Female Genotypes Affect Sperm Displacement in Drosophila

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ABSTRACT

Differential success of sperm is likely to be an important component of fitness. Extensive variation among male genotypes in competitive success of sperm in multiply mated females has been documented for *Drosophila melanogaster*. However, virtually all previous studies considered the female to be a passive vessel. Nevertheless, under certain conditions female fitness could be determined by her role in mediating use of sperm from multiple males. Here we ask whether females differ among genotypes in their tendency to exhibit last-male precedence. Competition of sperm from two tester male genotypes (bw^D and B3-09, a third-chromosome isogenic line from Beltsville, MD) was quantified by doubly mating female lines that had been rendered homozygous for *X*, second, or third chromosomes isolated from natural populations. The composite sperm displacement parameter, *P2′*, was highly heterogeneous among lines, whether or not viability effects were compensated, implying the presence of polymorphic genes affecting access of sperm to eggs. Genetic variation of this type is completely neutral in the absence of pleiotropy or interaction between variation in the two sexes.

GENETIC variation in fertility components of fitness may be more common than variation in viability in natural populations (Sved and Ayala 1970; Sved 1971). One area in which genetic analysis can be brought to bear on the question of the evolutionary forces acting on fertility variation is in representation of sperm from first-mated *vs.* second-mated males among progeny of multiply mated Drosophila females. Drosophila females often have sperm from two or more males present simultaneously in their reproductive tract (Cobbs 1977; Levine *et al.* 1980; Griffiths *et al.* 1982; Harshman and Clark 1998). Remating by the female results in fewer offspring sired by the first male than if the female had not remated. Theoretically, genetic variation affecting interejaculate competitive ability (*i.e.*, sperm precedence) could have interesting population dynamics, including the possibility of stable polymorphism (Prout and Bundgaard 1977; Prout and Clark 1996).

Clark *et al.* (1995) measured genetic variation in the male component of sperm displacement by scoring parentage in offspring from large numbers of females doubly mated with a tester male and males from a series of 152 chromosome-replacement lines. *P2*, the fraction of progeny sired by the second male, varied widely, ranging from 1.0 (complete second male precedence) to 0.5. In addition to this "offense" component, Clark *et al.* (1995) found genetic variation in the ability of first-mated males to "defend" against displacement from subsequent matings. Hughes (1997) also found differences among males in both first and second male precedence among isogenic chromosome *III* lines extracted from a laboratory population; however, the variation was primarily nonadditive.

Evolution of sperm precedence in the laboratory has occurred as a correlated response to artificial selection on other traits. Selection for delayed senescence resulted in greater sperm defense in older males (Service and Fales 1993). The offense component of sperm displacement did not exhibit any correlated response in this study. Furthermore, females mated to males from the short-generation selection laid eggs at a faster rate than they did after being mated to long-generation males. The short-generation males apparently induced a shorter refractory period, as females remated faster after mating with short-generation males than they did after mating with long-generation males (Service and Vossbrink 1996). Additional experiments showed that the effect was caused by components in the seminal fluid, not the sperm itself.

The importance of nonsperm components of seminal fluid in sperm competition was also demonstrated clearly when Harshman and Prout (1994) showed that spermless males reduce the number of offspring sired by a previously mating male. The distinction is important because it decouples the component of sperm that affects fertilization from a component that may affect female behavior. Rice (1996) demonstrated that male components of sperm precedence evolve quickly when the female genotype is fixed and that females are nor-

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mally evolving in response to male variation in these traits.

These observations raise the long-standing and central question of population genetics: if sperm precedence is an important fitness component, how is so much variation in the trait maintained in natural populations? All else being equal, the most strongly competitive sperm alleles should go to fixation. Of course, all else may not be equal. For example, theory shows that pleiotropic effects can counterbalance the effects of sperm displacement (Prout and Clark 1996), provided the pleiotropic effects are antagonistic. The explanatory power of antagonistic pleiotropy for the maintenance of genetic variation is a subject of considerable debate (Curtsinger *et al.* 1994; Leroi *et al.* 1994). In any case, empirical studies of flies have revealed positive correlations between sperm competitive ability and fecundity (Clark *et al.* 1995; Eberhard 1996). Though such studies cannot rule out strong antagonistic effects on unmeasured fitness components, they certainly diminish the attractiveness of antagonistic pleiotropy as an explanation for maintenance of variation in sperm precedence.

