Sperm numbers, their storage and usage in the fly *Dryomyza anilis*

MERJA OTRONEN

Department of Zoology, Uppsala University, Villavägen 9, S-752 36 Uppsala, Sweden
(merja.otronen@zoologi.uu.se)

SUMMARY

In the fly *Dryomyza anilis* females have two kinds of sperm storage organs: one bursa copulatrix and three spermathecae (two spermathecae with a common duct form the doublet, and the third is a singlet spermathecal unit). At the beginning of a mating the male deposits his sperm in the bursa copulatrix. After sperm transfer the male taps the female's abdomen with his claspers. This behaviour has been shown to increase the male's fertilization success. After mating, the female discharges large quantities of sperm before oviposition. To find out where the sperm remaining in the female are stored, I counted the number of sperm in the droplet and in the female's sperm storage organs after different types of mating. I carried out three mating experiments. In experiment 1, virgin females were mated with one male and the matings were interrupted either immediately after sperm transfer or after several tapping sequences. The results show that during male tapping more sperm moved into the singlet spermatheca. In addition, the total number of sperm correlated with sperm numbers in all sperm storage organs, and male size was positively related to the number of sperm remaining in the bursa. In experiment 2, females mated with several males. The number of sperm increased with increasing number of matings only in the doublet spermatheca. No increase in the number of sperm in the singlet spermatheca during consecutive matings suggests that sperm were replaced or did not reach this sperm storage organ. In experiment 3, virgin females were mated with a single male and half of them were allowed to lay eggs. The experiment showed that during egg-laying, females primarily used sperm from their singlet spermatheca. The results from the three experiments suggest that sperm stored in the singlet spermatheca is central for male fertilization success and male tapping is related to sperm storage in the singlet spermatheca. The different female's sperm storage organs in *D. anilis* may have separate functions during sperm storage as well as during sperm usage.

1. INTRODUCTION

In many groups of animals, females possess distinct sperm storage organs, where sperm transferred by males during copulation are placed (Smith 1984). These organs may play an important role in post-copulatory sexual selection. For example, in insects, female sperm storage organs vary greatly in shape and number among species, and it has been suggested that the shape of the organs could be used to predict male sperm precedence (Walker 1980). Although this hypothesis was rejected in a comparative analysis (Ridley 1989), it may only indicate that the relationship between the female's sperm storage organs and male fertilization success is a very complex one.

Sperm precedence has been studied in numerous insect species, the most common pattern being last male sperm precedence (Smith 1984). Yet very little is known of the actual mechanisms resulting in this pattern, or how and where sperm from multiple matings are stored. The relative influence of males and females on sperm storage is also poorly understood (Walker 1980; Birkhead & Møller 1993; Eberhard

1994). Existing studies on insects show, for example, that in some species males can remove previously stored sperm (e.g. Waage 1984; Ono *et al.* 1989), whereas in many other species the primary sperm storage organs are inaccessible to males, emphasizing the importance of female interests in sperm storage (Walker 1980; Siva-Jothy & Tsubaki 1989; Ward 1993). Furthermore, although males in many species may interfere with sperm storage, they are less likely to interfere directly with sperm usage. In some species it has been shown that the usage of sperm during oviposition can differ from the storage pattern. For example, females can selectively use sperm by choosing the sex of their offspring (Mange 1970) or using sperm from males genetically different from themselves (Olsson *et al.* 1996). In insects it has been shown that females, via their spermathecal muscles, can control the filling of sperm storage organs and/or sperm release during oviposition (Wilkes 1966; Khan & Musgrave 1969; Tombes & Roppel 1972; Villavaso 1975; Rodríguez 1994). Ward (1993) has suggested that in insects, females have evolved several sperm storage organs to have control over which male's sperm are

Table 1. *The average number of sperm (mean $\pm$ s.e.) in the droplet and the female's sperm storage organs in the three experiments*

	droplet	bursa	doublet	singlet	<i>n</i>
experiment 1					
copulation only	31 869 $\pm$ 6765	6967 $\pm$ 3095	1542 $\pm$ 943	655 $\pm$ 312	20
tapping sequences	27 436 $\pm$ 6016	9283 $\pm$ 3374	473 $\pm$ 151	1218 $\pm$ 391	17
experiment 2					
several matings	—	1299 $\pm$ 211	3955 $\pm$ 779	1355 $\pm$ 252	35
experiment 3					
no oviposition	—	940 $\pm$ 196	2057 $\pm$ 551	1526 $\pm$ 534	11
after oviposition	—	1006 $\pm$ 238	1184 $\pm$ 293	301 $\pm$ 154	12

used in fertilization. When examining sperm precedence patterns, it is therefore important to consider both where sperm are stored in the female sperm storage organs as well as how they are used during fertilization.

