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Molecular Social Interactions: *Drosophila melanogaster* Seminal Fluid Proteins as a Case Study

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Abstract

Studies of social behavior generally focus on interactions between two or more individual animals. However, these interactions are not simply between whole animals, but also occur between molecules that were produced by the interacting individuals. Such "molecular social interactions" can both influence and be influenced by the organismal-level social interactions. We illustrate this by reviewing the roles played by seminal fluid proteins (Sfps) in molecular social interactions between males and females of the fruit fly *Drosophila melanogaster*. Sfps, which are produced by males and transferred to females during mating, are involved in inherently social interactions with female-derived molecules, and they influence social interactions between males and females and between a female's past and potential future mates. Here, we explore four examples of molecular social interactions involving *D. melanogaster* Sfps: processes that influence mating, sperm storage, ovulation, and ejaculate transfer. We consider the molecular and organismal players involved in each interaction and the consequences of their interplay for the reproductive success of both sexes. We conclude with a discussion of the ways in which Sfps can both shape and be shaped by (in an evolutionary sense) the molecular social interactions in which they are involved.

While studies of social behavior generally focus on observable interactions between individuals, additional "hidden" social interactions occur on the molecular level. These molecular interactions can be considered social in two ways. First, observable social interactions are influenced by molecular interactions (Ellison & Gray 2009). Second, molecules from different individuals can interact in what we call here "molecular social interactions". The molecular biology of social behavior has thus far been focused primarily on the former: molecular interactions within an animal that either induce or result from social interactions. This approach has successfully identified molecular interactors in rodent and avian affiliative behavior (e.g., reviewed in Keverne and Curley 2004; Adkins-Regan 2009), nematode feeding behavior (e.g., reviewed in de Bono and Maricq 2005), eusocial behavior (e.g., Smith *et al.* 2008), and Drosophila courtship (e.g., reviewed in Villela and Hall 2008; Dickson 2008). However, a complete molecular understanding of social behavior necessitates an understanding not just of how molecules interact within a social animal, but also how "social molecules" interact among animals. Here, we present a case study of such "molecular social interactions" that involves *D. melanogaster* seminal fluid proteins (Sfps) that are produced in the male reproductive tract, and transferred to the female along with sperm during mating. In the case of *Drosophila melanogaster* Sfps, the molecular social interactions are extensive, as gene products in seminal fluid induce short- and long-term changes in females' behavior, physiology, and gene expression, and these changes require interactions of Sfps with female-derived molecules and physiology (e.g., muscle, circulatory, and neural systems). Thus, the male- and female-derived molecules are involved

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in an inherently social interaction—that is an interaction between two individuals of the same species. Molecular social interactions affect the outcome of individual matings and occur directly between males and between males and females, and indirectly between multiple males that have mated with a given female. As we will discuss, molecular social interactions both shape, and are shaped by, observable behavioral interactions between conspecifics to affect lifetime reproductive success.

Following mating, female *D. melanogaster* display a number of behavioral and physiological changes that impact both male and female reproductive success. For example, after mating, females increase their rates of oogenesis, ovulation, egg laying, and food intake (e.g., reviewed in Chapman 2001; Chapman & Davies 2004; Wolfner 2009). Sperm from the male are stored in specialized sperm storage organs (Fig. 1), and this process may be facilitated by changes in uterine shape beginning at the onset of mating (Adams & Wolfner 2007; Avila & Wolfner, in press). For several days, mated females are less likely to accept suitors, actively fleeing or kicking any persistent male (Spieth & Ringo 1983; Ringo 1996). Within hours after mating, the female increases expression of several known anti-microbial peptide genes (Lawniczak and Begun 2004; McGraw *et al.* 2004; Peng *et al.* 2005b; Mack *et al.* 2006; Kapelnikov *et al.* 2008b), yet the realized immune response that protects the female from infections is reduced (Fedorka *et al.* 2007). The lifespan of Drosophila females is also reduced by mating (Fowler & Partridge 1989; Chapman *et al.* 1995; Civetta & Clark 2000b; Wigby & Chapman 2005; Barnes *et al.* 2008).

These changes in behavior, physiology, and gene expression may be brought about by the behavioral act of mating, by the transfer of sperm, or by other contents of the seminal fluid. Since males that do not produce sperm still elicit post-mating responses in their partners (albeit, weaker and more short-term; Manning 1962, 1967; Kalb *et al.* 1993; Xue & Noll 2000), non-sperm components of the seminal fluid must be involved in the induction of these responses. In fact, males that transfer sperm but do not transfer Sfps produced in their accessory glands (Fig. 1) fail to elicit most post- mating responses in females (Kalb *et al.* 1993, Xue & Noll 2000). It is known that the ejaculatory duct and ejaculatory bulb also produce secreted proteins that constitute part of seminal fluid, and that some of these proteins are necessary for post-mating responses (e.g., Gilbert *et al.* 1981; Meikle *et al.* 1990; Ludwig *et al.* 1991; Samakovlis *et al.* 1991; Lung & Wolfner 2001; Lung *et al.* 2001; Iida & Cavener 2004; Bretman *et al.* submitted). These results together demonstrate that sperm and Sfps are both required to induce long-term post mating responses in females (Manning 1962, 1967; Kalb *et al.* 1993; Heifetz *et al.* 2001; Kubli 2003).

Sfps comprise an elaborate intraspecific signaling system. Of the more than 180 predicted extracellular proteins present in the reproductive secretory glands of male *D. melanogaster*, over 100 have been confirmed to be transferred to the female along with sperm (e.g., reviewed in Ravi Ram & Wolfner 2007a and Chapman 2008; see also Walker *et al.* 2006; Chintapalli *et al.* 2007; Findlay *et al.* 2008, 2009; Takemori & Yamamoto 2009). Many of the transferred proteins fall into conserved protein classes found in the seminal fluid of most animals studied to date and include proteases, protease inhibitors, acid lipases, cysteine rich secretory proteins (CRISPs), and lectins (Mueller *et al.* 2004; Ravi Ram & Wolfner 2007a). Other, less-expected, classes of Sfps such as odorant binding proteins suggest a possible role for small molecules in inducing female post-mating responses (Findlay *et al.* 2008). Odorant binding proteins are known to shuttle pheromones or other small molecules to odorant receptors in the olfactory system (e.g., reviewed in Pelosi *et al.* 2005). Presence of predicted odorant-binding proteins in the seminal fluids suggests that they may play a similar shuttling role for molecules once within the female reproductive tract. The wide variety of protein classes present in the seminal fluid suggests that Sfps take part in a complex series of interactions within the mated female and do not just fulfill a single simple role.

Upon transfer to females, Sfps target to specific tissues which are likely to relate to their function within the mated female (e.g., Meikle *et al.* 1990; Betram *et al.* 1996; Lung & Wolfner 1999; Heifetz *et al.* 2000; Peng *et al.* 2005a; Ravi Ram *et al.* 2005; Fig. 1). For example, proteins associated with sperm storage and retention have been detected in the female sperm storage organs, and ovulin, which stimulates ovulation, targets to the base of the ovaries (Heifetz *et al.* 2000; Ravi Ram *et al.* 2005). Several Sfps, including ovulin, have also been detected in the circulatory system of mated females from where they can gain access to the brain and/or endocrine systems (Meikle *et al.* 1990; Lung & Wolfner 1999; Ravi Ram *et al.* 2005; Pilpel *et al.* 2008) and thus, potentially, affect female behavior. Further studies of the targets of Sfps may help to uncover their functions in the mated female.

Drosophila melanogaster Sfps provide an excellent model system in which to investigate molecular social interactions, due to the powerful tools available in this species. Mutant or transgenic males in which Sfps are increased, decreased, or eliminated can be used to dissect the effect(s) of particular Sfps on female post-mating responses (e.g., Gilbert *et al.* 1981; Herndon *et al.* 1995; Neubaum & Wolfner 1999; Chapman *et al.* 2003; Liu & Kubli 2003; Iida & Cavener 2004; Ravi Ram *et al.* 2006; Ravi Ram & Wolfner 2007b; Mueller *et al.* 2008; Wong *et al.* 2008a, Bretman *et al.*, submitted). A large collection of freely available genomic databases (e.g., FlyBase; Fly atlas, Chintapalli *et al.* 2007) facilitate rapid progress as well. These techniques and tools, along with studies associating allelic variation in Sfps with variation in their effects, have led to a greater understanding of the molecular social interactions taking place between all of the players involved in Drosophila mating (e.g., reviewed in Wolfner 2009). Furthermore, studies of *D. melanogaster* Sfps are likely to provide insights into the molecular social interactions of other species given that Sfps impact female post-mating responses across a wide taxonomic range (e.g., reviewed in Gillott 2003; Poiani 2006).

We will use two particularly well-studied Sfps, the sex peptide (SP) and ovulin, as examples in the following sections to illustrate the way in which Sfps act as molecular mediators for social interactions. SP is a small peptide that affects female response to male courtship, oogenesis, ovulation, immune response, feeding, and juvenile hormone production (Moshitzky *et al.* 1996; Chapman et al 2003; Kubli 2003; Liu & Kubli *et al.* 2003; Carvalho *et al.* 2006; Domanitskaya *et al.* 2007). Ovulin is a large pro-hormone that increases ovulation during the first 24 hours after mating. Further details of both these proteins, as well as the social context in which they exert their functions, are discussed herein.

While over 180 known or putative *D. melanogaster* Sfps have been identified, only one female receptor to an Sfp is known: the sex peptide receptor, a G protein-coupled receptor expressed in the female reproductive tract and nervous system (Yapici *et al.* 2008). However, we expect that many Sfps interact with female-derived proteins. Some female-derived proteins that play a role in female post-mating behavior and physiology have been identified and will be discussed in this review, but their interactions with Sfps remain speculative at this time.

Several approaches have been used to identify genes in females whose products mediate response to, are regulated by, or otherwise interact with, Sfps. Proteins produced in the female sperm storage organs have been identified and have the potential to interact with Sfps (Lawniczak & Begun 2007; Allen & Spradling 2008; Prokupek *et al.* 2008, 2009). Microarray data from whole flies, heads, or reproductive tract tissues have shown that different aspects of mating, including Sfps, cause a transcriptional response in the female after mating (McGraw *et al.* 2004, 2008, 2009; Lawniczak & Begun 2004, 2007; Peng *et al.* 2005b; Mack *et al.* 2006; Kapelnikov *et al.* 2008b; Innocenti & Morrow 2009), though it is

not likely that most post-mating responses are due to mating-induced transcription. Transcriptional changes of the largest magnitude are seen by about 6 to 8 hours after mating, a time by which most Sfps are no longer detectable in the female. Therefore, Sfps may set into motion the transcriptional modification of the female, but the genes regulated by these modifications are less likely to encode Sfp-interacting proteins than the genes expressed by the female prior to mating. Nevertheless, these mating-regulated genes likely are players in the next steps of the molecular social interactions. To fully understand the molecular social interactions in which Sfps are involved, we must identify female interactors, their functions, and how they have co-evolved with their male-derived partners.

