therefore probably deposited in the bursa and males can presumably only directly influence rival sperm only here, as also seems to be the case for *Dryomyza anilis* (Otronen and Siva-Jothy 1991).

Materials and methods

Flies used in the experiments were derived from adults collected in a meadow near Fehraltorf, Switzerland. Generally, methods followed those used by Ward and Simmons (1991). Females were judged gravid on the basis of the extension of the abdomen; with a full batch of eggs ready to lay the intersegmental membranes are very stretched. Batches of dung were made for each experiment. The dung from several cows was collected fresh and thoroughly mixed before being divided into sub-batches. All sub-batches were frozen, and defrosted and used as required during experiments.

Females were allowed to lay eggs in small portions of dung. The eggs were then gently removed from the dung and placed on moist filter paper with a very small portion of dung in small Petri dishes. Eggs were incubated at room temperature and hatched within 24 h. Freshly-hatched larvae crawled into the dung where they could feed and remain moist until they were transferred to the rearing bottles, always on the day of hatching. Larvae were transferred in groups of up to five into approximately 10 g of dung and incubated at 19° C until adults emerged, after around 3 weeks. This was an adequate amount of dung to ensure high survival. Adults were maintained in isolation on sugar, water and *Drosophila* or *Musca* ad lib until sexual maturity, about 6 weeks. Flies to be dissected were fully anaesthetised with CO_2 before the head and thorax were crushed.

Copulae were conducted by placing a small portion of dung in a bottle, adding a male and then a female. The flies were observed continuously for 1 h and copula duration taken as the duration of genital contact. Flies which did not copulae within this time were discarded. The dung portions and males were then removed and the females kept isolated overnight. If a female was to be double-mated, she was put in a bottle containing a small dung portion and the second male added the following day. The female was then again isolated overnight. The following day the female was offered a small portion of dung in which to oviposit. The rationale behind this procedure is that it exaggerates the effects of a female's ability to control the fate of the sperm she contains.

Factors affecting sperm storage and the hatching success of eggs. Virgin females were mated with randomly-chosen males. Copulae were interrupted at 1 min, 5 min or 15 min, or allowed to be completed normally. The following day the females were offered dung in which they could oviposit. After 3 h, the dung portions were removed and the females were dissected the same day. Each spermatheca was examined for the presence or absence of sperm. The hatching successes of the clutches were noted the following day.

In the next experiment, virgin females were each mated for 20 min with a male, where the males varied in body size (measured as the length of a hind tibia). Hatching successes were again noted.

Sperm numbers in once- and double-mated females. Virgin females were each mated with a randomly-chosen male for 20 min; the males varied in hind tibia length. Once-mated females were dissected the day after mating and double-mated females the day after their second mating. The three spermathecae were gently separated and placed individually in wells in a depression dish containing 0.05 ml of insect Ringer solution. Each spermatheca was crushed with a glass rod and the mixture mixed for 1 min. The depression was observed under $\times 16$ magnification to ensure the spermathecae were completely crushed. The mixture was mixed with a Pasteur pipette for a further 1 min before sub-samples were pipetted onto a cell counter. The numbers of sperm were counted under $\times 400$ magnification in four sub-samples and the total number of sperm in each spermatheca calculated from the dilution factor.

The numbers of sperm in the double-mated females was analysed by using the logarithms of the total numbers of sperm, the numbers of spermathecae containing sperm and the arcsin square root transformed proportions of sperm in the singlet spermatheca as dependent variables; the sizes of the three animals, the female and the two males, as covariates; and whether the second male was larger or smaller than the first male as a factor. The influence of the covariates was considered first to remove the effect of the males' absolute sizes before the effect of their relative sizes was considered.

The use of stored sperm. Laboratory lines of flies were bred which were homozygous for different allelles of the enzyme phosphoglucomutase (PGM). One line was homozygous for the allele most common in field collections in Switzerland and the other for the next most common variant (unpublished data). Flies were raised in different amounts of dung so that within each line the males varied in size. Virgin females were mated for 20 min with a male from each line, where the males differed in body size.