In *Dryomyza anilis*, females have two different kinds of sperm storage organs. During copulation, the male transfers his sperm into the bursa copulatrix (Otronen & Siva-Jothy 1991), and from there sperm moves into the spermathecae. Females have three spermathecae dorsally to the common oviduct. From the front, the spermathecae are situated two on the right side (doublet) and one on the left side (singlet) of the body. The spermathecae are connected with the common oviduct approximately above the place where the bursa copulatrix is situated ventrally to the common oviduct.

In *D. anilis*, a mating starts with sperm transfer into the bursa copulatrix, after which the male withdraws his genitalia. After a while he will start tapping movements, tapping the external genitalia of the female with his claspers. After tapping sequences but before oviposition, females discharge a large sperm droplet. An experiment with radiolabelled sperm showed that tapping affected sperm storage in the female. In matings with several tapping sequences, more of the last male's sperm stayed in the female when compared with mating with no tapping sequences (Otronen & Siva-Jothy 1991). Tapping sequences also increase male fertilization success. For example, the fertilization success for the last mating was about 18% after an intromission, but increased with the increasing number of tapping sequences and was about 70% after 25 tapping sequences (Otronen 1990). Many matings in *D. anilis* include several copulation bouts (i.e. an intromission followed by tapping sequences) with intervening oviposition bouts, which may also increase male fertilization success (see Otronen 1994a). This study concentrates on matings with one copulation bout followed by oviposition.

The paper examines where sperm are stored during mating and how they are used during oviposition in the fly *D. anilis*. The first experiment examines the effect of tapping sequences on sperm distribution in the sperm storage organs by comparing matings interrupted after sperm transfer with those with several tapping sequences. The second experiment shows how sperm are stored after several consecutive matings. The third experiment compares sperm numbers in females that have laid their eggs and females without egg-laying,

and shows from which sperm storage organs sperm are used during egg-laying.

2. MATERIAL AND METHODS

Individuals used in sperm count experiments were reared in the laboratory. Males and females were separated on the day they emerged as adults and then kept in single-sex cages with about 20 individuals in each. After 3 weeks feeding on yeast, sugar and water *ad libitum*, virgin females had mature eggs.

(a) *Experiment 1. Does the distribution of sperm in the female's sperm storage organs differ in matings with tapping sequences compared with matings interrupted after sperm transfer?*

In *D. anilis* copulation and sperm transfer take about 1 min (Otronen 1990), after which the male withdraws his genitalia. The matings were either interrupted immediately after copulation ($n = 20$), or males were allowed to have several tapping sequences after copulation before the mating was interrupted ($n = 17$). The total time for sperm storage was 25–30 min in both treatments. Females in matings interrupted after copulation were placed alone in a tube for 30 min and allowed to discharge a sperm droplet. They discharged the droplet 11.3 ± 1.8 min (mean $\pm$ s.e.) after the end of copulation. In matings with tapping sequences, the males were allowed to tap for 20 min before being separated from the females. These females discharged the sperm droplet 4.5 ± 0.9 min after separation, and were killed immediately thereafter. The average number of tapping sequences was 4.9 ± 0.6 , and the total time before the females were killed was 24.9 ± 1.6 min. Sperm in the droplet, bursa copulatrix, doublet and singlet spermathecae were counted as outlined below. In addition, male wing length was measured.

(b) *Experiment 2. Does the number of sperm in the female's sperm storage organs increase with the number of matings?*

Females were allowed to mate with 1 ($n = 13$), 2 (2), 3 (4), 4 (2), 5 (4), 6 (4), 7 (2), 8 (2) or 10 (2) males (the total number of females = 35). Each mating was interrupted after 30 min if the pair had not separated before that. The number of tapping sequences was not counted. After each mating, the male was removed and the female was left alone for about 10 min to allow her to discharge sperm. After that she was mated with the next male. Females were randomly allocated to different numbers of matings. After all matings females were kept overnight in tubes containing wet tissue paper and were killed the next morning. The number of sperm in the bursa and in the spermathecae was counted.