Drosophila seminal fluid proteins and molecular social interactions: An interplay in four acts

In this section, we present four examples of molecular social interactions in the format of acts in a play involving *D. melanogaster* Sfps and those molecules with which they interact, derived from different actors (a female and her past, present, and potential future mates), that occur at different times (before, during, and after copulation), in different settings (outside and inside the female's body, within the female reproductive tract, nervous and circulatory systems), and on different time scales (immediately and over the course of evolution). We also discuss the potential influences of each of these interactions on male and female reproductive success. For many of the processes that we describe, there are other known molecular actors, but they are not known to interact with Sfps and therefore are not included in this review for simplicity. The curtain is rising.

Setting the stage: Transfer and fate of sperm and seminal fluid proteins

Copulation duration—Copulation duration is generally ~ 20 min in *D. melanogaster* (Gilchrist & Partridge 2000) and is influenced by several factors that include: female cuticular hydrocarbons (Marcillac & Ferveur 2004), female mating history (Friberg 2006; Singh& Singh 2004), male and female body size (LeFranc & Bundgaard 2000), and the social environment before and during copulation (see below; Bretman *et al.* 2009; Wigby *et al.* 2009). Copulation duration in *D. melanogaster* is generally longer than necessary for sperm transfer and may be subject to sexual conflict (Gilchrist & Partridge 2000; Mazzi *et al.* 2009).

Mating plug—Shortly after the start of mating, Sfps from the male's ejaculatory bulb begin to form a mating plug in the female's uterus (Ludwig *et al.* 1991; Bretman *et al.*, submitted). By the end of mating, Sfps from the male accessory glands will have completed the mating plug (Lung & Wolfner 2001). The purpose of the mating plug is unknown: it is unlikely that it physically and permanently blocks subsequent inseminations of the female as it is only present for a few hours, yet recent evidence suggests that an Sfp in the mating plug may prevent females from re-mating shortly after their initial mating (Bretman *et al.*, submitted). The mating plug may also be a way to assist with sperm storage, including potentially to protect the male's ejaculate from being expelled by the female before sperm have been stored.

Sperm—The time of sperm transfer varies between individual matings and between different fly strains (Fowler 1973), but appears to be complete by eight minutes after the start of mating (Fowler 1973; Lung & Wolfner 2001; Gilchrist & Partridge 2000). Sperm transfer itself takes only about 1 minute to complete (Gilchrist & Partridge 2000). Upon transfer to the female, sperm move through the uterus and into specialized sperm storage organs, the paired spermathecae and the seminal receptacle. The sperm storage process begins during mating and continues for a few hours (Bloch Qazi *et al.* 2003). Sperm can

remain viable in the female's sperm storage organs for approximately two weeks (Kaufmann & Demerec 1942; Lefevre & Jonsson 1962). Sperm from multiple males can co-occur in the sperm storage organs of females because *D. melanogaster* females, as in other animal species, commonly mate with multiple males in nature (e.g., see Boorman & Parker 1979; Birkhead & Møller 1998; Harshman & Clark 1998; Imhof *et al.* 1998; Simmons 2001). When two males mate successively with the same female, there is an opportunity for sperm competition as the gametes of the different males compete to fertilize the female's eggs.

Act 1: To mate or not to mate

Setting: The mating arena

Time: Before copulation

Organismal actors: Male, female, female's previous mate (in absentia)

Known molecular actors: SP and SPR, four supporting Sfps (cuticular and ejaculatory bulb pheromones which also participate in this Act will not be discussed; for review see e.g., Hall 1994; Ferveur 1997; Greenspan & Ferveur 2000; Markow & O'Grady 2005; Dickson 2008; Villella & Hall 2008).

Effect: Sfps received from one mate affect the outcome of subsequent intersexual interactions

When a male and female *D. melanogaster* encounter each other, the outcome is often a series of courtship steps (Hall 1994; Greenspan & Ferveur 2000) followed by copulation. But, what factors determine whether a male will court and attempt to copulate and how a female responds to the solicitations by males? In *D. melanogaster*, the decision by males to court and the response of females to male courtship depends, in part, on female mating status: males that have previously courted mated females are less likely subsequently to court a mated female than an unmated female, and mated females are less likely than unmated females to copulate with a courting male. The difference in male courtship behavior in response to female mating status is due, in part, to molecular social interactions between the male, the female, and the female's previous mates (e.g. Tompkins *et al.* 1983; Scott 1986; Ferveur 1997; Greenspan & Ferveur 2000; Markow & O'Grady 2005; Ejima *et al.* 2007; Dickson 2008; Villella & Hall 2008; Yew *et al.* 2009), but male courtship intensity does not depend on whether a female received Sfps from her previous mate (Tram & Wolfner 1998).

Mating-dependent changes in female responses to male courtship (i.e., whether the female copulates or not) have also been attributed to molecular social interactions. For example, females that received the Sfp SP during mating were much more likely to exhibit rejection behaviors (e.g., extrude ovipositor; Fig. 2) and less likely to copulate with a courting male than females that did not receive SP (Chen *et al.* 1988; Aigaki *et al.* 1991; Chapman *et al.* 2003; Liu & Kubli 2003; Soller *et al.* 2006; Yapici *et al.* 2008). Recently, another protein, PebII, has been found to reduce female receptivity immediately after mating (Bretman *et al.*, submitted). SP interacts with a G protein-coupled receptor (sex peptide receptor or SPR) in the female nervous system to effect female response to male courtship (Yapici *et al.* 2008). SP and SPR act through approximately 6–8 fruitless- and pickpocket-expressing multidentritic sensory neurons (Hasemeyer *et al.* 2009; Yang *et al.* 2009) located along the reproductive tract. Without SPR, females do not show the SP-induced post-mating changes in egg-laying or in rejection behavior, indicating the primary role for this molecule in these aspects of the female's post-mating responses (Yapici *et al.* 2008).

The effects of SP are long-lasting because SP is maintained in the mated female's sperm storage organs for days after mating and is gradually released (Peng *et al.* 2005a). SP binds

to sperm, which apparently stabilizes the peptide. As long as the female contains sperm with bound SP, she is likely to display rejection behavior in response to mating attempts by other males. This long-term effect of SP allows a male to affect social interactions, in absentia, for several days after mating has ended (Chapman *et al.* 2003; Liu & Kubli 2003). At least four additional Sfps are necessary for this facet of SP function. These Sfps, the predicted cysteine rich secretory protein CG17575, the predicted protease CG9997 and the gene duplicate lectins CG1652 and CG1656 work in an interdependent network to localize SP to the sperm storage organs of the females and/or bound to sperm (Ravi Ram & Wolfner 2009). In contrast to the SP, these four Sfps do not persist in females for days. Rather, they exert their effects soon after mating and then disappear. It is the persistence of SP that maintains the female's post-mating responses of egg-laying and re-mating rejection. If SP, or any of the network Sfps, is not transferred to a female, she resumes being willing to mate with a subsequent courting male within a day of her first mating (Ravi Ram & Wolfner 2007b).

Whether, when, and how frequently a female *D. melanogaster* re-mates affects not only the reproductive success of her previous and potential subsequent mates but also her own reproductive success. On the male side, it is generally in a female's previous mate(s)' interest that she either delays or forgoes re-mating (so that his sperm will not be displaced), while it is in her current suitor's interest that she does re-mate (so that he can sire offspring). On the female side, as in other species, repeated mating can be costly in terms of lifetime reproductive success (Fowler & Partridge 1989; Arnqvist & Nilsson 2000; Chapman *et al.* 1995, 2000), but repeated matings can also increase the reproductive success of a female's daughters (Priest *et al.* 2008a) or benefit the female in other ways (e.g., avoiding infertile matings or genetic incompatibility; Arnqvist & Nilsson 2000). Because matings can be costly to females (Fowler & Partridge, 1989), there is the potential for intersexual conflict between females and their suitors over re-mating (Arnqvist & Rowe 2005).

The example described in Act 1 demonstrates that the transfer of molecules during mating serves as a means of interaction between a female and her mate, between a female and the subsequent males she encounters, and between the female's multiple mating partners. Thus, the outcome of an encounter between male and female can be said to be influenced by both inter- and intrasexual molecular social interactions and can potentially result in both intra- and intersexual conflict over female re-mating.

Act 2: Should sperm stay or should they go?

Setting: The female reproductive tract

Time: During and after copulation

Organismal/cellular actors: Female, sperm

Known molecular actors: Acp36DE and other Sfps, proteins from the female sperm storage organs

Effect: Sfps affect conformation of uterus, sperm storage, and success in sperm competition

Once copulation begins, a series of molecular social interactions unfolds within the female's reproductive tract. A subset of these interactions determines the fate of sperm in the female reproductive tract and, thereby, influences male reproductive success in two ways: (i) storage and subsequent release of sperm from storage determine which male's sperm fertilize a female's eggs and (ii) sperm storage is necessary for the persistence of post-mating changes in female behavior (e.g., by serving as a source of SP; see Act 1 above; Manning 1962, 1967; Peng *et al.* 2005a; Bloch Qazi *et al.* 2003 for review). Thus, getting sperm into storage is of vital importance to males, and male contributions appear to play an

active role in making sure this happens. Time is also of the essence: sperm that remain unstored in the female's uterus when she lays her first egg post-mating may be expelled from the female (Fowler 1973; Gilbert 1981), creating a potential tradeoff between the timing of sperm storage and egg output.

The mechanism by which sperm move through the female reproductive tract presents some unusual considerations. Unlike mammalian sperm, *D. melanogaster* sperm are extremely long relative to the female's body size (roughly half the length of the entire female; Pitnick *et al.* 1995) and thus may be constrained from propelling through the female reproductive tract. Molecular social interactions appear to play a role in solving this conundrum. Recent evidence suggests that sperm movement through the female reproductive tract is mediated by uterine conformational changes that themselves are mediated by Sfps (Adams & Wolfner 2007; Avila & Wolfner, In press). Before mating, the female reproductive tract is in a "closed" conformation: the lumen is tightly compacted and folded into an S-shape and the entrance to the sperm storage organs is physically blocked by a flap of the uterine wall (Adams & Wolfner 2007). Within the first few minutes of mating, the posterior uterus begins to open up and eventually the entrance to the sperm storage organs becomes unblocked, with these changes continuing for approximately 45 minutes after the start of mating until the entire uterine lumen is open. During this time, the sperm mass is moved up the reproductive tract (perhaps as a result of the conformational changes) and the sperm mass becomes situated at the site from which sperm can enter storage.