The females were then maintained and allowed to lay three clutches, with around a week between clutches. This allowed me to follow the use of sperm over several clutches. Only data from females which laid three clutches were used. The small data set

only allowed the relative sizes of the males to be examined and not their absolute sizes.

All hatching larvae were placed in dung. When the adult offspring emerged, they were fed for 1 day on sugar and *Drosophila* ad lib before being killed. Their PGM genotype was determined by agarose electrophoresis (modified after Harris and Hopkinson 1978), allowing unambiguous identification of paternity.

The proportion of eggs which hatched was arcsin square root transformed before analysis, as recommended for proportions (Sokal and Rohlf 1981). The P2 values were similarly transformed.

Results

Factors affecting sperm storage and the hatching success of eggs

Twenty females laid eggs but 26 did not, even though they had been judged to be mature. This result was used to classify the females into those which really were gravid and those which probably required a few more days to become so. The numbers of spermathecae containing sperm were analysed by analysis of variance, with whether or not the female had laid eggs and whether or not the copula ended naturally as factors and copula duration as a covariate. The effect of copula duration was highly significant and the raw regression coefficient positive (slope = 0.051, $F_{1,41} = 28.83$, $P < 0.001$), i.e. the longer a female copulated the more spermathecae subsequently contained sperm. The female's laying status and the type of copula ending were also significant ($F_{1,41} = 4.94$, $P = 0.01$ and $F_{1,41} = 5.42$, $P < 0.05$, respectively). Females which laid eggs had sperm in more spermathecae than those which did not lay (means of 1.25 and 0.50 spermathecae respectively; Fig. 2). Females had sperm in more spermathecae when the copula had ended naturally than when it had been interrupted (means of 0.95 and 0.72 spermathecae respectively). As spermathecae were examined after some sperm had been used, these effects may be underestimates. As copulae as short as 5 min normally result in hatching of over 95% of eggs laid immediately (Parker et al. 1990), it is extremely unlikely that the non-laying females had received insufficient sperm to stimulate oviposition. The hatching success of a female's clutch was positively related to how long she had copulated ($y = 0.19\,x + 0.070$, $F_{1,18} = 8.09$, $P < 0.02$).

In the next experiment, where all 18 copulae were of 20 min, there was a significant positive regression between the size of a female's mate and the hatching success of her subsequent clutch ($y = 0.041\,x - 1.10$, $F_{1,16} = 10.95$, $P < 0.01$). This is probably because the larger males transferred more sperm (Simmons and Parker 1991; and see below).

Sperm numbers in once- and double-mated females

In 29 once-mated females, the logarithms of the total numbers of sperm stored were related to the number of spermathecae which contained sperm (analysed as a factor) ($F_{2,25} = 28.20$, $P < 0.001$) and the size of the female's mate (analysed as a covariate) ($F_{1,25} = 5.76$, $P < 0.03$). Figure 3 shows that if a female stored sperm in more spermathecae, she stored more sperm in total and that more sperm were stored as the male's size increased (raw regression coefficient = 0.593).

The dispersion of sperm over the three spermathecae was examined by calculating the index of dispersion, the variance to mean ratio (Sokal and Rohlf 1981), using the numbers of sperm observed in each spermatheca. There was a significant positive relationship between the index of dispersion and the female's mate's hind tibia length (Kendall's tau = 0.278, $P < 0.04$; Fig. 4), showing