(c) Experiment 3. From which sperm storage organ do females use sperm when laying eggs?

Females with mature eggs were allowed to mate with one male for 30 min; each mating included several tapping sequences. The experiment included two treatments. Half the females were placed in tubes containing a piece of fish meat and allowed to lay their eggs overnight ($n = 11$). The other half of the females were kept overnight in similar tubes containing only wet tissue paper ($n = 12$). All females were killed the following morning and dissected. The bursa copulatrix and spermathecae were removed and sperm in them counted.

(d) Sperm counting

In all experiments the females were killed with CO_2 and the bursa copulatrix and the three spermathecae were removed immediately. In experiment 1, females discharged the sperm droplet on the wall of a clean plastic tube, and the droplet was removed with forceps. Droplets are sticky and therefore easily removed. The sperm droplet, bursa copulatrix and the three spermathecae were broken separately with a glass rod in 10 μl of insect ringer, and more solution was added to make the volume up to 2 ml (droplet and bursa copulatrix) or 500 μl (spermathecae). After shaking, two 10 μl drops per sample were placed on a microscope slide, 5 μl of DAPI fluorescent stain (Sigma; concentration of 1 mg ml^{-1}) was added and the drops were allowed to dry in the dark. The number of sperm was then counted under epifluorescence. In each case the estimated number of sperm is based on the mean of the two drops counted.

3. RESULTS

(a) Experiment 1. Does the distribution of sperm in the female's sperm storage organs differ in matings with tapping sequences compared with matings interrupted after sperm transfer?

In both treatments most of the males' sperm came out with the droplet. In matings interrupted after copulations about 80% of the sperm came out with the droplet and in matings with several tapping sequences the figure was about 69% (table 1).

Logistic regression was performed to find out which variables best separate the treatments in experiment 1. This was done by asking if the proportion of sperm found in the droplet, the bursa copulatrix, and the doublet and singlet spermathecae could be used to predict which treatment a mating belonged to. The logistic regression model including all four variables was significant ($\chi^2 = 9.872$; d.f. = 4; $p = 0.042$) and it classified 73% of the matings correctly. Of the four variables, the singlet spermatheca significantly contributed to the model (Wald statistic = 4.548; d.f. = 1; $p = 0.033$, partial regression coefficient, $r = 0.223$). Table 1 shows that if females had tapping sequences with the male, the proportion of sperm in the singlet spermatheca increased in comparison with matings interrupted after sperm transfer. Of the other variables the doublet spermatheca was very close to being significant (Wald statistic = 3.686; d.f. = 1; $p = 0.054$; $r = -0.182$) indicating that a larger proportion of sperm was stored in the doublet if the female did not

have tapping sequences (table 1). Both the droplet and bursa copulatrix were not significant (Wald statistic = 0.404 and 0.737, respectively; in both, d.f. = 1). In the treatment with tapping sequences, the time before females were killed varied due to the variation in time it took for females to discharge sperm. However, this variation in time was not related to the proportion of sperm found in the bursa copulatrix (multiple regression: $t = -0.398$), doublet ($t = 1.098$) or singlet spermatheca ($t = 0.207$). (In all three, d.f. = 19, n.s.; the whole model: $F = 1.053$; d.f. = 3, 16, n.s.)

Male size was not related with the total number of sperm in an ejaculate (regression: $t = 0.961$; d.f. = 36, n.s.). To examine whether male size was related to sperm numbers in the droplet and the different sperm storage organs, male size was analysed against the log-transformed sperm numbers, including treatment (several tapping sequences or no tapping) as a categorical variable. All interactions between treatment and sperm numbers were examined, but as none was significant, they were excluded from the model. In the final model, the number of sperm in the bursa copulatrix was significantly related to male size ($F = 6.118$; d.f. = 1, 30; $p = 0.020$), indicating that the bursa copulatrix contained more sperm if the mating male was large. The other variables were not significant (droplet: $F = 0.139$; doublet: $F = 0.298$; singlet: $F = 0.041$; and treatment: $F = 0.538$; in all d.f. = 1, 30, n.s.).

