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Mating system evolution in sperm-heteromorphic *Drosophila*

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Abstract

In *Drosophila* species of the *obscura* group, males exhibit sperm-heteromorphism, simultaneously producing both long sperm, capable of fertilization, and short sperm that are not. The production of multiple sperm types calls into question whether mating system correlates, such as sperm length and number trade-offs and female remating behavior, are the same as previously described in sperm-monomorphic systems. We examine three *obscura* group species, *D. pseudoobscura*, *D. persimilis*, and *D. affinis* that differ significantly in the lengths of their long fertilizing sperm, to test predictions about the relationship between sperm length and four mating system characters: male age at sexual maturity; sperm number; female remating; and male reproductive output. In *D. affinis*, where males produce the longest fertilizing sperm, their sexual maturity is delayed and they produce fewer long sperm compared to the other two species, as predicted if long sperm are costly to produce. Female *D. affinis*, although they receive fewer sperm than females of the other two species, do not remate more frequently or produce fewer progeny from a single mating. Different responses between sperm-heteromorphic and sperm-monomorphic systems underscore the complex nature of the coevolution between male and female mating system characters.

Keywords: Mating system; Sperm-heteromorphism; *Drosophila*; Sperm production; Egg production

1. Introduction

Species within the genus *Drosophila* exhibit the greatest variation in sperm length observed for any animal taxon. Sperm lengths range from 77 μm in *D. persimilis* (Snook, 1997) to 58,290 μm in *D. bifurca* (Pitnick et al., 1995). Although the biological implications and significance of sperm size variation in *Drosophila* are not well understood, several mating system traits are strongly correlated with sperm size variation. There are significant costs to males for making long sperm that are reflected in two characters: male age at reproductive maturity and sperm limitation. Male reproductive maturity is significantly delayed in species with "giant" sperm (Pitnick and Markow, 1994a; Pitnick et al., 1995), in part due to the demands of growing the large testes required to produce the long sperm (Pitnick, 1996). These demands appear to limit the number of sperm made and transferred to females (Pitnick, 1993, 1996; Pitnick and Markow, 1994b). Potential female fitness also reflects sperm length variation. Females of giant sperm species tend to remate rapidly, perhaps to maintain an adequate supply of sperm for oviposition since they receive fewer sperm from one mating (Markow, 1996). Moreover, because these females receive few sperm, they produce few progeny from a single mating (Pitnick, 1993; Pitnick and Markow, 1994b).

In contrast, *Drosophila* species in which males make "short" sperm attain reproductive maturity faster. These males produce more sperm, females subsequently receive more sperm during a single mating, and tend to produce more progeny from that single mating in comparison to longer sperm species (Markow, 1996). Additionally, their remating intervals may be longer as remating appears related, at least in part, to the depletion of sperm from female sperm storage organs (Manning, 1967; Gromko et al., 1984; Schwartz and Boake, 1992; Gromko and Markow, 1993; Pitnick 1993, 1996; Pitnick and Markow, 1994b; although see Snook, 1998).

Yet another extreme form of sperm length variation exists in *Drosophila*, the puzzling phenomenon of

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sperm-heteromorphism in the *obscura* group. Males of all *obscura* group species examined simultaneously produce two kinds of nucleated sperm, a long and a short morph (Beatty and Sidhu, 1970; Joly and Lachaise, 1994; Snook, 1997). Both the lengths and the relative proportions of each morph vary among species (Beatty and Sidhu, 1970; Snook et al., 1994; Bressac and Hauschteck-Jungen, 1996; Snook, 1997). Males transfer and females store both sperm types (Beatty and Sidhu, 1970; Snook et al., 1994; Bressac and Hauschteck-Jungen, 1996) but only the long morph has been observed to fertilize eggs (Snook et al., 1994; Snook and Karr, 1998).