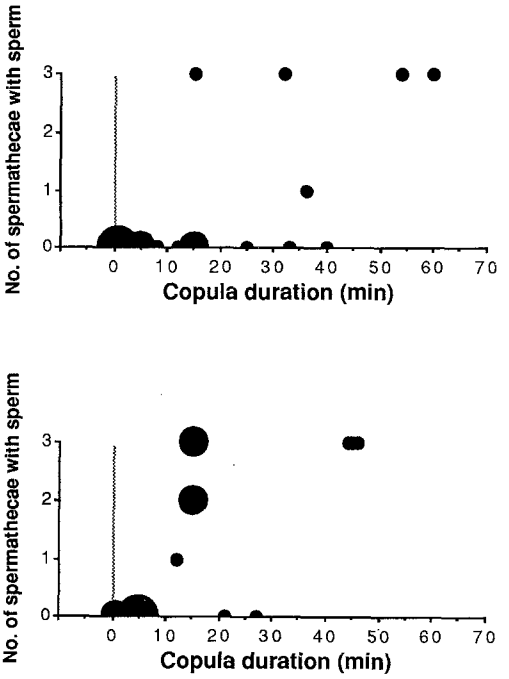

Fig. 2. The storage of sperm in spermathecae with respect to copula duration and female condition. *Upper graph*: females that did not lay eggs; *lower graph* females that did. *Larger circles* reflect the degree of overlap in the data points

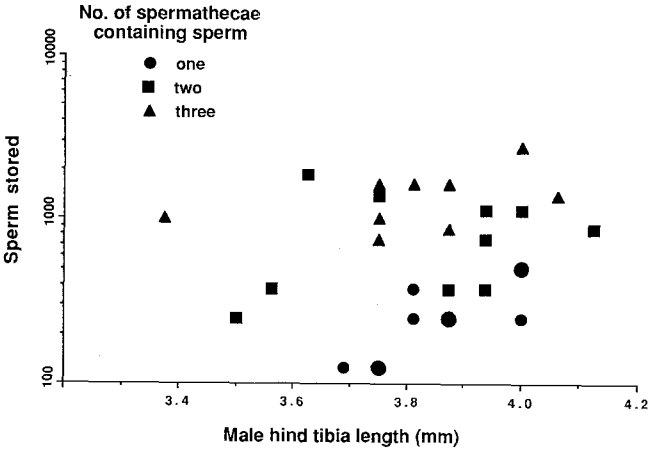

Fig. 3. The numbers of sperm stored with respect to the size of the female's mate and the number of spermathecae containing sperm (*circles*, one; *squares*, two; *triangles*, three). *Larger circles* reflect the degree of overlap in the data points

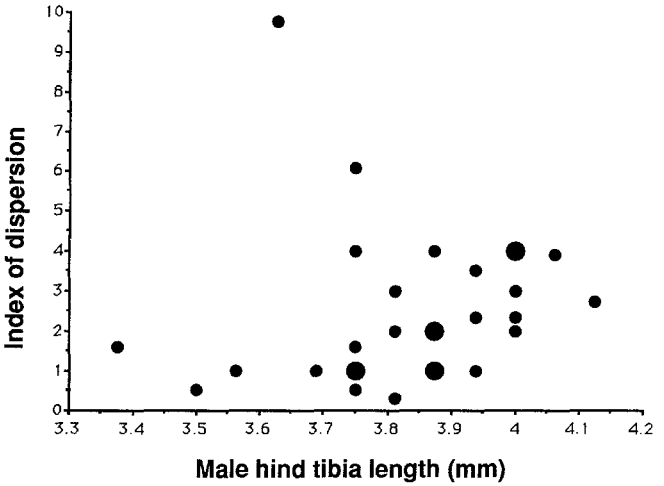

Fig. 4. The degree of clumping of sperm stored from males of different sizes. *Larger circles* reflect the degree of overlap in the data points

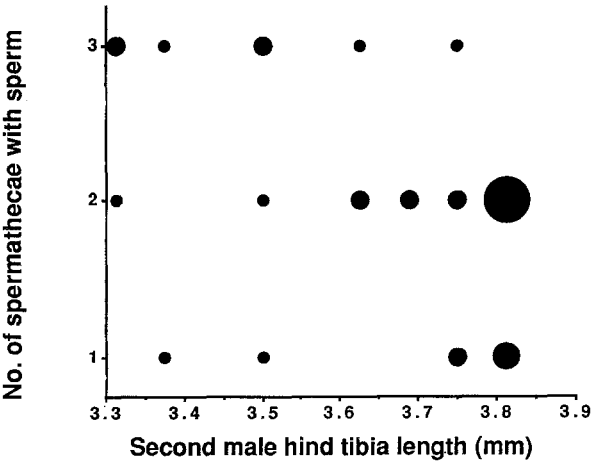

Fig. 5. The relationship between the size of a female's second mate and the number of spermathecae containing sperm after two matings. *Larger circles* reflect the degree of overlap in the data points