Total number of sperm in an ejaculate did not correlate significantly with the proportion of sperm staying in the female. Spearman rank correlations between total number of sperm and the proportion of sperm found in the bursa copulatrix ($r_s = -0.061$), doublet (0.055), or singlet spermatheca (0.225; in all, $n = 37$) were not significant. However, large total number of sperm in an ejaculate resulted in a significantly larger number of sperm being found in all sperm storage organs (bursa copulatrix: 0.403; doublet: 0.371; and singlet spermatheca: 0.448; in all three, $p < 0.05$). A sequential Bonferroni test was applied to all correlations above. Thus, passing a large ejaculate resulted in a large number of sperm being stored in all sperm storage organs, but ejaculate size did not affect the proportion of sperm stored in them.

(b) Experiment 2. Does the number of sperm in the female's sperm storage organs increase with the number of matings?

In this experiment, females mated with one to ten males, and the average number of sperm found in the females is shown in table 1. The number of matings was regressed against the sperm numbers in the three sperm storage organs. There was no significant increase in sperm numbers in the bursa copulatrix ($t = 1.123$; d.f. = 34, n.s.) or in the singlet spermatheca ($t = -1.431$; d.f. = 34, n.s.). However, the number of sperm in the doublet spermatheca significantly increased with increasing number of copulations (figure 1) ($t = 2.603$; d.f. = 34; $p = 0.014$; the whole model: $F = 3.255$; d.f. = 3, 31; $p = 0.035$).

Figure 1. Number of sperm in the doublet spermatheca against the number of matings.

(c) Experiment 3. From which sperm storage organ do the females use sperm when laying eggs?

The number of sperm found in the female sperm storage organs before and after oviposition is shown in table 1. To examine the differences between the two treatments (i.e. females that mated and oviposited, and females that only mated) I made a logistic regression. I examined whether the number of sperm in the three sperm storage organs could be used to predict to which treatment the females belonged. The logistic regression including all three variables classified correctly 74 % of the observations and was significant ($\chi^2 = 8.65$, d.f. = 3, $p = 0.034$). Of the three sperm storage organs, the number of sperm in the singlet spermatheca significantly contributed to the separation of the treatments (Wald statistic = 3.858, d.f. = 1, $p = 0.049$, $r = -0.242$). Bursa copulatrix and doublet spermatheca were not significant (Wald statistic = 0.008 and 0.040, respectively; in both d.f. = 1). Table 1 shows that when the females were allowed to lay their eggs, the number of sperm decreased both in the doublet and singlet spermatheca. The decrease was most pronounced in the singlet spermatheca where the number of sperm after oviposition was only about 20 % of the number before oviposition. In the doublet spermatheca this figure was about 60 %.

Experiments 1 and 3 both included matings with several tapping sequences without any egg-laying (see table 1; experiment 1: tapping sequences and experiment 3: no oviposition). In experiment 1, females were dissected immediately after mating whereas in experiment 3 they were dissected next morning, about 18 h after mating. A logistic regression showed that the distribution of sperm in the female's sperm storage organs was different between the matings in the two experiments ($\chi^2 = 24.9$, d.f. = 3, $p = 0.001$; the model classified correctly 81 % of the matings). Sperm numbers were significantly different in the bursa (Wald statistic = 4.890, d.f. = 1, $p = 0.027$, $r = -0.261$) and in the doublet spermatheca (Wald statistic = 4.106, d.f. = 1, $p = 0.042$, $r = 0.223$), but not in the singlet spermatheca (Wald statistic = 0.995; d.f. = 1, n.s.). Table 1 shows that when the females were dissected immediately after the mating they contained more sperm in the bursa copulatrix and less sperm in the

doublet spermathecae than females dissected on the following morning.

4. DISCUSSION

In *D. anilis*, females stored sperm in all sperm storage organs even without male tapping. However, the number of sperm found in the different sperm storage organs varied between matings. In matings with male tapping, a larger proportion of the male's sperm went into the singlet spermathecae compared with matings without male tapping. Differences in sperm storage between the singlet and doublet spermathecae were also evident from the experiment with multiple matings. The number of sperm in the doublet spermatheca increased with the number of matings, whereas there was no increase in the singlet spermatheca. This suggests that sperm movements in and out of the singlet and doublet spermathecae occur independently from each other. The experiments did not show what happens to the rival sperm in multiple matings. No increase in sperm numbers in the singlet spermatheca after several matings suggests that either rival sperm did not enter the singlet spermatheca or sperm from the previous mating were replaced during the following matings.