Among sperm-heteromorphic species, inter-specific variation in the lengths of short and long sperm is under the influence of different selective forces (Snook, 1997), but the adaptive significance of short, nonfertilizing sperm remains unidentified (Snook and Markow, 1996; Snook, 1998). While nonfertilizing gametes in lepidopterans have been suggested to serve as nutrient contributions to either the female or offspring (Silberglied et al., 1984), this is not the case in *D. pseudoobscura* (Snook and Markow, 1996). Nonfertilizing sperm have also been suggested to serve in sperm competition, as either cheap filler to subsequently decrease a female's propensity to remate or to displace a previous male's sperm from female sperm storage organs (Silberglied et al., 1984). In *D. pseudoobscura*, however, female remating behavior is unassociated with the numbers of short sperm in storage and thus they do not function as cheap filler (Snook, 1998).

Also unclear is whether in sperm-heteromorphic *Drosophila* species the production of substantial numbers of short, nonfunctional gametes mitigates the correlations between sperm length and those mating system characters observed in sperm-monomorphic species summarized above. Specific questions include: (1) is male sexual maturity delayed in sperm-heteromorphic species with longer fertilizing sperm?; (2) is there a trade-off between the length and number of fertilizing sperm (i.e. longer sperm producing species are sperm-limited)?; (3) do females mate more frequently in species producing longer fertilizing sperm as a result of this sperm limitation?; and (4) do females experience a fitness cost associated with sperm limitation?

In the present study we address these questions in three species of the *obscura* group. In *D. pseudoobscura*, short and long sperm are 90 and 360 μm in length, respectively, while in its sister species, *D. persimilis* (Dobzhansky and Powell, 1975), they are 77 and 320 μm. In contrast, in *D. affinis* the corresponding sperm lengths are 100 and 500 μm (Snook, 1997). Thus, the longer sperm morphs of *D. persimilis* and *D. affinis* differ by 30%. If longer sperm are more costly to produce in sperm-heteromorphic species, we predict, based upon findings in sperm-monomorphic species, that male sexual maturity will be delayed in *D. affinis* relative to the other two species. We also predict that because *D. affinis* males make the longest long sperm they will produce a smaller proportion of them. Due to the lower number of fertilizing sperm being produced, females will receive and store fewer long sperm and from a single mating will produce fewer eggs and progeny. Given that *D. affinis* females are predicted to store fewer sperm, they may also have shorter remating intervals compared to *D. pseudoobscura* and *D. persimilis*.

2. Materials and methods

2.1. Fly maintenance

Drosophila pseudoobscura was cultured from a mass collection of flies caught on fallen citrus in Tempe, AZ in 1990 and 1991. *Drosophila persimilis* was kindly provided by A.T. Beckenbach (Simon Fraser University, Burnaby, British Columbia) and *D. affinis* (14012-0141.2) was obtained from the National Drosophila Species Resource Center, Bowling Green, OH. All cultures were maintained on standard cornmeal–agar–molasses food with yeast and kept at room temperature, 22–25°C, with an approximate 12 h/12 h light/dark cycle. We collected virgins upon eclosion and stored 10 of each sex in eight dram yeasted food vials. All flies used, except in experiments determining age of reproductive maturity, were 5 days old.

2.2. Age of reproductive maturity

We determined the age of female and male reproductive maturity as the earliest age from eclosion in which at least 80% of the experimental individuals copulated. Experimental males were under the additional criterion of having to transfer sperm during copulation (Markow, 1996). One virgin test individual was placed in a yeasted food vial with two virgins of the opposite sex that were 5–7 days old (thus, assumed to be reproductively mature based on other experiments in the laboratory). Vials were observed for copulation for 2 h in the morning. If copulation was observed, the mated female was dissected and the reproductive tract removed to assay for the presence of sperm. Ages examined for reproductive maturity differed between species as did sample size (see Table 1).