Table 1. The analysis of variance results for (a) the log of the total number of sperm stored, (b) the number of spermathecae containing sperm and (c) the proportion of sperm in the singlet spermathecae for females mated twice

	Source of variation	*df*	MS	*F*	*P*
(a) Total sperm	Female size	1	0.36	2.17	0.16
	First male size	1	0.05	0.28	0.60
	Second male size	1	0.11	0.64	0.43
	Class	1	0.34	2.04	0.17
	Error	22	0.17		
(b) Spermathecae	Female size	1	1.63	5.70	0.03
	First male size	1	0.25	0.87	0.36
	Second male size	1	3.25	11.36	<0.01
	Class	1	3.17	11.06	<0.01
	Error	22	0.29		
(c) Proportion in singlet	Female size	1	0.37	1.81	0.19
	First male size	1	0.02	0.09	0.77
	Second male size	1	1.42	6.93	0.02
	Class	1	0.15	0.71	0.41
	Error	22	0.21		

The factor class refers to whether the second mate was smaller or larger than the first. Sizes were measured as hind tibia length (mm)

that the sperm of larger males were stored more unevenly amongst the spermatecae than those of smaller males. There was no pattern to the proportion of sperm in the singlet spermatheca.

In 27 double-mated females, there were no significant influences on the logs of the total numbers of sperm stored 1 day after the second mating (Table 1a). The numbers of spermathecae containing sperm were significantly influenced by the size of the female, the size of the second male and the relative sizes of the two males; the size of the first male was not significant (Table 1b). The larger the female, the more spermathecae contained sperm (raw regression coefficient = 1.77). The larger the second male, the *fewer* spermathecae contained sperm (Fig. 5; raw regression coefficient = −2.00). Finally, when the second male was the smaller mate, *more* spermathecae contained sperm than when the second male was the larger (means of 2.33 and 1.73 spermathecae respectively).

The proportion of sperm in the singlet spermatheca was significantly influenced only by the size of the second male (Table 1c). The larger the second male the smaller the proportion of sperm in the singlet spermathecae (Fig. 6; raw regression coefficient = −1.32).

The use of stored sperm

The numbers of eggs, hatching success and P2 from six females were analysed using repeated-measures analysis of variance, as appropriate when each female provided three clutches. The numbers of eggs laid were not affected by clutch number or the relative sizes of the males (Table 2a). The overall mean clutch size was 46.39 eggs.

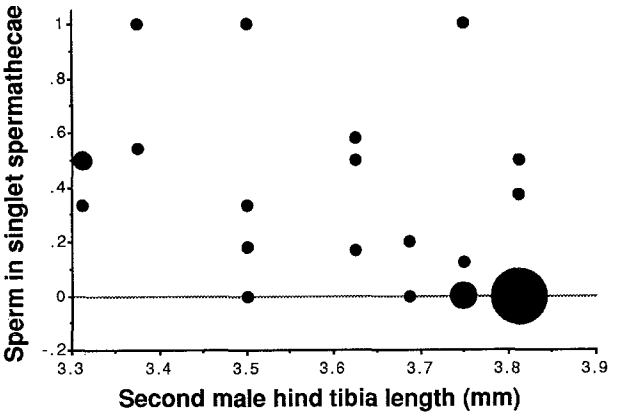

Fig. 6. The proportion of sperm in the singlet spermatheca (transformed) and the size of the female's second mate. *Larger circles* reflect the degree of overlap in the data points