The changes in uterine conformation are not simply the result of the mechanics of mating, nor do they require sperm to take place (Adams & Wolfner 2007). Sfps, and not sperm, are required for these conformational changes to occur (Adams & Wolfner 2007). One particular Sfp, the glycoprotein Acp36DE, is needed for the reproductive tract to proceed past the mid-stages (Avila & Wolfner, in press). In the absence of Acp36DE, the female tract only opens part way, retaining a constriction near the anterior end of the uterus, which appears to prevent the majority of sperm from being actively stored. Consistent with this hypothesis, Acp36DE is also required for efficient and complete sperm storage, with only 10–50% of the sperm stored in a wild-type mating being stored when females mate to Acp36DE null males (Neubaum & Wolfner 1999; Bloch Qazi & Wolfner 2003). Presumably as a result of its effects on sperm storage, Acp36DE also impacts a male's ability to compete for fertilizations (Chapman *et al.* 2000).

The female molecules and physiology involved in the uterine conformational changes are currently unknown. One plausible mechanism by which Sfps effect uterine conformational changes is by stimulating the female's nervous system to control the release of neuromodulators that induce muscle contraction or relaxation. The uterus is surrounded by both circular and longitudinal muscles (Heifetz & Wolfner 2004; Middleton *et al.* 2006; Adams & Wolfner 2007). Mating enhances muscle differentiation and innervation in the female reproductive tract and causes vesicle release from nerve termini that innervate the female reproductive tract (Heifetz & Wolfner, 2004: Kapelnikov *et al.* 2008a). Sfps, especially Acp36DE, should be tested for a role in inducing such vesicle release.

Uterine conformational changes are not the only way in which male and female molecules may impact sperm storage. For example, glucose dehydrogenase, a protein produced in the reproductive tracts of both males and females, affects both the number and distribution of sperm stored in the paired spermathecae (Iida & Cavener 2004). Another Sfp, Esterase-6, influences the timing and rate of sperm storage (Gilbert *et al.* 1981). Sperm proteins, such as a polycystin-2 like protein found on the flagella, are also necessary for the storage of sperm that have been transferred to the female (Gao *et al.* 2003; Watnick *et al.* 2003).

The influence of molecular social interactions on sperm use patterns does not end with sperm entry into storage. Sperm must be maintained in storage and then released at an appropriate time to fertilize eggs that are released from the ovary into the oviducts and uterus (see Act 3 below). Females must receive Sfps to use sperm from a male. Females that do not receive Sfps during mating will store a small number of sperm, but those sperm will not be used to fertilize eggs (Hihara 1981; Xue & Noll 2000). Interestingly, Sfps received by a female in a previous or subsequent mating can partially restore the fertilizing ability of another mate's sperm (Xue & Noll 2000; Chapman *et al.* 2000). In *in vitro* assays, *D. melanogaster* seminal fluid also helps to maintain sperm viability over a short-time scale (1hr, Holman 2009), but it is not known whether this is also the case within the mated female. We do not yet know the male and female molecules involved in maintaining sperm viability in the female but secretions from the spermathecae appear to be necessary for prolonged sperm viability (Anderson 1945; Boulétreau-Merle 1977; Allen & Spradling 2008). For example, sperm release from storage depends, in part, on spermathecal proteins (Iida & Cavener 2004) and on the presence of eggs in the female reproductive tract: the transition from sperm storage to sperm release is delayed in egg-less females (Bloch Qazi & Wolfner 2003). Retention and release of stored sperm also depends, in part, on Sfps. Six Sfps have been identified that are necessary for retention or release of sperm from the female sperm storage organs (Gilbert *et al.* 1981; Ravi Ram & Wolfner 2007b; Wong *et al.* 2008a). Interestingly, four of the Sfps that affect sperm release are the same proteins described in Act 1 that are needed to get SP into the female's sperm storage organs (Ravi Ram & Wolfner 2009). Mechanistic connections (if any) between the multiple roles of these proteins are not yet known. Furthermore, the interactions between male- and female-derived molecules that influence sperm release have yet to be explored.

The storage, maintenance, and timely release of sperm in the female's sperm storage organs have important consequences for both male and female reproductive success. Clearly, a male will have the opportunity to fertilize a female's eggs only if his sperm are stored, maintained, and released when appropriate. For a female, storing sperm allows her eggs to be fertilized for at least two weeks (the duration of sperm storage) even if she does not encounter a male (Kaufmann & Demerec 1942; Lefevre & Jonsson 1962). Sperm storage also allows a female to delay egg-laying until she finds suitable environmental conditions for offspring development. Females can also benefit by storing sperm of multiple males, which provides opportunities for both sperm competition (Boorman & Parker 1979; Simmons 2001) and cryptic female choice (Eberhard 1996).

Act 3: Pas de deux

Setting: The female reproductive tract

Time: During and after copulation

Organismal/cellular actors: Eggs, neurons, muscles

Known molecular actor: Ovulin and Supporting Sfps, Octopamine, OAMB

Effect: Processing of an ovulation-stimulating Sfp requires both male and female contributions

In insects, ovulation behavior is especially sensitive to male-female interactions, as ovulation dramatically increases after mating. Unmated *D. melanogaster* females will lay a few unfertilized eggs per day (~2), whereas a mated female will lay several dozen eggs within the first day after mating (~50). This change in number of eggs laid is due to changes in both oogenesis and ovulation and both of these processes are influenced by Sfps (Prout & Clark 2000; Chapman 2001). The stimulation of oogenesis appears to result, in part, from an SP-induced increase in juvenile hormone levels and from oogenesis progressing past an

arrest point; these will not be discussed further here (Moshitzky *et al.* 1996; Soller *et al.* 1999). The changes in ovulation rate are influenced by a different Sfp, ovulin, and are discussed below.

During ovulation, mature oocytes are released from the ovary and pass through the oviduct via muscle contractions requiring coordination among the nervous system, muscle, and epithelium of the female reproductive tract (Middleton *et al.* 2006; Rodríguez-Valentín *et al.* 2006; Lange 2009). This coordination ultimately results in oviposition on the food substrate (Yang *et al.* 2008). Female molecules necessary for the movement of eggs through the female reproductive tract have been identified. For example, the biogenic amine octopamine and one of its receptors (OAMB) are required for ovulation (Monastirioti 1996, 2003; Lee *et al.* 2003, 2009; Cole *et al.* 2005). Neurons producing neuromodulators (e.g., octopamine) and neuropeptides (e.g., ILP7) innervate the muscles and epithelium of the female reproductive tract (Monastirioti 2003; Middleton *et al.* 2006; Rodríguez-Valentín *et al.* 2006; Lee *et al.* 2009; Yang *et al.* 2009). Sfps modulate release of vesicles from nerve termini in the female reproductive tract (Heifetz & Wolfner 2004), which may include levels of octopamine release. Thus, Sfps may regulate ovulation by modulating the octopamine system. Furthermore, vesicle transport proteins in the p24 family, are necessary for oviposition in females. In female *D. melanogaster* mutant for these genes, failure to oviposit results in eggs remaining lodged in the uterus, with further mature eggs backing up in the upper reproductive tract (Carney & Taylor 2003; Bartoszewski *et al.* 2004; Boltz *et al.* 2007). Whether and how these proteins interact with Sfps is currently unknown.

On the male side, the transfer of sperm and Sfps is required to trigger the full response of increased egg-laying (Heifetz et al 2001). Ovulin contributes to the dramatic increase in egg-laying rates observed within 24 hours after mating (Herndon & Wolfner 1995; Heifetz *et al.* 2000). Specifically, as described in the introduction, ovulin increases ovulation rate (Heifetz *et al.* 2000). Ovulin's mechanism of action is currently an area of active study. After mating, most of the transferred ovulin remains in the reproductive tract, and targets to the base of the ovaries (Monsma *et al.* 1990; Heifetz *et al.* 2000). However, a substantial amount (about 10% of the ovulin that the female receives) crosses a special transiently-permeable region of the reproductive tract wall to enter the circulatory system (Lung & Wolfner 1999; Monsma *et al.* 1990). In principle, ovulin could act from either place to stimulate ovulation. From its site at the base of the ovary it might directly stimulate muscle contraction or release or action of neuromodulators such as octopamine (Lee *et al.* 2003, 2009; Monastirioti 2003); from the circulatory system, it could act from the outside of the musculature to effect changes in contraction or could act indirectly by binding to neural or endocrine targets removed from the reproductive system and causing activity in those that then affect the reproductive tract.

Ovulin is the object of a particularly interesting molecular social interaction that may affect ovulation. This interaction appears to be a pas de deux between male and female molecules resulting in the systematic processing of ovulin into smaller pieces (Monsma & Wolfner 1988; Park & Wolfner 1995). Upon transfer, ovulin within the female reproductive tract is processed into several cleavage products (as are several other Sfps; Bertram *et al.* 1996; Ravi Ram *et al.* 2006; Fig. 3). It is unknown what role processing of ovulin plays in its effect on ovulation, but indirect evidence suggests that, as with prohormones in other species (e.g., Hook *et al.* 1994; Chen & Raikhel 1996; Garden *et al.* 1998), processing may increase ovulin's activity: expression of ovulin's cleavage products in unmated females stimulates a higher rate of ovulation than does expression of full-length ovulin (Heifetz *et al.* 2005). One of these bioactive cleavage products contains regions with sequence similarities to the egg-laying hormone of the mollusk *Aplysia* (Monsma & Wolfner 1988). The different ovulin cleavage products appear in the same pattern and over approximately the same time scale in

matings between wild-type flies, beginning ~10 minutes after the start of mating and ending about 2 hours later, by which time ovulin is fully processed (Park & Wolfner 1995). Thus, this time-dependent processing of ovulin could potentially provide a mechanism by which ovulation rate is regulated.