2.3. Sperm production and transfer by males, and sperm storage by females

To assess sperm transfer and storage patterns, we determined the proportions of each sperm type produced by males, transferred to females, and stored by females in *D. persimilis* and *D. affinis*. Sperm production, trans-

Table 1
Age (days) when males or females were tested for reproductive maturity in each species examined. NE indicates newly eclosed (<12 h old) test individuals. The numerator for the number mated is the number that mated and/or transferred sperm and the denominator is the total number of flies tested

		Number mate		Percent	
Species	Age	♂	♀	♂	♀
D. affinis	1	0/30	0/30	0.0	0.0
	2	2/30	0/30	6.7	0.0
	3	21/25	24/37	84.0	64.9
	4	28/29	26/30	96.5	86.7
D. persimilis	1	0/30	1/30	0	3.0
	2	23/27	1/30	85.2	3.0
	3	25/28	21/30	89.3	70.0
	4	25/27	27/29	93.1	93.1
D. pseudoobscura	NE	0/30	0/30	0.0	0.0
	1	24/91	30/93	26.4	32.3
	2	88/103	60/95	88.0	63.2
	3	66/77	59/65	85.7	90.8

fer and storage were ascertained by dissecting virgin males or mated females at various times after copulation as described elsewhere for *D. pseudoobscura* (Snook et al., 1994; see Table 2 and Fig. 1 for sample sizes).

2.4. Remating, reproductive output and ovariole number

To examine the relationship between sperm length, mating and reproductive output, we determined fecundity and productivity and female remating behavior using singly and multiply mated females of each *obscura* group species tested. To examine singly mated females, we placed one virgin female in a yeasted food vial with two virgin males. Once copulation began, the noncopulating male was aspirated from the vial and after copulation the mating male was also removed. Females were transferred to new yeasted food vials every day for the first 15 days and then every other day until death. The same design was employed for multiply mated females, except females were given twice daily opportunities to remate, 2 h in the morning and 2 h in the afternoon (periodic interaction design; Pyle and Gromko, 1978), for 5 consecutive days. After flies were transferred to new vials, we immediately counted the number of eggs oviposited in the prior vial and saved all vials for subsequent adult progeny counts. We performed 2–6 replicates for each treatment in each species and tested if replicates were homogeneous by independent t-tests or one-way ANOVA. If replicates were homogeneous, data were pooled. Data for individual females were discarded if females in the multiple mating group did not remate or if females in either treatment failed to produce progeny. In *D. pseudoobscura*, we dissected females to assay for the presence of sperm if they had not produced adult progeny after five consecutive vial changes. We did not dissect *D. persimilis* or *D. affinis* because most females had died before we could determine if females had produced adult progeny in all of five prior vial changes (see Table 2 for sample sizes). Data on multiply mated females were used to determine remating behavior, including percentage of females remating, the average number of mates, and the average remating interval between mates to test for any association between these behaviors and sperm length.

We also examined ovariole number in the three species to determine the relationship between potential and actual reproductive output. Females of the different species were collected from their respective stock bottles and stored 10 per food vial with dry yeast. After 5 days, the ovaries were dissected in phosphate buffer solution and examined for ovariole number (n=5 for each species).

All statistical analyses were performed using Systat (version 5.03, Systat, Inc.; Wilkinson, 1990).

Table 2
Proportion of short sperm produced and transferred by males. Data are means±SE. Sample sizes in parantheses

Species	Produced	Transferred
D. affinis	0.75±0.01 (14)	0.72±0.01 (14)
D. persimilis	0.51±0.02 (15)	0.50±0.02 (15)
D. pseudoobscura	0.44±0.02 (12)	0.48±0.02 (11)

3. Results

3.1. Age of reproductive maturity

Males of *D. pseudoobscura* and *D. persimilis* were reproductively mature at 2 days post-eclosion while *D.*