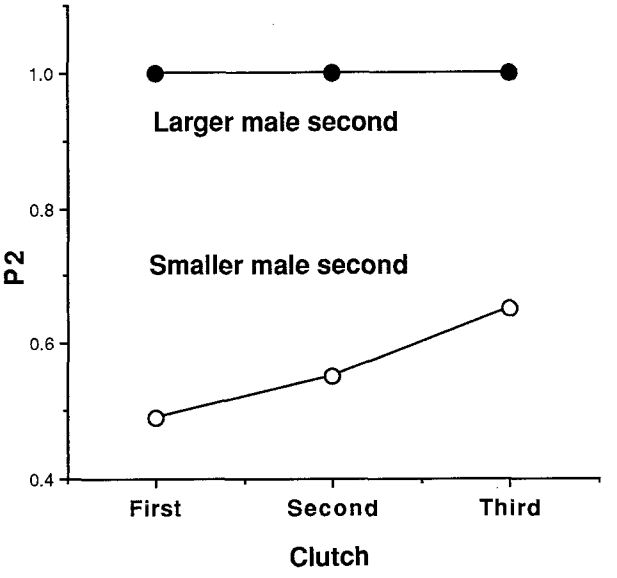

Fig. 7. The P2 values for first to third clutches according to the relative sizes of the mating males

The proportion of eggs hatching was influenced by the relative sizes of the males but not by the clutch number (Table 2b). When the second male was the smaller mate, more eggs hatched than when the second male was the larger (mean proportions of 0.83 and 0.38 respectively). The interaction of clutch number and relative size of males was significant for the transformed P2 values (Table 2c), showing both factors were important. When the second male was larger, all the offspring were from him but when the second male was smaller the P2 value increased with clutch number (Fig. 7).

Discussion

Larger male *S. stercoraria* transfer more sperm to a female in a 20-min copula than do smaller males (Simmons and Parker 1992). Females are here shown to store more sperm after mating with larger males, which could be a non-selective process but the storage is likely to be under female control for two reasons. Firstly, the male is no longer present and could only indirectly control storage by the placement of his sperm. However, the exits to the spermathecal ducts lie very close together, especially compared to the size of the bursa. Secondly, the spermathecal duct musculature is clearly under female control. Female control of the storage process is also indicated by female condition influencing sperm storage and the sperm from larger males being stored more unevenly amongst the spermathecae than those from smaller males, as all males will have the same interests. Furthermore, males do not alter their copula duration according to a female's size (Ward and Simmons 1991) or mating status (Parker et al. 1993). They are therefore probably unable to react to subtle differences in sperm storage caused by the female.

When females had mated twice, the number of spermathecae with sperm was smaller the larger the female's second mate; again probably because the sperm from

Table 2. The analysis of variance results for (a) the number of eggs laid, (b) the proportions which hatched and (c) the P2 values for females mated twice which laid three clutches. The factor class refers to whether the second mate was smaller or larger than the first

	Source of variation	*df*	MS	*F*	*P*
(a) Number of eggs	Class (A)	1	93.39	0.13	0.73
	Subjects within groups	4	694.89		
	Clutch number (B)	2	182.39	0.67	0.53
	AB	2	132.06	0.49	0.63
	B × subjects within groups	8	271.81		
(b) Proportion of eggs hatching	Class (A)	1	1.55	12.90	0.02
	Subjects within groups	4	0.12		
	Clutch number (B)	2	0.17	2.40	0.15
	AB	2	0.05	0.76	0.50
	B × subjects within groups	8	0.07		
(c) P2 values	Class (A)	1	1.89	4.79	0.09
	Subjects within groups	4	0.40		
	Clutch number (B)	2	0.02	5.44	0.03
	AB	2	0.02	5.44	0.03
	B × subjects within groups	8	0.003		

larger males were stored more unevenly amongst the spermathecae than those from smaller males. The sperm from larger males were also more likely to have been stored in the two spermathecae which lay together in the female. This suggests that females store sperm from more desirable mates where they can most easily be used together later. Females were thus able to influence the paternity of their offspring in successive clutches. Females whose second mate was larger than their first had fewer eggs hatch but all of these were fathered by the larger male. Females where the smaller male was second had better hatching success and appear to have used progressively more sperm from the smaller male, possibly as they ran out of sperm from the larger male.