Ovulin cleavage requires male contributions but only occurs after ovulin has been transferred to the female (Park & Wolfner 1995), suggesting that female contributions or infrastructure are necessary for processing to occur. One molecule necessary for ovulin's cleavage is the male-derived Sfp CG11864 (Ravi Ram *et al.* 2006). This protein is a predicted metalloprotease that itself is cleaved from a predicted inactive form to the predicted active form while in transit to the female, by one or more other Sfps (Ravi Ram, LaFlamme, Wolfner, unpubl. data). Though CG11864 is thought to be active while still in the male, there is no evidence that it can cleave ovulin before it is in the female's reproductive tract. As yet unidentified female-derived factors may also be required for ovulin's cleavage. This processing represents a fascinating example of a highly coordinated molecular social interaction (and biochemical pathway) that begins in the male, ends within the female, and may impact both male and female reproductive success. But, our knowledge of ovulin processing also raises more questions. Why is this process so tightly regulated? Are the contributions of males and female to ovulin processing acting in conflict or in cooperation? How does this molecular social interaction affect the reproductive success of both sexes?

Before we can fully understand the molecular social interactions that occur during the proteolytic processing of ovulin, we must identify the female factors that regulate this processing. The most promising molecular candidates for ovulin processing are proteolysis regulators. Several studies of female reproductive tract proteins have discovered female-secreted proteases and protease inhibitors that are regulated in response to mating (Arbeitman *et al.* 2002; Swanson *et al.* 2004; Lawniczak & Begun 2004, 2007; McGraw *et al.* 2004; Mack *et al.* 2006; Chintapalli *et al.* 2007; Kapelnikov *et al.* 2008b). It is likely that some of these are involved in proteolytic pathways that begin in the male and end in the female, such as that for the processing of ovulin, and these proteins are currently under investigation. Other female-derived influences on ovulin processing could potentially include small molecules and the physiological environment of the female reproductive tract (e.g., pH and other ionic conditions).

Irrespective of the specific molecular actors involved, it is clear that both females and their mates influence the female's rate of ovulation shortly after mating. Thus, it is not surprising that ovulation rate at this time has important consequences for both sexes. At this point, it is important to recall from Act 2 that the first egg that moves through the uterus after mating appears to push out sperm that have not moved into storage. Therefore, ovulation shortly after mating influences not only progeny production but also subsequent male fertilization success. In a female's first mating, the interest of the sexes in relation to ovulation rate may be congruent, as ovulation shortly after mating appears to clear stale eggs from the uterus, stimulate oogenesis, and coordinate egg production and sperm storage (Chapman *et al.* 2001). However, in subsequent matings, ovulation rate shortly after mating could be the subject of intersexual conflict. Males may benefit by temporarily delaying ovulation to maximize sperm storage, whereas females may benefit by adjusting the timing of ovulation to influence the subsequent fertilization success of her mate (e.g., by ovulating quickly to eject a male's sperm or delaying ovulation to maximize sperm storage). Thus far, ovulation rate has only been examined after a female's first mating. Future research should test for evidence of cryptic female choice (Eberhard 1996) and sexual conflict (Arnqvist & Rowe 2005) over ovulation rate in subsequent matings. Certainly, the fact that the proportion of unfertilized eggs laid increases after a female's subsequent mating (Prout & Clark 2000)

suggests that coordination of the release of sperm and eggs is being modulated by the female, the male, or both.

Act 4: Two is company, three is a crowd

Setting: The mating arena

Time: During and after copulation

Organismal actors: Entire cast!

Known molecular actors: SP, SPR, ovulin, probably other Sfps and female-derived molecules

Effect: Social environment and female mating status affect mating duration, amount of Sfps transferred, and male competitive reproductive success

Although *D. melanogaster* copulations may look very much the same to the outside observer, copulations can vary substantially in the amount of Sfps transferred (Sirot *et al.* 2009). The causes and consequences of this variation have only recently begun to be explored in *D. melanogaster*, where the social environment influences the amount of Sfps transferred (Friberg 2006; Linklater *et al.* 2007; Bretman *et al.* 2009; Wigby *et al.* 2009). In particular, the quantity of Sfps transferred by a male depends on whether other males are present before and during copulation: males transfer more of the two Sfps tested (SP and ovulin; Fig. 4) when they are in the presence of another male before and during copulation than when they are alone with the female (Wigby *et al.* 2009). Thus, males can adjust the amount of Sfps they transfer in response to the potential level of sperm competition. Males also appear to adjust Sfp allocation in response to another metric of sperm competition level: whether the male perceives that the female has previously mated or not (Friberg 2006). Such "strategic allocation" of Sfps is predicted by theory (Cameron *et al.* 2007) and has also been demonstrated for the sperm component of the ejaculate in many species (reviewed in Wedell *et al.* 2002). The influences of other social environmental variables (e.g., operational sex ratio, female and male density) on Sfp transfer have yet to be systematically explored, but some of the variation in Sfp transfer by males results from depletion with successive matings and replenishment thereafter (DiBenedetto *et al.* 1990; Sirot *et al.* 2009).

Variation in Sfp transfer appears to result in corresponding variation in female post-mating responses. In the extreme situation in which females receive no Sfps during mating, females show little or no changes in post-mating behavior and males fertilize no eggs (Xue & Noll 2000). In less extreme cases, for example when males transfer more Sfps in response to potential sperm competition (Bretman *et al.* 2009; Wigby *et al.* 2009) or selection for large accessory gland size (Wigby *et al.* 2009), female post-mating responses are more pronounced (e.g., higher egg production, longer delay in re-mating) and males sire more offspring. Similarly, females that receive lower-than-normal amounts of Sfps because their mates had recently mated show less pronounced post-mating responses than females mated to unmated males (Hihara 1981).

The consequences of variation in Sfp transfer for male reproductive success appear clear: males experience higher competitive reproductive success when they transfer more Sfps (Wigby *et al.* 2009). The consequences of variation in Sfp transfer for females are more complex. Lifetime offspring production of females that repeatedly receive SP is lower than that of females that mate but do not receive SP (Wigby & Chapman 2005). However, females that receive the full suite of Sfps produce daughters with higher lifetime offspring production than females that mate but do not receive Sfps (Priest *et al.* 2008b; but see also Long *et al.* 2009; Priest *et al.* 2009). More research is needed to determine how subtle

variation in amount of Sfps received affects female offspring and grand-offspring production.

Finally, it is important to point out that, since Sfp production appears to be both costly (Wigby *et al.* 2009) and limiting (Hihara *et al.* 1981; Sirot *et al.* 2009), male investment in individual ejaculates may be greater than previously appreciated (Cordts & Partridge 1996). These costs, together with the costs of courtship (Cordts & Partridge 1996), may help to explain the benefits to males of selectively pursuing (or not pursuing) certain females based on phenotype or mating status (e.g., Byrne & Rice 2006) and relates to the question posed in Act 1: to mate or not to mate.

Behind the scenes: Evolutionary Dynamics

Behind the scenes of the main mating arena, a number of important factors have been setting the stage. Some of these are developmental genes that determine the sex of the fly, such as Sxl (e.g., reviewed in Cline & Meyer 1996; Christiansen, *et al.* 2002; Manoli, *et al.* 2006) or its downstream effectors, such as dsf and fru, that are needed for sex-specific behavior and neural development (e.g., reviewed in Manoli, *et al.* 2006, Shirangi & McKeown 2006; Yamamoto 2007; Dickson 2008). Other genes are required for proper development of the genital tract and fertility; examples include: HR39 (Allen & Spradling 2008) or lz (Anderson 1945) in females, and a late function of prd (Xue & Noll 2002) in males. Though the actions of these genes are thus ultimately important in mating interactions of Drosophila, they have a limited role in the types of molecular social interactions we have discussed and will not be discussed further here. On the other hand, evolutionary processes shape all genes in the genome and have a specific role in affecting mating behavior and related social interactions in Drosophila. These are discussed further, below.

Molecular social interactions, such as those discussed earlier, influence not only the individual interacting flies but also the evolution of the interacting molecules. Over time, evolutionary responses to the interactions between Sfps and female molecules may leave signatures on the genes' sequences themselves (some examples in Drosophila include: Clark 2002; Begun & Lindfors 2005; Mueller, *et al.* 2005; Clark, *et al.* 2006; Schully & Hellberg 2006; Haerty, *et al.* 2007; Kelleher, *et al.* 2007; Wong, *et al.* 2008b; see Swanson & Vacquier 2002, Panhuis, *et al.* 2006 for reviews). Genes encoding Drosophila Sfps, like reproduction-related genes across a wide range of taxa, are more likely to show evidence of positive selection than are most other groups of genes, with the notable exception of immunity-related genes (Swanson, *et al.* 2001; Clark 2002; Begun and Lindfors 2005; Mueller *et al.* 2005; Schully & Hellberg 2006; Haerty *et al.* 2007; Kelleher, *et al.* 2007; Wong, *et al.* 2008b; Dean *et al.* 2008; See Clark *et al.* 2006; Swanson & Vacquier 2002; Panhuis, *et al.* 2006 for review). It has been suggested that rapid evolution of these genes may result from sexual selection (e.g., cryptic female choice, sperm competition) and/or sexual conflict (reviewed in Swanson & Vacquier 2002).

An example of the interplay between mechanisms of action and evolutionary dynamics is ovulin. Earlier, we presented ovulin processing as an example of a highly coordinated process between the sexes that begins in the male and ends in the female. Thus, ovulin's action to increase ovulation soon after mating would appear to benefit both sexes, but further consideration raises some questions. Is ovulin a mechanism by which the male controls the female's physiology, or does the female take advantage of the male's ovulin contribution for her own benefit? Has ovulin's evolution been driven by sexual conflict? Or, is ovulin's function truly beneficial to both sexes? Though we currently cannot answer many of the important questions about ovulin, such as why it is processed or how processing of this

protein affects its function, we may be able to gain some clues from the evolutionary history of the protein.

Consistent with its important function in reproduction, ovulin contains short regions that are highly-conserved between Drosophila species (Wong *et al.* 2006, and submitted), with its C-terminus in particular containing several conserved motifs. Genetic and biochemical studies indicate that these motifs mediate self-association of ovulin molecules, evidenced by observations of ovulin multimers *in vivo* in *D. melanogaster*, as well as *in vitro* between ovulin molecules from different species that contain these motifs (Wong *et al.* 2006 and submitted). These self-association domains are within a portion of ovulin that has ovulation-inducing activity, although it is as yet unknown whether self-association is needed for ovulin's function.