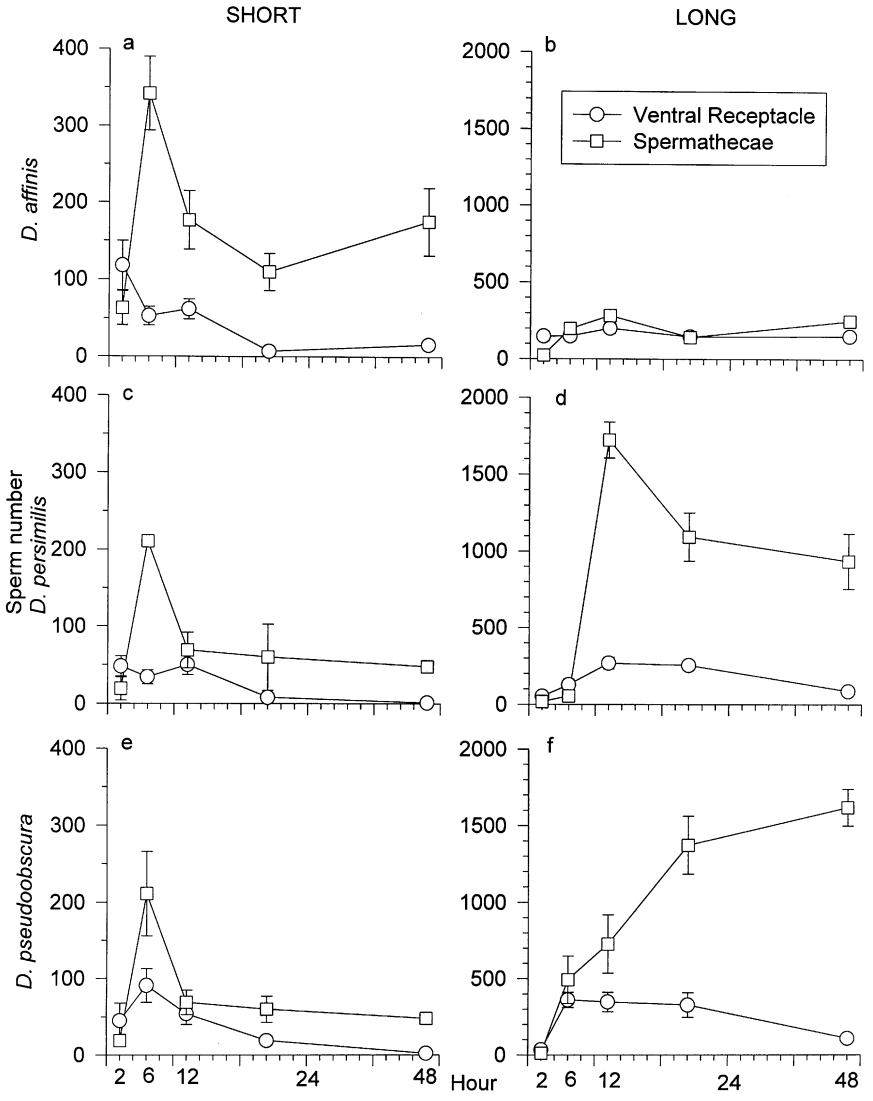

Fig. 1. Total number of short (a, c, e) and long (b, d, f) sperm stored in either the ventral receptacle or spermathecae for D. affinis (a, b), D. persimilis (c, d), and D. pseudoobscura (e, f) at various times post-copulation. Sample sizes are as follows. For D. pseudoobscura ventral receptacle, 2 hr = 13, 6 hr = 15, 12 hr = 13, 24 hr = 8, 48 hr = 15; spermathecae, 2 hr =11, 6 hr = 15, 12 hr = 13, 24 hr =8, 48 hr = 15. For D. persimilis ventral receptacle, 2 hr = 13, 6 hr = 12, 12 hr = 13, 24 hr = 12, 48 hr = 12; spermathecae, 2 hr = 13, 6 hr = 12, 12 hr = 13, 24 = 12, 48 hr = 13. For D. affinis for both ventral receptacle and spermatheacae, 2 hr = 14, 6 hr = 13, 12 hr = 14, 24 hr = 14, 48 hr = 11