I suggest that Parker and Simmons (1991) have investigated events occurring in the bursa during and immediately after copula followed by immediate oviposition. This would also explain Parker's (1970b) main result, that P2 values stay approximately constant over a female's successive clutches; but, because second copulae were immediately after first ones, females may only have been able to store the same mixture of sperm from different males in each spermatheca. The situation may be more complicated when females have better control over sperm storage. Parker (1970b) also conducted an experiment with long intervals between a female's matings. A male's success was again approximately constant over several egg batches. Unfortunately however, no analysis of the overall data was conducted, as was usual in 1970. Nonetheless, there was a 23% difference in success for males mating with females which had had eight previous mates compared to males mating with virgins; i.e. a female's mating history may have had a large influence on a male's success. Parker and Simmons (1991) acknowledge that it is unlikely that sperm could be pumped freely in the spermathecal ducts as required by their model.

I propose that female influence on sperm storage and use is as follows. A female mates with her first mate and stores more sperm the larger he is, and these sperm are stored more unevenly amonst the spermathecae, but the female does not favour any particular spermatheca. When she has received sperm from her second mate, she assesses his size, and possibly the relative sizes of the two, and stores sperm differently according to whether he is smaller or larger than the first male. If he is smaller, then his sperm are undesirable and the female may not want to store any. However, sperm will be strongly selected to reach the spermathecae and so the female may not be able to prevent some sperm gaining access, probably to random spermathecae. If the second male is larger, the female preferentially stores his sperm in the double spermathecae. However, as she does not have more sperm as a result than the first female above, this suggests that she flushes at least some of the sperm she has already stored from the first male, probably from the singlet spermathecae: no sperm were detected here in many females mated to the largest males. As second males do appear to flush sperm from the female (Parker and Simmons 1991), she would only have to move the sperm from the spermathecae to the bursa or oviduct and leave their removal from there to the second male. If the number of sperm stored from the larger second male minus those of the first male flushed away was roughly equal to the number stored unwillingly when the second male was smaller, this could be why the total numbers of sperm stored did not vary according to mating order.

The differences in the hatching success of double-mated females are also consistent with this process. When the second male was the larger mate, the female may have used sperm only from the double spermathecae, resulting in low hatching success but high P2. When the second male was smaller, the female may not have had favoured sperm so concentrated and perhaps used sperm from all spermathecae, resulting in better hatching success but lower P2. The normal situation would be that the bursa and oviduct would be full of fresh sperm from the last male to mate and these could fertilise most of the eggs. The low hatching successes here are thus probably an artefact of the method used to examine how females influence sperm storage and use.

The significance of these results for *S. stercoraria* in the field cannot at present be estimated. Females in the field will have many fresh sperm in the oviduct as the eggs are fertilised and laid and this would seem to give the sperm of the last male to mate a positional advantage. As the results on *D. anilis* (Otronen and Siva-Jothy 1991) also show however, females may be able to manipulate sperm between copula and mating and this has not yet been directly examined in field-collected females of *S. stercoraria*.

Ridley (1989) found the comparative data on P2 values in insect tentatively supported the hypothesis that the P2 value is related to female mating frequency: in monandrous species P2 was significantly lower than in polyandrous ones. Ridley did not consider that Diptera are unusual in normally having multiple spermathecae. The mean species P2 for Diptera is 0.51 from Ridley's data and the mean for other orders, using a species mean for each order, is 0.70. From a female's point of view, there may be no consistent pattern in whether desirable mates are first or second, but if females could exclusively use sperm from desirable males and the mating order was random, we could expect a mean P2 of 0.5. In species with one spermatheca, females may be unable to exercise the same degree of control and the P2 values found would be more influenced by last-male interests, generally a higher P2. Unfortunately, the data are inadequate for a formal test of the difference but it is possible that multiple spermathecae in the Diptera, and a few other insects (Matsuda 1976), have evolved to give females better control over the use of the sperm they have stored.