Interspersed around the highly-conserved backbone of ovulin are rapidly-evolving regions. In fact, ovulin is one of the most rapidly-evolving proteins in the Drosophila genome (Aguadé *et al.* 1992; Aguadé 1998; Tsaur and Wu 1997; Tsaur, *et al.* 1998). Amino acid divergence between ovulin from *D. melanogaster* and its close relative, D. simulans, is about 15%, compared to a 1–2% average for all other genes (Kern *et al.* 2004; Andolfatto 2005; Tamura, *et al.* 2004). There is evidence that some of ovulin's amino acid divergence results from positive selection, but the selective forces have not been identified (Aguadé *et al.* 1992; Aguadé 1998; Tsaur & Wu 1997; Tsaur, *et al.* 1998; Fay & Wu 2000). The ovulin-type pattern of highly divergent protein regions on an otherwise conserved backbone may be the result of sexual conflict, resulting from sexual selection, acting on proteins required for essential biological processes, such as those discussed in Acts 2 and 3.

Given the importance of Sfp genes to reproductive success, positive selection should drive the most advantageous alleles to fixation. We would therefore expect any variation in genes under positive selection to be transient. However, in contrast to this prediction, nucleotide variation is maintained in some Sfps, like ovulin (Aguadé, *et al.* 1992; Tsaur, *et al.* 1998), and in Sfp-mediated traits, like sperm competition (Clark, *et al.* 1995; Clark *et al.* 2000; Chapman 2001 for review). Why is so much polymorphism maintained?

The answer may be that there is no single "most advantageous" allele for a particular Sfp. What is "best" may depend on the genotypes of the individuals involved in the interaction. Using sperm competition as an example, within the mated female a male's sperm must compete for fertilizations with sperm from other males with whom the female had mated or will mate. In the case of three different genotypes (A, B, and C), Male A may outcompete male B, and male B may outcompete male C, but this does not mean that male A will outcompete male C (A>B, B>C, but C>A), when female genotype is held constant. This property of sperm competition, known as nontransitivity (Clark, *et al.* 2000), tells us that the outcome of a sperm competition interaction depends on the particular Sfp alleles of the competing males. Antagonistic pleiotropy may also lead to maintenance of polymorphism among Sfp genes (Fiumera, *et al.* 2005, 2007). For example, a polymorphism in the Sfp CG17331 is associated both with offensive sperm competitive ability and female refactoriness. When males carry the "C" allele, they are better able to prevent their mates from re-mating, but are poorer at displacing a previous mate's sperm (Fiumera, *et al.* 2005). The dependence of female post-mating behavior on the specific genotypic combinations of her multiple mates makes it difficult for one Sfp gene allele to "win".

The female's genotype, along with the interaction between male and female genotypes, also determines the outcome of sperm competition (Figure 5; Clark & Begun 1998; Clark *et al.* 1999). Complicating matters further, there is no genetic correlation between male-derived and female-derived effects on sperm competition (Civetta & Clark 2000a). When the

genotypes of competing males are held constant, a change in female genotype can determine which male is the "winner". The complex interactions between the genotypes of competing males and between male and female genotypes would make it nearly impossible for a particular male genotype to persevere in sperm competition. In fact, artificial selection even fails in the face of this complexity (Bjork *et al.* 2007).

Males must compete against each other for fertilization success and against the female's sometimes conflicting interests. For example, one might expect males to benefit from inhibiting females from remating, whereas females may benefit from receiving the sperm of multiple males. It is also likely, given the differing magnitudes of investment in progeny production between males and females, that they have different optima for the level of post-mating effects (such as ovulation rate) (Chippindale, *et al.* 2001). Conflict between the sexes due to these different optima has been interpreted to suggest a kind of evolutionary "arms race" (Figure 6). First, male-male competition selects for alleles conferring higher male competitive reproductive success. These alleles may lower a female's lifetime progeny production for unknown reasons, resulting in selection for females to counter-adapt to overcome harmful male alleles (Rice 2000; Chippindale, *et al.* 2001; Gavrilets, *et al.* 2001; Civetta & Singh 2003). At any given time in a population, this scenario of adaptation and counter-adaptation would occur simultaneously for many traits, leading to high levels of allelic polymorphism at the loci involved. Consistent with this prediction, we see that maintenance of variation in male-derived proteins depends on female genotype (reviewed in Chapman 2001). Furthermore, variation in Sfp-mediated female post-mating traits, such as female sperm usage patterns (determined by sperm competition experiments), is also dependent on female genotype (Clark *et al.* 1999; Clark 2002; Lawniczak & Begun 2005; McGraw *et al.* 2009).

Experimental evolution studies support the hypothesis that evolution of Sfps is driven, in part, by sexual selection. When monogamy is enforced on *D. melanogaster* for multiple generations, males become less harmful and females become less resistant to harm than polygamous controls (Holland & Rice 1999). In *D. pseudoobscura* populations selected under monogamy, normal levels of promiscuity, or elevated levels of promiscuity, males differed in their ability to prevent remating of their mates (Crudgington, *et al.* 2005). Counterintuitively, *D. pseudoobscura* males selected under monogamy induced greater refactory periods in females, suggesting that the males selected under promiscuous female conditions invested more in pre-copulatory competition than in post-copulatory competition. In another experiment, when female *D. melanogaster* were prevented from co-evolving with a male population that was allowed to adapt to the static female genome, these males were able to induce higher remating rates in females and were better at the "defense" component of sperm competition (Rice 1996). Though we do not know for certain whether any of these results are due to changes in Sfps (either at the sequence or expression levels), the Wigby *et al.* (2009) study described in Act 4 suggests that Sfps are involved in these types of adaptations because in that study changes in levels of transferred Sfps correlated with changes in post-copulatory traits in females (see also Bretman *et al.* 2009).

Female contribution to molecular social interactions

Since many Sfps are rapidly evolving, we might expect parallel patterns of evolution for the many female proteins (yet to be identified, with the exception of SPR) with which they interact. In line with this prediction, there are rapidly-evolving genes expressed in the female reproductive tract (Swanson, *et al.* 2004; Lawniczak & Begun 2007; Prokupek, *et al.* 2008), although this group as a class is not enriched for rapidly-evolving genes (Haerty, *et al.* 2007). However, molecular evidence for the type of "arms race" between the sexes, mediated by Sfp-female interactor pairs, is lacking. The only female interactor of an Sfp that

has been identified, the G-coupled protein receptor SPR, has not undergone rapid evolution; in fact, it is strikingly conserved across evolutionarily distant lineages (Yapici, *et al.* 2008). Studies that have identified rapidly-evolving female reproductive tract proteins found that they are enriched for serine proteases, not receptors as might have been predicted (e.g. Swanson *et al.* 2001, Clark *et al.* 2009), though several rapidly evolving female genes also have unknown functions. Some serine proteases expressed in female reproductive tracts are differentially expressed in response to mating (Lawniczak & Begun 2007; Prokupek, *et al.* 2009), suggesting a possible direct or indirect interaction with Sfps.

Much work needs to be done to determine whether SPR is the norm in Drosophila, or an exception. Examples can be found from many other species where, unlike SP and SPR, variation in a male or female molecule seems to drive rapid evolution at its partner from the opposite sex. For instance, the abalone sperm lysin protein has undergone rapid diversification which is thought to be a response to sequence changes in its receptor on the egg (Swanson & Vacquier 1998; Swanson, *et al.* 2001; Clark, *et al.* 2009). Before we can test the prediction that changes in male reproductive proteins cause changes to female molecules (or vice versa) and downstream behaviors, more male/female molecule pairs must be identified. Transcriptional profiling studies of females after mating (McGraw, *et al.* 2004, 2008; Mack, *et al.* 2006; Kapelnikov, *et al.* 2008b), in conjunction with evolutionary studies will likely lead to identification of further promising candidates.

Discussion and Future Directions

We now know a great deal about how Sfps affect social interactions related to mating in *D. melanogaster*. However, the current body of research on Sfps is only the tip of the iceberg when it comes to understanding how the social interactions themselves affect Sfp usage and evolution. Future experiments can explore new social contexts in *D. melanogaster* for their effect on the overall amount of Sfps transferred, the effect on specific Sfps, and the ultimate reproductive consequences for each individual involved. Experimental evolution studies may test for changes in Sfp production or mating-induced transcriptional responses in response to altered population dynamics.

One of the greatest challenges to elucidating the relationship between organismal level interactions and individual Sfps (and their female counterparts) is the difficulty in performing experiments under “natural” conditions. Advantageous reproductive traits no doubt depend on environmental conditions. For example, the larval environment (McGraw, *et al.* 2007) and adult male nutrition (Fricke, *et al.* 2008) have been shown to affect post-mating traits in *D. melanogaster*. In the lab, there are no predators and flies do not experience the dangers they might in nature, such as depletion of food supply, extreme temperatures, or desiccation. Simulating as wide an array of conditions as possible will provide valuable information on which social interactions are really important in nature, and how these interactions affect Sfp dynamics and post-mating traits.

Several studies have considered at least one of the important conditions experienced in nature: genetic variation among individuals. While most mechanistic studies of Sfps have been carried out using standard lab strains, variation in sperm competition and other post-mating traits have been linked to variation in male and female genotypes (Clark, *et al.* 1995; Prout & Clark 1996; Clark, *et al.* 1999; Fiumera, *et al.* 2006; Fiumera, *et al.* 2007; McGraw, *et al.* 2009). Currently, the genomes of nearly 200 inbred strains derived from wild-caught *D. melanogaster* are being sequenced (Mackay, *et al.* 2008). This unprecedented genomic tool will provide a means for identifying the genetic basis of any number of complex traits, including behaviors affected by Sfps. Future experiments will be able to tease apart the effects of different genotypic combinations on social interactions such as receptivity to

mating and sperm competition. From here, we can finally start to understand how females, not just males, act to control their reproductive success and to affect the reproductive success of their mates. Only then will we be able to comprehend how all levels of mating interactions come together to affect the complex molecular social interactions between *Drosophila* individuals.