affinis males were 3 days old (Table 1). Females of *D. pseudoobscura* were mature at 3 days and females of the other two species were mature at 4 days post-eclosion (Table 1). We performed Fisher exact tests for each species to determine whether sexual dimorphism at the first day in which one sex reached sexual maturity existed. We found that *D. affinis* exhibited no significant sexual dimorphism in age at reproductive maturity on day 3 (df=1, 60, P=0.14) when males first became reproductively mature. In contrast, in *D. persimilis* and *D. pseudoobscura*, where sperm length is shorter compared to *D. affinis*, as predicted males of both species mature significantly earlier than females (*D. persimilis*: df=1, 55, $P<0.0001$; *D. pseudoobscura*, df=1, 196, P=0.0005).

3.2. Sperm production

Drosophila affinis males, known to produce the longest fertilizing sperm of the three species (Snook, 1997), were predicted to produce them in the lowest proportion. Relative proportions of sperm types produced and transferred to females are given in Table 2. *Drosophila pseudoobscura* and *D. persimilis*, with similar sperm lengths, produced approximately 50% of each sperm type (Table 2). As predicted, if long sperm are more costly to produce, *D. affinis* males produced these in the lowest proportion. Males of all three species transferred approximately the same proportion of each sperm type as they produced (Table 2).

3.3. Sperm storage

A corollary to the prediction that *D. affinis* would produce a smaller proportion of fertilizing sperm was the expectation that *D. affinis* females would receive and subsequently store fewer sperm compared to the other two species. To test this, we examined the proportion of sperm in the uterus and the sperm storage organs following mating and the number of both sperm types stored prior to and after oviposition.

We found that the proportion of sperm in the uterus following mating remained fairly constant in the three species until the onset of oviposition and reflected the proportion of sperm types transferred. Between 24 and 48 h after copulation at the start of oviposition, however, the proportion of short sperm found in the uterus drastically decreases (Table 3). The change in sperm ratios in the uterus at the time of oviposition reflected concurrent changes in sperm ratios found in the female sperm storage organs during this time (described below; Fig. 1).

Two hours following sperm transfer, *D. affinis* females began storing sperm (Fig. 1a and b). The number of short sperm in the spermathecae and ventral receptacle at 2 h post-copulation was approximately the same (Fig. 1a). After 6 h, this relationship changed such that the highest number of short sperm was stored in the spermathecae (Fig. 1a). The number of short sperm found in both sperm storage organ types 12–48 h after copulation remained relatively constant (Fig. 1a). The number of long sperm found in the spermatheacae and ventral receptacle remained equal and relatively constant at all times post-copulation (Fig. 1b).

Patterns of sperm storage by *D. persimilis* and *D. pseudoobscura* females were similar (Fig. 1c–f). At 2 h post-copulation, the ventral receptacle and spermathecae had approximately the same number of short sperm (Fig. 1c and e; similar also to *D. affinis*). At 6 h post-copulation the spermathecae stored the majority of short sperm (as with *D. affinis*). Between 12 and 48 h after copulation the number of short sperm decreased, until relatively few to no short sperm remained in storage. Long sperm of *D. pseudoobscura* and *D. persimilis* also began to be stored 2 h after mating in both sperm storage organ types. Unlike in *D. affinis*, the number of long sperm in the sperm storage organs began to increase substantially such that by 48 h after copulation mainly only long sperm were in storage.

3.4. Female remating behavior

Given that *D. affinis* females store fewer sperm, we predicted that *D. affinis* females would remate more frequently than the other two species that store more sperm. We examined three remating indices: percentage of females remating; average number of mates; and the average remating interval between those mates. In all three measures, *D. pseudoobscura* females displayed the highest indices of remating (Table 4; number of mates: F=25.89, df=2144; remating interval: F=4.39, df=2144). Female *D. affinis* were characterized by intermediate values for the percentage of females remating and number of mates, but their remating interval did not differ significantly from female *D. pseudoobscura* (Table 4). Female *D. persimilis* showed the lowest values for all indices (Table 4). The lack of a strikingly higher mating frequency in *D. affinis* females relative to both other species does not support the predicted sperm limitation in these females.