Another potential factor that may allow stable maintenance of male variation is the presence of interactions with female genotypes, such that a male's sperm is most competitive in only certain female genotypes. Evidence for courtship during copulation (Eberhard 1994), for postcopulatory choice in insects (Eberhard 1996), and the mere fact that females of many species can store sperm for extended periods shows that females can be active participants in patterns of sperm storage and use. Similarly, incorporation of substances from the ejaculate into the female's somatic and ovarian tissue can be of direct nutritional value and may provide the female with strong cues regarding male quality (Pitnick *et al.* 1997). Nevertheless, we are unaware of any studies designed to ascertain whether genetic variation among females and/or genetic interaction effects between females and males can mediate gamete success in natural animal populations. Here we describe a study designed to answer the question: Is there genetic variation among females in the tendency to favor the use of the second male's sperm?

MATERIALS AND METHODS

Drosophila cultures: Females were drawn from a set of chromosome-extracted lines from Winters, California, Beltsville, Maryland and Raleigh, North Carolina. We examined 52 *X*-chromosome lines, 27 second-chromosome lines, and 38 third-chromosome lines. Lines from Beltsville were extracted in the laboratory of Brian Charlesworth (University of Chicago) and were kindly provided to us by Dr. Charlesworth. Lines from Raleigh, NC were extracted in the laboratory of Trudy Mackay (North Carolina State University) and were kindly provided by Dr. Mackay. Lines from Winters, California were collected in Summer 1995, and immediately extracted in the laboratory of Chuck Langley (University of California, Davis). A laboratory stock of bw^D (kindly supplied by Mel Green, University of California, Davis) and the third chromosome isogenic line, B3-09, derived from the Beltsville collection, were used as the source of male flies throughout the experiment. B3-09, a line previously characterized by Clark *et al.* (1995) was selected for this experiment because it had moderate "offense" and "defense" phenotypes. Our rationale was that such a line would provide more power to detect female variation than a randomly selected line from Clark *et al.* (1995), which would probably exhibit very high *P2* values. B3-09 is marked with the fourth chromosome recessive allele $sparkling^{poliert}$ as a guard against contamination.

Sperm displacement tests: Sperm displacement by a pair of male genotypes was tested in both mating orders, with the order (bw^D, B3-09) labeled experiment 1, and the order (B3-09, bw^D) experiment 2. In experiment 1, virgin 4- to 5-day-old females from each extracted line were mated first to same-aged, virgin bw^D males *en masse* for 2 hours. Females were then aspirated into individual shell vials, where they were allowed to oviposit for 2 days. These vials were designated as vial 1. Then two or three males of the same extracted line were placed in each vial for the second mating and left overnight. Second males were then removed and females were transferred by aspiration to vial 2. After 4 days females were transferred again without anesthesia to vial 3, and 1 wk later females were discarded. All three vials were scored for eye color phenotype (wild *vs.* bw^D) on the 17th day after oviposition began. If females mated with the first male, offspring from that mating would be scored in vial 1, and we could infer that the female had mated with both males from the offspring in vials 2 and 3. The fraction of the progeny in vials 2 and 3 that were sired by the second male (conditional on the female having mated with both males) is designated as the statistic *P2′*, which is potentially different from the usual statistic *P2* (Boorman and Parker 1976; Gromko *et al.* 1984a). *P2* measures the fraction of offspring sired by the second male when the matings are observed. *P2′* may differ from this in that matings are inferred to occur from the offspring counts. It is possible that differential viability and sampling effects will make *P2′* differ from *P2* (Gilchrist and Partridge 1997), so this distinction is important to bear in mind. The total count of progeny produced by each female summed over her oviposition vials is referred to as her fecundity. This too is a compound fitness component, in that differences in absolute viability of offspring will affect this measure of fecundity.

Experiment 2 is the same as experiment 1 except females were mated to B3-09 males first. The *P2′* values from the first and second experiment are labeled $P2^{+\prime}$ and $P2^{bw\prime}$, respectively. $P2^{+\prime}$ is the proportion of progeny sired by B3-09 in experiment 1 (where B3-09 is mated second), while $P2^{bw\prime}$ is the proportion of progeny sired by bw^D in experiment 2 (where bw^D is mated second).