Previous results on the distribution of sperm in the female sperm storage organs in *D. anilis* showed that most of the sperm belonging to the mating male came out with the droplet (Otronen & Siva-Jothy 1991). The radiolabelling experiments suggested that about 70–90 % of the last male's sperm came out with the droplet. In addition, more of the last male's sperm came out in matings interrupted after copulation compared with matings with several tapping sequences. The current results on sperm counting support these observations. About 80 % of the male's sperm came out with the droplet in matings without tapping sequences and the figure in matings with several tapping sequences was 69 %. These results indicate that only a few per cent of the total number of sperm that males pass to the female during intromission are stored and used for fertilization.

In experiment 1, females were killed, on average, 5 min earlier in matings with tapping sequences than in matings interrupted after copulation. It is unlikely, however, that this difference could have caused the observed differences in sperm distribution between the two treatments. In the treatment with tapping sequences, there was several minutes' variation in the time before females were killed as a result of the variation in the time before females discharged sperm, but this variation in time did not affect sperm distribution.

Why do males in *D. anilis* pass so many sperm per ejaculate if only a small fraction are stored and used by the female? In many species males transfer many more sperm than females actually store, and the phenomenon is common in many groups of animals (Birkhead & Møller 1993). For example, in *Drosophila melanogaster* only about 20 % of the deposited sperm are stored (Lefevre & Jonsson 1962). In a theoretical paper Parker (1990) has argued that large sperm numbers

per ejaculate are related to male sperm competition because of the raffle principle. According to this principle, the male with the largest number of sperm will fertilize most of the eggs. In *D. anilis* the proportion of sperm stored in different sperm storage organs did not correlate with the total number of sperm transferred. However, large number of sperm in an ejaculate resulted in a large number of sperm in all sperm storage organs. Although male–male competition via sperm numbers does not affect the distribution of sperm between the female sperm storage organs, males with large ejaculates could have an advantage in sperm competition within the individual sperm storage organs of the female. Thus, in *D. anilis* males may compete not only by tapping but also with their sperm numbers. Large number of sperm per ejaculate could also be advantageous after a takeover, which often results in two ejaculates in the female's bursa copulatrix (see Otronen 1994b). The results presented here also showed that during oviposition females used sperm mainly from the singlet spermatheca. After one egg batch the singlet spermatheca contained only about 20% of the number of sperm before oviposition. Sperm stored in the singlet spermatheca had priority over sperm stored in the doublet spermatheca or in the bursa copulatrix. In matings with several tapping sequences, more sperm moved into the singlet spermatheca compared with matings without tapping. Thus, the three experiments together showed the relationship between male tapping, sperm storage and sperm usage: male tapping was correlated with sperm movement into the female's sperm storage organs and, in particular, into the singlet spermatheca, which is likely to be the one that females primarily use during fertilization.

The number of sperm in the bursa also varied between mating experiments. In the first experiment, where females were killed about 30 min after the start of mating, there were many more sperm in the bursa than in the experiment where females were killed about 18 h after mating. The large number of sperm in the bursa copulatrix immediately after mating in *D. anilis* could indicate that these sperm are also used in fertilization (see, for example, Siva-Jothy & Tsubaki 1994). However, sperm from the bursa disappeared even without egg-laying, unlike the sperm in the spermathecae, suggesting that they were superfluous. Further studies are necessary to determine the role of sperm in the bursa copulatrix during oviposition. In *D. anilis*, unlike in some other species, spermathecae are situated above the common oviduct, separately from the bursa copulatrix, making it possible to use sperm from them independently.

In conclusion, the different female sperm storage organs in *D. anilis* may have separate functions during sperm storage as well as during sperm usage. During mating, male tapping is related to changes in sperm distribution in the female sperm storage organs. However, males may interfere with sperm storage in the female spermathecae only indirectly via tapping, thus female control over sperm storage and usage cannot be excluded. Differential distribution of sperm in the female sperm storage organs after different types

of matings or before and after oviposition suggests that multiple sperm storage organs, common in many insect species, are closely related to the control over fertilization.