3.5. Reproductive output and ovariole number

As a corollary to reduced proportion of fertilizing sperm in storage, we expected that *D. affinis* females would produce fewer eggs and progeny from a single mating compared to the other two species and that as a result of sperm limitation, multiple mating should increase female reproductive output. We subsequently examined fecundity and productivity in singly and multiply mated females to assess reproductive output patterns and the relationship, if any, to sperm length. Replicate experiments for *D. affinis* fecundity and productivity under either singly or multiply mated conditions were homogeneous for each trait, so data were pooled. Singly mated *D. affinis* females did not differ from multiply mating females in either fecundity or productivity (Table 5). The ratio of progeny to egg production averaged 0.44, irrespective of the number of mates (Table 5).

Replicates within singly and multiply mated treatments for *D. pseudoobscura* were homogeneous for both the number of eggs and progeny so the data were pooled. Singly and multiply mated *D. pseudoobscura* females produced equivalent numbers of eggs and progeny (Table 5). As with *D. affinis*, the ratio of progeny to egg production was less than one, averaging ca. 0.62 (Table 5).

Table 3
Proportion of short sperm found in the uterus by females (2–48 h) after copulation. Data are means±SE. Sample sizes in parentheses

Species	2 h	6 h	12 h	24 h	48 h
affinis	0.65±0.02 (14)	0.76±0.02 (13)	0.72±0.02 (14)	0.49±0.03 (9)	0.28±0.1 (10)
persimilis	0.60±0.01 (13)	0.57±0.03 (12)	0.63±0.03 (13)	0.64±0.08 (12)	0.28±0.14 (8)
pseudoobscura	0.44±0.04 (15)	0.45±0.03 (15)	0.55±0.03 (13)	0.51±0.08 (8)	0.09±0.04 (11)

Table 4
The percent of females remating, and of those remating their mean number of mates and mean remating interval for each of three *obscura* group species. Data are means±SE. Different letters in parentheses indicate species that significantly differ from each other[a]

Species	Percent remating	Number of mates**	Remating interval*
D. affinis (*n*=29)	89.6	2.6±0.14 (B)	2.0±0.14 (B)
D. persimilis (*n*=54)	85.2	2.1±0.05 (A)	2.5±0.11 (A)
D. pseudoobscura (*n*=79)	96.2	3.0±0.09 (C)	2.1±0.1 (B)

[a] * p=0.01; ** p=<0.0001.

Table 5
Egg and progeny production for singly (# mates=1) or multiply mated (one remating: # mates=2; two rematings: # mates=3) *obscura* group species. Data are means±SE. Sample sizes in parentheses. F (or t) and P values are presented from one-way ANOVAs examining differences in egg or progeny production between females having a different number of mates. ND=Not determined

Species	# mates=1		# mates=2		# mates=3		F (or t)		P	
	Egg	Progeny	Egg	Progeny	Egg	Progeny	Eggs	Progeny	Eggs	Progeny
affinis	410±34 (29)	212±17	450±46 (13)	188±22	459±53 (11)	254±21	0.42	1.83	0.66	0.17
persimilis	202±15 (28)	114±9	225±14 (40)	112±8	214±30 (6)	98±18	ND	0.23	ND	0.79
pseudo-obscura	610±34 (52)	351±21	602±80 (10)	386±46	638±35 (42)	416±22	0.19	2.25	0.83	0.11

In *D. persimilis*, replicates within the multiple mating treatment for fecundity were heterogeneous (F=3.550; df=5, 40; P=0.009), but replicates within the singly mated treatment were homogeneous (F=0.989; df=2, 25; P=0.3). Due to the nonhomogenity in multiply-mated fecundity, we did not test if the total number of eggs differed between treatments but present the mean number of eggs as if they were homogeneous (Table 5). All replicates were homogeneous for productivity. There was no difference in productivity between multiply and singly mated females (Table 5). The ratio of progeny to eggs produced was ca. 0.51.