Acknowledgements. I thank C. Müller, T. Vogel and, especially, P. Abegglen for help with the laboratory work and C. Goater, R. Semlitsch, L.W. Simmons, G.A. Parker and three referees for comments on the manuscript.

References

Borgia G (1979) Sexual selection and the evolution of mating systems. In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition in insects. Academic, New York, pp 19–80

Birkhead TR, Hunter FM (1990) Mechanisms of sperm competition. Trends Ecol Evol 5:48–52

Eberhard WG (1985) Sexual selection and animal genitalia. Harvard UP, Harvard

Eberhard WG (1990) Inadvertant machismo? Trends Ecol Evol 5:263

Harris H, Hopkinson DA (1978) Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing, Amsterdam

Kloet GS, Hincks WD (1975) A check list of British insects. Royal Entomological Society, London

Knowlton N, Greenwell SR (1984) Male sperm competition avoidance mechanisms: the influence of female interests. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic, New York, pp 61–84

Matsuda R (1976) Morphology and evolution of the insect abdomen. Pergamon, Oxford

Otronen M, Siva-Jothy MT (1991) The effects of postcopulatory male behaviour on ejaculate distribution within the female sperm storage organs of the fly, *Dryomyza anilis* (Diptera: Dryomyzidae). Behav Ecol Sociobiol 29:33–37

Parker GA (1970a) Sperm competition and its evolutionary consequences in the insects. Biol Rev Cambridge Phil Soc 45:525–567

Parker GA (1970b) Sperm competition and its evolutionary effect on copula duration in the fly *Scatophaga stercoraria*. J Insect Physiol 16:1301–1328

Parker GA (1978) Searching for mates. In: Krebs JR, Davies NB (eds) Behavioural ecology, 1st edn. Blackwell, Oxford, pp 214–244

Parker GA (1979) Sexual selection and sexual conflict. In: Blum MS, Blum NA (eds) Sexual selection and reproductive competition in insects. Academic, New York, pp 123–166

Parker GA (1984) Sperm competition and the evolution of animal mating strategies. In: Smith RL (ed) Sperm competition and the evolution of animal mating systems. Academic, New York, pp 1–60

Parker GA (1992) Marginal value theorem with exploitation time costs: diet, sperm, and optimal copula duration in dung flies. Am Nat 139:1237–1256

Parker GA, Simmons LW (1991) A model of constant random sperm displacement during mating: evidence from *Scatophaga*. Proc R Soc London B 246:107–115

Parker GA, Simmons LW, Kirk H (1990) Analysing sperm competition data: simple models for predicting mechanisms. Behav Ecol Sociobiol 27:55–65

Parker GA, Simmons LW, Ward PI (1993) Optimal copula duration in dungflies: effects of frequency dependence and female mating status. Behav Ecol Sociobiol 32:157–166

Ridley M (1989) The incidence of sperm displacement in insects: four conjectures, one corroboration. Biol J Linn Soc 38:349–367

Sigurjonsdottir H, Parker GA (1980) Dung fly struggles: evidence for assessment strategy. Behav Ecol Sociobiol 8:219–230

Simmons LW, Parker GA (1992) Individual variation in sperm competition success of yellow dung flies, *Scatophaga stercoraria*. Evolution 46:366–375

Simmons LW, Ward PI (1991) The heritability of sexually dimorphic traits in the yellow dung fly *Scathophaga stercoraria* (L.). J Evol Biol 4:593–601

Smith RL (ed) (1984) Sperm competition and the evolution of animal mating systems. Academic, New York

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, San Francisco

Ward PI, Simmons LW (1991) Copula duration and testes size in the yellow dung fly, *Scathophaga stercoraria* (L.): the effects of diet, body size, and mating history. Behav Ecol Sociobiol 29:77–85