Statistical analysis: Two-way analysis of variance was used to test the null hypotheses that the degree of sperm displacement was the same across all lines of females. Tests were done separately for sets of *X*, second, and third chromosome lines. Location of the tests (Davis *vs.* Penn State) was treated as a crossed classification (along with line), because medium and humidity were different in the two locations, and line × location interactions are equivalent to biologically interesting gene × environment interactions. Each cross was well replicated at each location. For hypothesis testing of sperm displacement effects, angular transformed *P2′* values were used to quantify mean squares within and between lines separately for experiments 1 and 2. Analysis of residuals revealed no significant trends, suggesting that the angular-transformation was appropriate. Spearman rank correlations among line means of *P2′*,

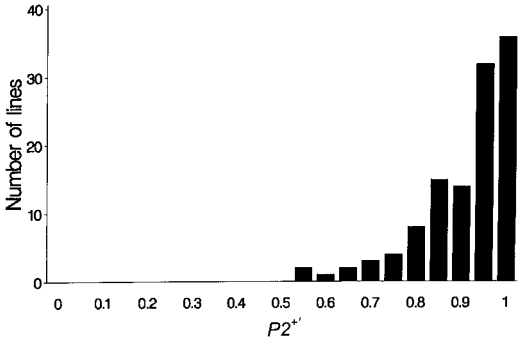

Figure 1.—Histogram of line means of $P2^{+'}$. The fraction of offspring of females that are wild type, when females mated with bw^D males followed by B3-09 males is shown. Inference of double matings is drawn from progeny phenotype arrays in three vials. A total of 119 different chromosome-extraction lines of females were examined.

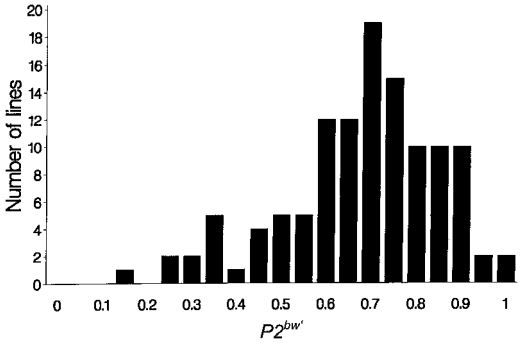

Figure 2.—Histogram of line means of $P2^{bw'}$. The fraction of offspring of females that are bw^D, when females mated with B3-09 males followed by bw^D males is shown.

fecundity, and remating tendency were also tested. Correlations between experiments 1 and 2 and between female effects seen in this study and the variation among males seen in Clark *et al.* (1995) were also tested. All statistical tests were done using the SAS STAT package version 6.03.

Variation among lines in the relative viability of $+/+$ and $bw^D/+$ progeny would confound estimates of $P^{2+'}$ and $P2^{bw'}$, so correction for viability variation was made as follows. The two orders of crossing with bw^D and B3-09 males each produce counts of *bw* and wild offspring, giving a total of 2 d.f., and both of these degrees of freedom are consumed to estimate $P^{2+'}$ and $P2^{bw'}$. However, we can correct for viability differences in a sequential manner, first estimating viabilities and then estimating sperm displacement parameters from the residuals of the viability model. This model attempts to minimize the variation attributable to sperm displacement, because it allows as much of the variation among lines to be explained by viability first and leaves only whatever variation among lines that remains to infer sperm displacement. In the absence of any sperm displacement effect, both orders of crosses produce the same ratio of *bw* : wild phenotype offspring. Let this ratio be v:1, and we estimate v as $n_{bw}/(n_+ + 1)$, where n_{bw} is the count of *bw* offspring and n_+ is the count of wild type summed over both crosses. Previability selected zygotic counts were then estimated by weighting observed counts with these viability estimates, and sperm displacement parameters were calculated from these previability estimates.

RESULTS

Distributions of $P2^{+'}$ and $P2^{bw'}$: The whole experiment entailed following the 492,036 offspring produced by 5638 single females in 16,914 vials. Figures 1 and 2 show the distributions of line means of the sperm displacement statistics, $P2^{+'}$ and $P2^{bw'}$. Females from some lines support strong displacement by B3-09 or bw^D, while other lines show little or no evidence of precedence. B3-09 males had higher mean $P2'$ values than did bw^D males (mean $P2^{+'}$ was 0.907 at Davis and 0.909 at Penn State; mean $P2^{bw'}$ was 0.724 at Davis and 0.683 at Penn State). The significance of $P2'$ differences among lines was tested by analysis of variance (Table 1) for each of the three major chromosomes. The analysis includes the variable "location" to account for possible heterogeneity between counts done at Davis and at Penn State. The location effect was nonsignificant in four of the six tests; significance of one of the two exceptions was only marginal. More importantly, none of the line × location interactions was significant, meaning that the rank order of line differences in $P2'$ estimates from Davis and Penn State were not significantly different. This consistency of results across campuses allows us to draw a very robust conclusion: highly reproducible phenotypic variation in females has major effects on patterns of sperm use among doubly-mated females. The phenotypic variation is caused by polymorphic genes on all three major chromosomes, but the mechanism whereby the variation affects sperm use remains unknown.