The differences between species in the ratio of progeny to eggs produced suggest either substantial gamete wastage or high developmental mortality. Snook and Karr (1998) found 97% of *D. affinis* and 91% of *D. pseudoobscura* eggs were fertilized compared with only ca. 51% in *D. persimilis*. These results indicate high developmental mortality in *D. affinis* and *D. pseudoobscura* and 50% gamete wastage in *D. persimilis*. The latter suggests that while *D. pseudoobscura* and *D. persimilis* have similar sperm storage patterns, sperm utilization, at least in the laboratory, is different.

Of the three species, *D. pseudoobscura* females produce the largest number of eggs and progeny and *D. persimilis* the lowest, producing 34% of the eggs and 28% of the progeny of its sister species. Differences in ovariole numbers do not explain the difference in reproductive output. One-way ANOVA indicates that species vary significantly in ovariole number (df=2,12; F=17.7; P<0.001). Tukey post-hoc test indicates *D. affinis* females have fewer ovarioles (27.0±1.9) than either *D. persimilis* (36.4±0.5) or *D. pseudoobscura* (34.6± 0.7), which did not differ from each other.

4. Discussion

In sperm-monomorphic *Drosophila* species, production of long sperm appears to correlate with a number of mating system characters, such as male age at reproductive maturity, sperm production and female fitness (Pitnick and Markow, 1994a,b; Pitnick et al., 1995; Pitnick, 1996). We tested whether the same correlates between sperm length and mating system traits occur in sperm-heteromorphic *Drosophila* species. *Drosophila affinis* males produce fertilizing sperm that are 30% longer than those of *D. persimilis*. Thus *D. affinis* males were predicted to require the most time to become reproductively mature and to produce and transfer fewer long, fertilizing sperm to females. Furthermore, because they received fewer sperm, *D. affinis* females were expected to remate more frequently and to have lower single-mating productivity than females of the other two species.

As predicted, *D. affinis* males took twice as long to become reproductively mature as *D. pseudoobscura* and *D. persimilis* and also produced and transferred significantly fewer long sperm. *Drosophila affinis* males produced 25% long, fertilizing sperm compared to the ca. 50% long sperm produced by *D. pseudoobscura* and *D. persimilis* males. Males transfer the same proportion of sperm as produced and thus, *D. affinis* females are expected to store fewer long sperm. Indeed, at the onset of oviposition, *D. affinis* females stored approximately

81% fewer long sperm than the other two species. These females also stored 30% more short, nonfertilizing sperm.

A striking difference existed between the three species in the primary sperm storage organ and the location in which both sperm types were stored. In *D. pseudoobscura* (Snook et al., 1994) and *D. persimilis*, the spermathecae stored the majority of long sperm whereas in *D. affinis* the ventral receptacle and spermathecae stored similar numbers of long sperm (Fig. 1). Females of another sperm-heteromorphic species, *D. subobscura*, store the majority of sperm in the spermathecae (Bressac and Hauschteck-Jungen, 1996). The number of long sperm being stored prior to oviposition increased in both *D. pseudoobscura* and *D. persimilis*, while in *D. affinis* the number of long sperm stored before egg laying remained constant. Females of *D. persimilis* and *D. pseudoobscura* had very few short sperm in either sperm storage organ whereas the relatively large number of short sperm stored by *D. affinis* females was found primarily in the spermathecae. Regardless of the species, the proportion of short sperm in storage was less than that predicted based on the proportion of sperm types received by females. The discrepancy between short sperm transferred vs stored is less in *D. affinis* than the other two species (75% vs 30% compared to 50% vs 2%).