I thank Mike Siva-Jothy and the anonymous referees for commenting on the manuscript, Lammi Biological Station for working facilities and Anne Ojala for donating DAPI.

REFERENCES

- Birkhead, T. & Møller, A. P. 1993 Female control of paternity. *Trends Ecol. Evol.* **8**, 100–104.
- Eberhard, W. G. 1994 Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. *Evolution* **48**, 711–733.
- Khan, N. R. & Musgrave, A. J. 1969 Observations on the functional anatomy of the reproductive organs of *Sitophilus* (Coleoptera: Curculionidae). *Can. J. Zool.* **47**, 665–669.
- Lefevre, G. & Jonsson, U. B. 1962 Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. *Genetics* **47**, 1719–1736.
- Mange, A. P. 1970 Possible nonrandom utilization of X- and Y-bearing sperm in *Drosophila melanogaster*. *Genetics* **65**, 95–106.
- Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996 Sperm selection by females. *Nature, Lond.* **383**, 585.
- Ono, T., Siva-Jothy, M. T. & Kato, A. 1989 Removal and subsequent ingestion of rival's semen during copulation in a tree cricket. *Physiol. Ent.* **14**, 195–202.
- Otronen, M. 1990 Mating behaviour and sperm competition in the fly *Dryomyza anilis*. *Behav. Ecol. Sociobiol.* **26**, 349–356.
- Otronen, M. 1994a Repeated copulations as a strategy to maximize fertilization in the fly *Dryomyza anilis* (Dryomyzidae). *Behav. Ecol.* **5**, 51–56.
- Otronen, M. 1994b Fertilization success in the fly *Dryomyza anilis* (Dryomyzidae): effects of male size and the mating situation. *Behav. Ecol. Sociobiol.* **35**, 33–38.
- Otronen, M. & Siva-Jothy, M. T. 1991 The effect of postcopulatory male behaviour on ejaculate distribution within the female sperm storage organs in the fly *Dryomyza anilis*. *Behav. Ecol. Sociobiol.* **29**, 33–37.
- Parker, G. A. 1990 Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. B* **242**, 120–126.
- Ridley, M. 1989 The incidence of sperm displacement in insects: four conjectures, one corroboration. *Biol. J. Linn. Soc.* **38**, 349–367.
- Rodrigues, V. 1994 Function of spermathecal muscle in *Chelymorpha alternans* Boheman (Coleoptera: Chrysomelidae: Cassidinae). *Physiol. Ent.* **19**, 198–202.
- Siva-Jothy, M. T. & Tsubaki, Y. 1989 Variation in copulation duration in *Mnais pruinosa pruinosa* (Calopterygidae; Odonata). I. Alternative mate-securing tactics and sperm precedence. *Behav. Ecol. Sociobiol.* **24**, 39–45.
- Siva-Jothy, M. T. & Tsubaki, Y. 1994 Sperm competition and sperm precedence in the dragonfly *Nanophya pygmaea*. *Physiol. Ent.* **19**, 363–366.
- Smith, R. L. (ed.) 1984 *Sperm competition and the evolution of animal mating systems*. New York: Academic Press.
- Tombes, A. S. & Roppel, R. M. 1972 Ultrastructure of the spermatheca of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Int. J. Insect Morphol. Embryol.* **1**, 141–152.
- Villavaso, E. J. 1975 Functions of the spermathecal muscle of the boll weevil, *Anthonomus grandis*. *J. Insect Physiol.* **21**, 1275–1278.

- Waage, J. K. 1984 Sperm competition and the evolution of Odonate mating systems. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 251–290. Orlando: Academic Press.
- Walker, W. F. 1980 Sperm utilization strategies in nonsocial insects. *Am. Nat.* **115**, 780–799.
- Ward, P. I. 1993 Females influence sperm storage and use in the yellow dung fly *Scatophaga stercoraria* (L.). *Behav. Ecol. Sociobiol.* **32**, 313–319.
- Wilkes, A. 1966 Sperm utilization following multiple inseminations in the wasp *Dahlbominus fuscipennis*. *Can. J. Genet. Cytol.* **8**, 451–461.

Received 14 January 1997; accepted 27 January 1997