Repeating the analysis of Table 1 on the viability-corrected data, we found that there was a significant line effect for all three chromosomes for both sperm displacement parameters, and none of the line × location interactions were significant. With the same degrees of freedom as those in Table 1, the F statistics for $P2^{+'}$ line effect for chromosomes *1*, *2*, and *3* were 1.60 ($P < 0.05$), 17.15 ($P < 0.0001$), and 3.77 ($P < 0.0001$), respectively. Similarly, the F statistics for the $P2^{bw'}$ line effect were 1.62 ($P < 0.01$), 8.34 ($P < 0.0001$), and 22.39 ($P < 0.0001$), respectively.

We can measure the correlation between $P2^{+'}$ and $P2^{bw'}$ to ask whether variation among females results from main effects of female genotype or from male-female interactions. For example, if B3-09 sperm is used preferentially by certain females regardless of whether B3-09 males mate first or second, then we expect such females to exhibit high $P2^{+'}$ and low $P2^{bw'}$, causing a negative correlation between $P2^{+'}$ and $P2^{bw'}$. Furthermore, if there were large differences among lines in relative viabilities of *bw* and wild phenotype flies, the uncorrected data would exhibit a negative correlation between $P2^{+'}$ and $P2^{bw'}$. Also, if female genotypes vary in some way that results in differential fertilization success of first *vs.* second males, then we expect a positive correlation between $P2^{+'}$ and $P2^{bw'}$. The Spearman rank

TABLE 1

Variation among female lines in sperm displacement

	$P2^{+'}$			$P2^{bw'}$		
	d.f.	Mean square	F	d.f.	Mean square	F
Chromosome 1						
Line	51	0.2178	2.13***	49	0.3911	2.88***
Location	1	0.0007	0.01	1	3.5218	25.94**
Line × Loc*	41	0.1132	1.11	41	0.1741	1.28
Chromosome 2						
Line	26	0.2329	1.90**	26	0.3682	2.39***
Location	1	0.0123	0.10	1	0.5546	3.59
Line × Loc*	2	0.2556	2.08	2	0.0715	0.46
Chromosome 3						
Line	37	0.0829	1.24	37	0.3844	3.13***
Location	1	0.1010	1.52	1	1.0116	8.25*
Line × loc*	6	0.0577	0.87	7	0.1645	1.34

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

correlation of line means of $P2^{+'}$ *vs.* $P2^{bw'}$ is 0.265 ($P < 0.004$; Table 2). The significant positive correlation implies the presence of genetic variation affecting the propensity of females to use sperm from first *vs.* second males (as opposed to a preference for one genotype of sperm over the other). Viability correction did not remove this positive correlation between $P2^{+'}$ and $P2^{bw'}$. Although this result demonstrates a strong female effect on sperm use, it does not rule out the presence of significant interaction between sexes, and additional experiments are needed to quantify such interactions.

In contrast to the results for sperm displacement, the female fecundities did differ widely between the two laboratories (Table 3). Differences between campuses in average fecundity were probably caused by variation in fly food (Davis used a cornmeal-agar-molasses recipe, while Penn State used cornmeal-semolina-agar-sucrose). In addition to the large main effect of location, different lines responded to the different media in different ways, producing a significant line × location interaction for fecundity in all experiments.

Correlation among sperm displacement, remating frequency and fecundity: Experiment 1 revealed significant positive correlations between $P2^{+'}$ *vs.* fecundity of doubly mated females and $P2^{+'}$ *vs.* the magnitude of increase of doubly- over singly-mated females in experiments where B3-09 males mated second (Table 2). This could result from higher viability of B3-09 males. If there is displacement then a remated female will have more wild-type offspring (higher viability) and a female may be more likely to remate with a wild-type male than a bw^D male if the wild-type male has higher courtship vigor. In addition, the fraction of females that mate twice is positively correlated with the strength of sperm displacement. This latter correlation may occur if females that remate do so because they have used up more sperm from the first male before second mating. These results imply that females that respond most to