In addition to sperm storage differences, sperm utilization patterns differed between species. As *D. affinis* females received fewer sperm relative to females of the other two species, singly-mated *D. affinis* females should have had the lowest reproductive output and multiple mating should have increased their productivity. Instead, female *D. affinis* produced an intermediate number of eggs and progeny compared to the other two species, and multiple mating did not significantly increase the number of eggs or progeny produced for any species. Differences between species in the number of eggs produced is not related to ovariole number as we found *D. affinis* had significantly fewer ovarioles than both *D. pseudoobscura* and *D. persimilis*. Previous studies, each conducted under different conditions, report an increase in *D. pseudoobscura* fecundity (Beckenbach, 1978) or progeny production (Pruzan-Hotchkiss et al., 1981; Turner and Anderson, 1983) following multiple mating whereas no advantage to multiple mating is observed for *D. affinis* (Bressac et al., 1991).

Here we found that *D. affinis* and *D. persimilis* females produce approximately twice as many eggs as progeny whereas in *D. pseudoobscura* 62% of eggs produced offspring. In the laboratory, females apparently exhibit either considerable egg wastage or high developmental mortality, depending upon the species. Gamete wastage appears to occur in *D. persimilis* since ca. 50% of the eggs laid are unfertilized (Snook and Karr, 1998) and here we found that 50% of eggs developed to adulthood. In comparison, fertilization in a laboratory strain of *D. affinis* is ca. 97% (Snook and Karr, 1998), yet we found less than 50% of eggs developed into adults, indicating high mortality. The egg to progeny production ratio from wild-caught *D. pseudoobscura* females is almost one (Snook and Markow, unpublished data) suggesting that females of this species do not waste their gametes in nature. The disparity between the number of eggs and progeny produced by laboratory compared to wild-caught females likely reflects the relative overproduction of eggs by yeast-fed laboratory females (Ushkumari and Ranganath, 1986; Robertson and Sang, 1944a,b; Minato, 1980).

Previous work in *Drosophila* has indirectly suggested that female remating behavior is mediated by sperm load, and that females may remate when the sperm load is decreased to a certain threshold (Manning, 1967; Gromko et al., 1984; Schwartz and Boake, 1992; Gromko and Markow, 1993; Pitnick, 1993, 1996; Pitnick and Markow, 1994b). If true, then *D. affinis* females, because they contain the fewest number of sperm, should have a shorter remating interval relative to the other two species. Instead, the remating intervals of female *D. affinis* and *D. pseudoobscura* were the same, and both were shorter than in *D. persimilis*.

The evolutionary significance of sperm-heteromorphism remains a conundrum (Snook and Markow, 1996; Snook, 1998). Short, nonfertilizing sperm have been suggested to delay female remating by serving as “cheap filler” in the female reproductive tract (Silberglied et al., 1984), assuming that female remating behavior is determined by sperm load. If true, then *D. affinis* females, because they contain the largest number of short sperm, should have a longer remating interval relative to the other two species. Contrary to this expectation, *D. affinis* females did not exhibit a greater remating interval compared to *D. pseudoobscura* and *D. persimilis*. Direct examination of sperm load in *D. pseudoobscura* females revealed that propensity to remate is unrelated to the number of either short or long sperm stored (Snook, 1998) or to oviposition (Snook and So, 2000). Short sperm are thus unlikely to function as cheap filler in the *obscura* group.

In conclusion, sperm-heteromorphic *Drosophila* appear to have similar reproductive trade-offs associated with sperm length for age at reproductive maturity and sperm number/length trade-offs that have been described for sperm-monomorphic *Drosophila* (for review see Markow, 1996). The other traits, female remating interval and female fitness, are not similar between sperm-heteromorphic and monomorphic mating systems. Intriguingly, the three *obscura* group species varied not only in sperm length, but also in sperm production, storage and utilization patterns. These patterns did not always reflect the degree of phylogenetic relatedness as the two sister species, *D. pseudoobscura* and *D. persim-*

ilis, differed in some mating system correlates. Future work examining mating system evolution in the sperm-heteromorphic *obscura* group should focus on phylogenetic studies expanding these results in an effort to further elucidate the association between mating system traits and gametic traits and the evolutionary persistence of nonfertilizing sperm.

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