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References

Adams EM, Wolfner MF. Seminal proteins but not sperm induce morphological changes in the *Drosophila melanogaster* female reproductive tract during sperm storage. Journal of Insect Physiology. 2007; 53:319–331. [PubMed: 17276455]

Adkins-Regan E. Hormones and sexual differentiation of avian social behavior. Developmental Neuroscience. 2009; 31:342–350. [PubMed: 19546571]

Aguadé M. Different forces drive the evolution of the Acp26Aa and Acp26Ab accessory gland genes in the *Drosophila melanogaster* species complex. Genetics. 1998; 150:1079–89. [PubMed: 9799260]

Aguadé M, Miyashita N, Langley CH. Polymorphism and divergence in the mst26a male accessory-gland gene region in drosophila. Genetics. 1992; 132:755–770. [PubMed: 1361475]

Aigaki T, Fleischmann I, Chen PS, Kubli E. Ectopic expression of sex peptide alters reproductive-behavior of female drosophila-melanogaster. Neuron. 1991; 7:557–563. [PubMed: 1931051]

Allen AK, Spradling AC. The Sf1-related nuclear hormone receptor Hr39 regulates Drosophila female reproductive tract development and function. Development. 2008; 135:311–321. [PubMed: 18077584]

Anderson RC. A study of the factors affecting fertility of lozenge females of *Drosophila melanogaster*. Genetics. 1945; 30:280–296. [PubMed: 17247158]

Arbeitman MN, Furlong EEM, Imam F, Johnson E, Null BH, Baker BS, Krasnow MA, Scott MP, Davis RW, White KP. Gene expression during the life cycle of *Drosophila melanogaster*. Science. 2002; 297:2270–2275. [PubMed: 12351791]

Arnqvist G, Nilsson T. The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav. 2000; 60:145–164. [PubMed: 10973716]

Arnqvist, G.; Rowe, L. Sexual conflict. Princeton University Press; Princeton: 2005.

Andolfatto P. Adaptive evolution of non-coding DNA in Drosophila. Nature. 2005; 437:1149–52. [PubMed: 16237443]

Avila FW, Wolfner MF. Acp36DE is required for uterine conformational changes in mated Drosophila females. Proc Natl Acad Sci USA. 2009 in press.

Barnes AI, Wigby S, Boone JM, Partridge L, Chapman T. Feeding, fecundity and lifespan in female *Drosophila melanogaster*. Proceedings of the Royal Society B-Biological Sciences. 2008; 275:1675–1683.

Bartoszewski S, Luschnig S, Desjeux I, Grosshans J, Nusslein-Volhard C. Drosophila p24 homologues eclair and baiser are necessary for the activity of the maternally expressed Tkv receptor during early embryogenesis. Mech Devel. 2004; 121:1259–1273. [PubMed: 15327786]

Begun DJ, Lindfors HA. Rapid evolution of genomic Acp complement in the melanogaster subgroup of Drosophila. Mol Biol Evol. 2005; 22:2010–21. [PubMed: 15987879]

Bertram MJ, Neubaum DM, Wolfner MF. Localization of the Drosophila male accessory gland protein Acp36DE in the mated female suggests a role in sperm storage. Insect Biochem Molec Biol. 1996; 26:971–980. [PubMed: 9014340]

Birkhead, TR.; Moller, AP. Sperm competition and sexual selection. Academic Press; San Diego: 1998.

Bjork A, Starmer WT, Higginson DM, Rhodes CJ, Pitnick S. Complex interactions with females and rival males limit the evolution of sperm offence and defence. Proc Roy Soc Lond B Bio. 2007; 274:1779–88.

Bloch Qazi MC, Heifetz Y, Wolfner MF. The developments between gametogenesis and fertilization: ovulation and female sperm storage in *Drosophila melanogaster*. Dev Biol. 2003; 256:195–211. [PubMed: 12679097]

Bloch Qazi MC, Wolfner MF. An early role for the *Drosophila melanogaster* male seminal protein Acp36DE in female sperm storage. J Exp Biol. 2003; 206:3521–3528. [PubMed: 12939382]

Boltz KA, Ellis LL, Carney GE. *Drosophila melanogaster* p24 genes have developmental, tissue-specific, and sex-specific expression patterns and functions. Dev Dynam. 2007; 236:544–555.

Boorman E, Parker GA. Sperm (ejaculate) competition in *Drosophila melanogaster*, and reproductive value of females to males in relation to female age and mating status. Ecol Entomol. 1976; 1:145–155.

Boulétreaumerle J. Role of spermathecae in sperm utilization and stimulation of oogenesis in drosophila-melanogaster. J Insect Physiol. 1977; 23:1099–1104.

Bretman A, Fricke C, Chapman T. Plastic responses of male *Drosophila melanogaster* to the level of sperm competition increase male reproductive fitness. Proc Roy Soc Lond B Bio. 2009:1705–1711.

Bretman A, Lawniczak MKN, Boone JM, Chapman T. A mating plug protein reduces early female remating in *Drosophila melanogaster*. J Insect Physiol. in revision.

Byrne PG, Rice WR. Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proc Roy Soc Lond B Bio. 2006; 273:917–922.

Cameron E, Day T, Rowe L. Sperm competition and the evolution of ejaculate composition. Am Nat. 2007; 169:E158–E172. [PubMed: 17479456]

Carney GE, Taylor BJ. logjam encodes a predicted EMP24/GP25 protein that is required for Drosophila oviposition behavior. Genetics. 2003; 164:173–186. [PubMed: 12750330]

Carvalho GB, Kapahi P, Anderson DJ, Benzer S. Allocrine modulation of feeding behavior by the sex peptide of Drosophila. Curr Biol. 2006; 16:692–696. [PubMed: 16581515]

Chapman T. Seminal fluid-mediated fitness traits in Drosophila. Heredity. 2001; 87:511–521. [PubMed: 11869341]

Chapman T. The soup in my fly: Evolution, form and function of seminal fluid proteins. PLoS Biol. 2008; 6:1379–1382.

Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, Smith HK, Partridge L. The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA interference. Proc Natl Acad Sci USA. 2003; 100:9923–9928. [PubMed: 12893873]

Chapman T, Davies SJ. Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. Peptides. 2004; 25:1477–1490. [PubMed: 15374649]

Chapman T, Herndon LA, Heifetz Y, Partridge L, Wolfner MF. The Acp26Aa seminal fluid protein is a modulator of early egg hatchability in *Drosophila melanogaster*. Proc Roy Soc Lond B Bio. 2001; 268:1647–1654.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature. 1995; 373:241–244. [PubMed: 7816137]

Chapman T, Neubaum DM, Wolfner MF, Partridge L. The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*. Proc Roy Soc Lond B Bio. 2000; 267:1097–1105.

Chen JS, Raikhel AS. Subunit cleavage of mosquito pro-vitellogenin by a subtilisin-like convertase. Proc Natl Acad Sci USA. 1996; 93:6186–6190. [PubMed: 8650241]

Chen PS, Stummzollinger E, Aigaki T, Balmer J, Bienz M, Bohlen P. A male accessory-gland peptide that regulates reproductive-behavior of female drosophila-melanogaster. Cell. 1988; 54:291–298. [PubMed: 3135120]

Chintapalli VR, Wang J, Dow JAT. Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. Nature Genet. 2007; 39:715–720. [PubMed: 17534367]

Chippindale AK, Gibson JR, Rice WR. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in Drosophila. Proc Natl Acad Sci USA. 2001; 98:1671–5. [PubMed: 11172009]

Christiansen AE, Keisman EL, Ahmad SM, Baker BS. Sex comes in from the cold: the integration of sex and pattern. Trends Genet. 2002; 18:510–6. [PubMed: 12350340]

Civetta A. Shall we dance or shall we fight? Using DNA sequence data to untangle controversies surrounding sexual selection. Genome. 2003; 46:925–929. [PubMed: 14663506]

Civetta A, Clark AG. Chromosomal effects on male and female components of sperm precedence in Drosophila. Genet Res. 2000a; 75:143–51. [PubMed: 10816972]

Civetta A, Clark AG. Correlated effects of sperm competition and postmating female mortality. Proc Natl Acad Sci. 2000b; 97:13162–13165. [PubMed: 11078508]

Clark AG. Sperm competition and the maintenance of polymorphism. Heredity. 2002; 88:148–53. [PubMed: 11932773]

Clark NL, Aagaard JE, Swanson WJ. Evolution of reproductive proteins from animals and plants. Reproduction. 2006; 131:11–22. [PubMed: 16388004]

Clark AG, Aguadé M, Prout T, Harshman LG, Langley CH. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. Genetics. 1995; 139:189–201. [PubMed: 7705622]

Clark AG, Begun DJ. Female genotypes affect sperm displacement in Drosophila. Genetics. 1998; 149:1487–93. [PubMed: 9649536]

Clark AG, Begun DJ, Prout T. Female x male interactions in Drosophila sperm competition. Science. 1999; 283:217–20. [PubMed: 9880253]

Clark AG, Dermitzakis ET, Civetta A. Nontransitivity of sperm precedence in Drosophila. Evolution. 2000; 54:1030–5. [PubMed: 10937275]

Clark NL, Gasper J, Sekino M, Springer SA, Aquadro CF, Swanson WJ. Coevolution of interacting fertilization proteins. PLoS Genet. 2009; 5:e1000570. [PubMed: 19629160]

Cline TW, Meyer BJ. Vive la différence: males vs females in flies vs worms. Annu Rev Genet. 1996; 30:637–702. [PubMed: 8982468]

Cole SH, Carney GE, McClung CA, Willard SS, Taylor BJ, Hirsh J. Two functional but noncomplementing Drosophila tyrosine decarboxylase genes. J Biol Chem. 2005; 280:14948–14955. [PubMed: 15691831]

Cordts R, Partridge L. Courtship reduces longevity of male *Drosophila melanogaster*. Anim Behav. 1996; 52:269–278.

Crudgington HS, Beckerman AP, Brüstle L, Green K, Snook RR. Experimental removal and elevation of sexual selection: does sexual selection generate manipulative males and resistant females? Am Nat. 2005; 165(Suppl 5):S72–87. [PubMed: 15795864]

Dean MD, Good JM, Nachman MW. Adaptive evolution of proteins secreted during sperm maturation: an analysis of the mouse epididymal transcriptome. Mol Biol Evol. 2008; 25:383–92. [PubMed: 18056076]

de Bono M, Maricq AV. Neuronal substrates of complex behaviors in *C. elegans*. Ann Rev Neurosci. 2005; 28:451–501. [PubMed: 16022603]

Dibenedetto AJ, Harada HA, Wolfner MF. Structure, cell-specific expression, and mating-induced regulation of a drosophila-melanogaster male accessory-gland gene. Dev Biol. 1990; 139:134–148. [PubMed: 2109712]

Dickson BJ. Wired for sex: the neurobiology of Drosophila mating decisions. Science. 2008; 322:904–909. [PubMed: 18988843]

Domanitskaya EV, Liu HF, Chen SJ, Kubli E. The hydroxyproline motif of male sex peptide elicits the innate immune response in Drosophila females. FEBS J. 2007; 274:5659–5668. [PubMed: 17922838]

Eberhard, WG. Female control: Sexual selection by cryptic female choice. Princeton University Press; Princeton: 1996.