TABLE 2

Spearman correlations among line means

	$P2^{bw'}$	Fec^{+}	ΔFEC^{+}	Fec^{bw}	ΔFec^{bw}	Fracdoub
$P2^{+'}$	0.265**	0.190*	0.200*	0.066	−0.007	0.227*
$P2^{bw'}$		−0.288**	−0.217*	−0.209*	−0.144	0.244**
FEC^{+}			0.516***	0.598***	0.168	0.229*
ΔFEC^{+}				0.209*	0.139	0.206*
FEC^{bw}					0.392[c]	0.129
ΔFEC^{bw}						−0.058

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

$P2^{+'}$ is the fraction of offspring sired by the wild-type B3-09 males when they are the second to mate, and $P2^{bw'}$ is the fraction of offspring sired by the bw^D males when they are the second to mate. Fec^{+} and Fec^{bw} are the fecundities of doubly-mated females in experiments 1 and 2, respectively, and ΔFec^{+} and ΔFec^{bw} are the proportion increase in fecundity of doubly mated over singly-mated females. Fracdoub is the fraction of females that mated both males pooled over both experiments.

TABLE 3

Variation among female lines in fecundity

	Fec^+			Fec^{bw}		
	d.f.	Mean square	F	d.f.	Mean square	F
Chromosome *1*						
Line	51	9,030	5.07***	49	9,888	5.00***
Location	1	1,440,942	808.58***	1	982,495	497.25***
Line × Loc*	41	8,672	4.87***	41	12,595	6.37***
Chromosome *2*						
Line	26	2,302	3.89***	26	3,460	4.77***
Location	1	40,630	68.72***	1	153,928	212.27***
Line × Loc*	2	2,821	4.77**	2	2,155	2.97
Chromosome *3*						
Line	37	13,618	6.04***	37	9,203	4.64***
Location	1	51,384	22.79***	1	11,530	5.81*
Line × loc*	6	9,147	4.06***	7	8,872	4.47***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

B3-09 males (by having a high probability of remating with them) both have higher total fecundity and a higher fraction of offspring sired by the B3-09 males.

Experiment 2 also showed a positive correlation between remating probability and sperm displacement (Fracdoub *vs.* $P2^{bw'}$ had $r = 0.244$, $P = 0.009$). The correlation between $P2^{bw'}$ and Fec^+ (fecundity of doubly mated females in the first experiment) was significantly negative, implying that females that support stronger displacement when bw^D males mate second have lower fecundity when remated by B3-09.

Correlations between male and female effects: Are there genetic correlations between male and female components of sperm precedence? We can ask this question by testing correlations between female effects scored in this study and the male effects on sperm displacement scored by Clark *et al.* (1995). The only such correlation that was significant was between the male defense component *P1* and the female discrimination component $P2^+$ ($r = -0.319$, $P = 0.029$). This correlation implies that the lines with males whose sperm is more apt to be retained in storage (better defenders) are the lines whose females support lower levels of second male precedence.

Theoretical considerations: Consider the simplest case where differences among female genotypes in sperm discrimination are controlled by one locus. Suppose that *AA* females are indiscriminant, such that after two matings half their offspring are sired by the first male and half are sired by the second male. Provided mating is at random and no other component of selection is operating, the successful male gametes will occur in the offspring of these females in proportions *p*:*q*. What would be the dynamics of an allele, *a*, causing females to exert a preference for the second male's sperm? Females bearing the mutation would still be mated by males of genotypes *AA*, *Aa*, and *aa* in proportions p^2:$2pq$:q^2. If the females remate, the genotypes of the second males would also occur in these same proportions. First, second, and subsequent males will all produce sperm with haplotypes *A* and *a* in proportions *p*:*q*. Regardless of the number of times a female mates and the degree of sperm displacement, the proportion of successful male gametes will still remain *p*:*q*. This means that if a mutation occurs that causes females to favor sperm from the second male, unless the mutation has a pleiotropic effect on some other component of fitness, it will be entirely neutral. Such variation in discrimination in females will no longer be neutral if the discrimination depends at all on the genotypes of the males. If the discrimination does depend on the male genotype, then a matrix of sperm displacement parameters must be made for all mating combinations, and the model becomes sufficiently complex that another study must be devoted to the problem.

DISCUSSION

For both physiological and evolutionary reasons it may not be surprising that female Drosophila are not all identical in their propensity to manifest sperm displacement. Any means by which males may seek to control the use of sperm (such as chemical cues in seminal fluid) requires that females receive and process the signal. Variation in signal reception is not particularly surprising. However, the magnitude of variation that was observed is remarkable, ranging from females that support virtually no sperm displacement for sperm from B3-09 and bw^D males to females that use the sperm from the second male nearly exclusively, regardless of the mating order.

The above result may be less surprising in the context

of other aspects of female reproductive behavior in Drosophila, which are also clearly not dictated by males. Artificial selection on remating speed by females gave a strong response and each of the three major chromosomes has factors that contribute to the variation in female mating behavior (Gromko and Newport 1988; Fukui and Gromko 1991). Van Vianen and Bijlsma (1993) found that female remating speed in a laboratory population varied significantly with female genotype and with male genotypes. Remating is also delayed in *lozenge* mutant females, possibly because this mutation results in spontaneous ovulation that may serve to confuse the normal signal causing refractoriness to mating (Fuyama 1995). All these studies demonstrate that female Drosophila exhibit genetic variation in several components of reproduction.

Previous observations suggest that female Drosophila are not passive in the process of deciding which sperm are used in fertilization. One possible case of differential sperm use mediated by storage ability is seen in *Drosophila pseudoobscura*, whose males produce two distinct sperm morphologies, long and short tailed. Both classes of sperm are transferred to the female in nearly equal numbers, but Snook *et al.* (1994) demonstrated that only the long sperm persist in the storage organs, and so are used preferentially. This observation is compatible with our data on sperm displacement, as viability of sperm after storage could be a property of both the male's genotype, mediated by seminal proteins and cytoplasmic provisioning, and of the environment that the female provides for sperm storage.

Recent evidence suggests that seminal proteins play a role in directing how sperm storage occurs. Bertram *et al.* (1996) showed that the seminal protein Acp36DE becomes associated with the wall of the oviduct just anterior to the openings to the sperm storage organs and that it is also associated with the leading edge of the sperm mass. Timing of the presence of Acp36DE coincides with a critical period for sperm to be stored successfully. These observations suggest that Acp36DE may play a role in "corraling" the sperm (Bertram *et al.* 1996; Wolfner 1997), reducing sperm wastage and assuring greater numbers make it to the storage organs. Antibody and gene knockout studies may provide a test of these ideas. Given that accessory gland proteins are a signal that must be "read" by the female, it is not difficult to imagine mechanisms whereby there might be variation in females that results in differential sperm success.

Another aspect of sperm competition that females control is the overall tendency for opportunities for sperm competition to occur in the first place. Female Drosophila generally are considered to be the rate limiters in remating, such that courting males can easily be rejected by females who are uninterested in mating. The female's decision to remate depends in part on amounts of stored sperm (Gromko *et al.* 1984b), and hence to this extent is based on the female's and not the male's reproductive needs. On the other hand, the male does play some role in determining female remating behavior by transmitting seminal proteins that make females refractory to remating. One seminal protein, sex peptide, has a pronounced effect on remating latency in females (Chen 1984). The observation of species specificity in responses to sex peptides suggests that this system of molecular signaling is coevolving in the two sexes (Chen 1996). Males further assure reproductive success by stimulating oviposition with seminal proteins, including sex peptide (Chen 1984) and *Acp26Aa* (Herndon and Wolfner 1995), and female responses to these chemical cues may be variable. Sex peptide, encoded by the gene *Acp70A*, exhibits an apparently nonneutral pattern of sequence variation with two deep clades defined by an amino-acid polymorphism (Cirera and Aguadé 1997). Evidence that selection has acted on *Acp26Aa* has been obtained by noting the excess nonsynonymous divergence in the protein product of this gene (Aguadé *et al.* 1992). In general, seminal proteins seem to be evolving faster than many other proteins, suggesting the possibility that the changes are adaptively driven (Coulhart and Singh 1988; Thomas and Singh 1992). Environmental variation can also influence the likelihood that a female will remate. In particular, low-nutrient conditions will make a female less receptive to remating, provided the female has an ample supply of stored sperm (Harshman *et al.* 1988). This again implies that the female remates when her own reproductive demands suggest it is necessary.

If male components of mating success are allowed to evolve in the absence of response in females, the result is lower remating times and reduced female survivorship (Rice 1996). This result demonstrates that remating behavior and sperm competition depend on factors in both sexes, and that populations harbor genetic variation in these features. The system of male-female signaling that is involved in decisions about remating and sperm use may be highly idiosyncratic in such a way that particular combinations of allelic variants are successful while others are less so. Our observation of high levels of variation among females, and Rice's observation that evolution in females does make a difference in the male's mating and sperm competitive ability, strongly motivate studies of the genetics of these male-female interactions.

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