Ejima A, Smith BPC, Lucas C, Van Naters WV, Miller CJ, Carlson JR, Levine JD, Griffith LC. Generalization of courtship learning in Drosophila is mediated by cis-vaccenyl acetate. Curr Biol. 2007; 17:599–605. [PubMed: 17363250]

Ellison, PT.; Gray, PB., editors. Enodcrinology of social relationships. Harvard University Press; Cambridge, MA: 2009.

Fay JC, Wu CI. Hitchhiking under positive Darwinian selection. Genetics. 2000; 155:1405–13. [PubMed: 10880498]

Fedorka KM, Linder JE, Winterhalter W, Promislow D. Post-mating disparity between potential and realized immune response in *Drosophila melanogaster*. Proc Roy Soc Lond B Bio. 2007; 274:1211–1217.

Ferveur JF. The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. Bioessays. 1997; 19:353–358. [PubMed: 9136633]

Findlay GD, MacCoss MJ, Swanson WJ. Proteomic discovery of previously unannotated, rapidly evolving seminal fluid genes in Drosophila. Genome Res. 2009; 19:886–896. [PubMed: 19411605]

Findlay GD, Yi XH, MacCoss MJ, Swanson WJ. Proteomics reveals novel Drosophila seminal fluid proteins transferred at mating. PLoS Biol. 2008; 6:1417–1426.

Fiumera AC, Dumont BL, Clark AG. Sperm competitive ability in *Drosophila melanogaster* associated with variation in male reproductive proteins. Genetics. 2005; 169:243–57. [PubMed: 15466425]

Fiumera AC, Dumont BL, Clark AG. Natural variation in male-induced 'cost-of-mating' and allele-specific association with male reproductive genes in *Drosophila melanogaster*. Philos Trans R Soc Lond, B, Biol Sci. 2006; 361:355–61. [PubMed: 16612893]

Fiumera AC, Dumont BL, Clark AG. Associations between sperm competition and natural variation in male reproductive genes on the third chromosome of *Drosophila melanogaster*. Genetics. 2007; 176:1245–60. [PubMed: 17435238]

Fowler GL. Some aspects of reproductive biology of drosophila - sperm transfer, sperm storage, and sperm utilization. Adv Genet. 1973; 17:293–360.

Fowler K, Partridge L. A cost of mating in female fruit flies. Nature. 1989; 338:760–761.

Friberg U. Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. Anim Behav. 2006; 72:1259–1268.

Fricke C, Bretman A, Chapman T. Adult male nutrition and reproductive success in *Drosophila melanogaster*. Evolution. 2008; 62:3170–7. [PubMed: 18786187]

Gao ZQ, Ruden DM, Lu XY. PKD2 cation channel is required for directional sperm movement and male fertility. Curr Biol. 2003; 13:2175–2178. [PubMed: 14680633]

Garden RW, Shippy SA, Li LJ, Moroz TP, Sweedler JV. Proteolytic processing of the Aplysia egg-laying hormone prohormone. Proc Natl Acad Sci USA. 1998; 95:3972–3977. [PubMed: 9520477]

Gavrilets S, Arnqvist G, Friberg U. The evolution of female mate choice by sexual conflict. Proc Roy Soc Lond B Biol. 2001; 268:531–9.

Gilbert DG, Richmond RC, Sheehan KB. Studies of esterase 6 in *Drosophila melanogaster* 5 Progeny production and sperm use in females inseminated by males having active or null alleles. Evolution. 1981; 35:21–37.

Gilchrist AS, Partridge L. Why it is difficult to model sperm displacement in *Drosophila melanogaster*: The relation between sperm transfer and copulation duration. Evolution. 2000; 54:534–542. [PubMed: 10937230]

Gillott C. Male accessory gland secretions: Modulators of female reproductive physiology and behavior. Ann Rev Entomol. 2003; 48:163–184. [PubMed: 12208817]

Greenspan RJ, Ferveur JF. Courtship in Drosophila. Ann Rev Genet. 2000; 34:205–232. [PubMed: 11092827]

Haerty W, Jagadeeshan S, Kulathinal RJ, Wong A, Kristipati RR, Sirot LK, Levesque L, Artieri CG, Wolfner MF, Civetta A, Singh RS. Evolution in the fast lane: rapidly evolving sex-related genes in Drosophila. Genetics. 2007; 177:1321–35. [PubMed: 18039869]

Hall JC. The mating of a fly. Science. 1994; 264:1702–1714. [PubMed: 8209251]

Harshman LG, Clark AG. Inference of sperm competition from broods of field-caught Drosophila. Evolution. 1998; 52:1334–1341.

Hasemeyer M, Yapici N, Heberlein U, Dickson BJ. Sensory neurons in the Drosophila genital tract regulate female reproductive behavior. Neuron. 2009; 61:511–518. [PubMed: 19249272]

Heifetz Y, Lung O, Frongillo EA, Wolfner MF. The Drosophila seminal fluid protein Acp26Aa stimulates release of oocytes by the ovary. Curr Biol. 2000; 10:99–102. [PubMed: 10662669]

Heifetz Y, Tram U, Wolfner MF. Male contributions to egg production: the role of accessory gland products and sperm in *Drosophila melanogaster*. Proc Roy Soc Lond B Bio. 2001; 268:175–180.

Heifetz Y, Vandenberg LN, Cohn HI, Wolfner MF. Two cleavage products of the Drosophila accessory gland protein ovulin can independently induce ovulation. Proc Natl Acad Sci USA. 2005; 102:743–748. [PubMed: 15640356]

Heifetz Y, Wolfner MF. Mating, seminal fluid components, and sperm cause changes in vesicle release in the Drosophila female reproductive tract. Proc Natl Acad Sci USA. 2004; 101:6261–6266. [PubMed: 15071179]

Herndon LA, Wolfner MF. A Drosophila seminal fluid protein, Acp26Aa, stimulates egg-laying in females for 1 day after mating. Proc Natl Acad Sci USA. 1995; 92:10114–10118. [PubMed: 7479736]

Hihara F. Effects of the male accessory gland secretion on oviposition and re mating in females of *Drosophilamelanogaster*. Zool Mag (Tokyo). 1981; 90:307–316.

Holland B, Rice WR. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. Proc Natl Acad Sci USA. 1999; 96:5083–8. [PubMed: 10220422]

Holman L. *Drosophila melanogaster* seminal fluid can protect the sperm of other males. Funct Ecol. 2009; 23:180–186.

Hook VYH, Azaryan AV, Hwang SR, Tezapsidis N. Proteases and the emerging role of protease inhibitors in prohormone processing. FASEB J. 1994; 8:1269–1278. [PubMed: 8001739]

Iida K, Cavener DR. Glucose dehydrogenase is required for normal sperm storage and utilization in female *Drosophila melanogaster*. J Exp Biol. 2004; 207:675–681. [PubMed: 14718510]

Imhof M, Harr B, Brem G, Schlotterer C. Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. Molec Ecol. 1998; 7:915–917. [PubMed: 9691492]

Innocenti P, Morrow EH. Immunogenic males: a genome-wide analysis of reproduction and the cost of mating in *Drosophila melanogaster* females. J Evol Biol. 2009; 22:964–973. [PubMed: 19228270]

Kalb JM, Dibenedetto AJ, Wolfner MF. Probing the function of *Drosophila melanogaster* accessory-glands by directed cell ablation. Proc Natl Acad Sci USA. 1993; 90:8093–8097. [PubMed: 8367469]

Kapelnikov A, Rivlin PK, Hoy RR, Heifetz Y. Tissue remodeling: a mating-induced differentiation program for the Drosophila oviduct. BMC Dev Biol. 2008a:8. [PubMed: 18218133]

Kapelnikov A, Zelinger E, Gottlieb Y, Rhrissorrakrai K, Gunsalus KC, Heifetz Y. Mating induces an immune response and developmental switch in the Drosophila oviduct. Proc Natl Acad Sci USA. 2008b; 105:13912–13917. [PubMed: 18725632]

Kaufmann P, Demerec M. Utilization of sperm by the female *Drosophila melanogaster*. Am Nat. 1942; 76:445–469.

Kelleher E, Swanson WJ, Markow TA. Gene duplication and adaptive evolution of digestive proteases in Drosophila arizonae female reproductive tracts. PLoS Genet. 2007; 3:e148. [PubMed: 17784792]

Kern AD, Jones CD, Begun DJ. Molecular population genetics of male accessory gland proteins in the Drosophila simulans complex. Genetics. 2004; 167:725–735. [PubMed: 15238524]

Keverne EB, Curley JP. Vasopressin, oxytocin and social behaviour. Curr Opin Neurobiol. 2004; 14:777–783. [PubMed: 15582383]

Kubli E. Sex-peptides: seminal peptides of the Drosophila male. Cellular & Molecular Life Sci. 2003; 60:1689–1704.

Lange AB. Tyramine: From octopamine precursor to neuroactive chemical in insects. Gen Comp Endocr. 2009; 162:18–26. [PubMed: 18588893]

Lawniczak MKN, Begun DJ. A genome-wide analysis of courting and mating responses in *Drosophila melanogaster* females. Genome. 2004; 47:900–910. [PubMed: 15499404]

Lawniczak MKN, Begun DJ. A QTL analysis of female variation contributing to refractoriness and sperm competition in *Drosophila melanogaster*. Genet Res. 2005; 86:107–14. [PubMed: 16356284]

Lawniczak MKN, Begun DJ. Molecular population genetics of female- expressed mating-induced serine proteases in *Drosophila melanogaster*. Molec Biol Evol. 2007; 24:1944–1951. [PubMed: 17573377]

Lee HG, Rohila S, Han KA. The octopamine receptor OAMB mediates ovulation via Ca2+/calmodulin-dependent protein kinase II in the Drosophila oviduct epithelium. PLoS One. 2009; 4:e4716. [PubMed: 19262750]

Lee HG, Seong CS, Kim YC, Davis RL, Han KA. Octoparnine receptor OAMB is required for ovulation in *Drosophila melanogaster*. Dev Biol. 2003; 264:179–190. [PubMed: 14623240]

Lefevre G, Jonsson UB. Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. Genetics. 1962; 47:1719–1736. [PubMed: 13929245]

LeFranc A, Bundgaard J. The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. Hereditas. 2000; 132:243–247. [PubMed: 11075519]

Linklater JR, Wertheim B, Wigby S, Chapman T. Ejaculate depletion patterns evolve in response to experimental manipulation of sex ratio in *Drosophila melanogaster*. Evolution. 2007; 61:2027–2034. [PubMed: 17683443]

Liu HF, Kubli E. Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 2003; 100:9929–9933. [PubMed: 12897240]

Long TAF, Stewart AD, Miller PM. Potential confounds to an assay of cross-generational fitness benefits of mating and male seminal fluid. Biol Lett. 2009; 5:26–27. [PubMed: 18812308]

Ludwig MZ, Uspensky II, Ivanov AI, Kopantseva MR, Dianov CM, Tamarina NA, Korochkin LI. Genetic control and expression of the major ejaculatory bulb protein (Peb-ME) in *Drosophila melanogaster*. Biochem Genet. 1991; 29:215–239. [PubMed: 1772395]

Lung O, Kuo L, Wolfner MF. Drosophila males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. J Insect Physiol. 2001; 47:617–622. [PubMed: 11249950]

Lung O, Wolfner MF. Drosophila seminal fluid proteins enter the circulatory system of the mated female fly by crossing the posterior vaginal wall. Insect Biochem Molec Biol. 1999; 29:1043–1052. [PubMed: 10612039]

Lung O, Wolfner MF. Identification and characterization of the major *Drosophila melanogaster* mating plug protein. Insect Biochem Molec Biol. 2001; 31:543–551. [PubMed: 11267893]

Mack PD, Kapelnikov A, Heifetz Y, Bender M. Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. Proc Natl Acad Sci USA. 2006; 103:10358–63. [PubMed: 16798875]

Mackay, T.; Richards, S.; Gibbs, R. Proposal to Sequence a Drosophila Genetic Reference Panel: A Community Resource for the Study of Genotypic and Phenotypic Variation. 2008. http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/DrosophilaGeneticReferencePanelWhitepaper4_9_08_final_combined.pdf

Manning A. Sperm factor affecting receptivity of *Drosophila melanogaster* females. Nature. 1962; 194:252.

Manning A. Control of sexual receptivity in female drosophila. Anim Behav. 1967; 15:239–250. [PubMed: 6030948]

Manoli DS, Meissner GW, Baker BS. Blueprints for behavior: genetic specification of neural circuitry for innate behaviors. Trends Neurosci. 2006; 29:444–451. [PubMed: 16806511]

Marcillac F, Ferveur JF. A set of female pheromones affects reproduction before, during and after mating in Drosophila. J Exper Biol. 2004; 207:3927–3933. [PubMed: 15472023]

Markow TA, O'Grady PM. Evolutionary genetics of reproductive behavior in Drosophila: Connecting the dots. Ann Rev Genet. 2005; 39:263–291. [PubMed: 16285861]

Mazzi D, Kesaniemi J, Hoikkala A, Klappert K. Sexual conflict over the duration of copulation in Drosophila montana: why is longer better? BMC Evol Biol. 2009:9. [PubMed: 19134215]

McGraw LA, Clark AG, Wolfner MF. Post-mating gene expression profiles of female *Drosophila melanogaster* in response to time and to four male accessory gland proteins. Genetics. 2008; 179:1395–1408. [PubMed: 18562649]

McGraw LA, Gibson G, Clark AG, Wolfner MF. Genes regulated by mating, sperm, or seminal proteins in mated female *Drosophila melanogaster*. Curr Biol. 2004; 14:1509–1514. [PubMed: 15324670]

McGraw LA, Gibson G, Clark AG, Wolfner MF. Strain-dependent differences in several reproductive traits are not accompanied by early postmating transcriptome changes in female *Drosophila melanogaster*. Genetics. 2009; 181:1273–80. [PubMed: 19237688]

Meikle DB, Sheehan KB, Phillis DM, Richmond RC. Localization and longevity of seminal-fluid esterase-6 in mated female *Drosophila melanogaster*. J Insect Physiol. 1990; 36:93–101.

Middleton CA, Nongthomba U, Parry K, Sweeney ST, Sparrow JC, Elliott CJH. Neuromuscular organization and aminergic modulation of contractions in the Drosophila ovary. BMC Biol. 2006:4. [PubMed: 16469111]

Monastirioti M. Biogenic amine systems in the fruit fly *Drosophila melanogaster*. Microsc Res Tech. 1999; 45:106–121. [PubMed: 10332728]

Monastirioti M. Distinct octopamine cell population residing in the CNS abdominal ganglion controls ovulation in *Drosophila melanogaster*. Dev Biol. 2003; 264:38–49. [PubMed: 14623230]

Monsma SA, Harada HA, Wolfner MF. Synthesis of 2 Drosophila male accessory-gland proteins and their fate after transfer to the female during mating. Dev Biol. 1990; 142:465–475. [PubMed: 2257979]

Monsma SA, Wolfner MF. Structure and expression of a Drosophila male accessory-gland gene whose product resembles a peptide pheromone precursor. Genes Dev. 1988; 2:1063–1073. [PubMed: 3142802]

Moshitzky P, Fleischmann I, Chaimov N, Saudan P, Klauser S, Kubli E, Applebaum SW. Sex peptide activates juvenile hormone biosynthesis in the *Drosophila melanogaster* corpus allatum. Arch Insect Biochem. 1996; 32:363–374.

Mueller JL, Ripoll DR, Aquadro CF, Wolfner MF. Comparative structural modeling and inference of conserved protein classes in Drosophila seminal fluid. Proc Natl Acad Sci USA. 2004; 101:13542–13547. [PubMed: 15345744]

Mueller JL, Kristipati RR, McGraw LA, Bloch Qazi MC, Siggia ED, Clark AG, Aquadro C, Wolfner MF. Cross-species comparison of Drosophila male accessory gland protein genes. Genetics. 2005; 171:131–43. [PubMed: 15944345]

Mueller JL, Linklater JR, Ram KR, Chapman T, Wolfner MR. Targeted gene deletion and phenotypic analysis of the *Drosophila melanogaster* seminal fluid protease inhibitor Acp62F. Genetics. 2008; 178:1605–1614. [PubMed: 18245332]

Neubaum DM, Wolfner MF. Mated *Drosophila melanogaster* females require a seminal fluid protein, Acp36DE, to store sperm efficiently. Genetics. 1999; 153:845–857. [PubMed: 10511562]

Panhuis TM, Clark NL, Swanson WJ. Rapid evolution of reproductive proteins in abalone and Drosophila. Philos Trans R Soc Lond, B, Biol Sci. 2006; 361:261–8. [PubMed: 16612885]

Park M, Wolfner MF. Male and female cooperate in the prohormone-like processing of a *Drosophila melanogaster* seminal fluid protein. Dev Biol. 1995; 171:694–702. [PubMed: 7556947]

Pelosi P, Calvello M, Ban LP. Diversity of odorant-binding proteins and chemosensory proteins in insects. Chem Sens. 2005; 30:I291–i292.

Peng J, Chen S, Busser S, Liu HF, Honegger T, Kubli E. Gradual release of sperm bound sex-peptide controls female postmating behavior in Drosophila. Curr Biol. 2005a; 15:207–213. [PubMed: 15694303]

Peng J, Zipperlen P, Kubli E. Drosophila sex peptide stimulates female innate immune system after mating via the Toll and Imd pathways. Current Biology. 2005b; 15:1690–1694. [PubMed: 16169493]

Pilpel N, Nezer I, Applebaum SW, Helfetz Y. Mating-increases trypsin in female Drosophila hemolymph. Insect Biochem Molec Biol. 2008; 38:320–330. [PubMed: 18252246]

Pitnick S, Spicer GS, Markow TA. How long is a giant sperm. Nature. 1995; 375:109–109. [PubMed: 7753164]

Poiani A. Complexity of seminal fluid: a review. Behav Ecol Sociobiol. 2006; 60:289–310.

Priest NK, Galloway LF, Roach DA. Mating frequency and inclusive fitness in *Drosophila melanogaster*. American Naturalist. 2008a; 171:10–21.

Priest NK, Roach DA, Galloway LF. Cross-generational fitness benefits of mating and male seminal fluid. Biol Lett. 2008b; 4:6–8. [PubMed: 17986427]

Priest NK, Roach DA, Galloway LF. Support for a pluralistic view of behavioural evolution. Biol Lett. 2009; 5:28–29.

Prokupek A, Hoffmann F, Eyun SI, Moriyama E, Zhou M, Harshman L. An evolutionary expressed sequence tag analysis of Drosophila spermatheca genes. Evolution. 2008; 62:2936–2947. [PubMed: 18752616]

Prokupek AM, Kachman SD, Ladunga I, Harshman LG. Transcriptional profiling of the sperm storage organs of *Drosophila melanogaster*. Insect Molec Biol. 2009; 18:465–475. [PubMed: 19453766]

Prout T, Clark AG. Seminal fluid causes temporarily reduced egg hatch in previously mated females. Proc Roy Soc Lond B Bio. 2000; 267:201–203.

Ravi Ram K, Ji S, Wolfner MF. Fates and targets of male accessory gland proteins in mated female *Drosophila melanogaster*. Insect Biochem Molec Biol. 2005; 35:1059–1071. [PubMed: 15979005]

Ravi Ram K, Sirot LK, Wolfner MF. Predicted seminal astacin-like protease is required for processing of reproductive proteins in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 2006; 103:18674–18679. [PubMed: 17116868]

Ravi Ram K, Wolfner MF. Seminal influences: Drosophila Acps and the molecular interplay between males and females during reproduction. Integr Comp Biol. 2007a; 47:427–445. [PubMed: 21672851]

Ravi Ram K, Wolfner MF. Sustained post-mating response in *Drosophila melanogaster* requires multiple seminal fluid proteins. PLoS Genet. 2007b; 3:2428–2438.

Ravi Ram K, Wolfner MF. A network of interactions among seminal proteins underlies the long-term postmating response in Drosophila. Proc Natl Acad Sci USA. 2009; 106:15384–15389. [PubMed: 19706411]

Rice WR. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature. 1996; 381:232–4. [PubMed: 8622764]

Rice WR. Dangerous liaisons. Proc Natl Acad Sci USA. 2000; 97:12953–5. [PubMed: 11087851]

Ringo J. Sexual receptivity in insects. Ann Rev Entomol. 1996; 41:473–494. [PubMed: 15012338]

Rodríguez-Valentín R, Lopez-Gonzalez I, Jorquera R, Laarca P, Zurita M, Reynaud E. Oviduct contraction in Drosophila is modulated by a neural network that is both, octopaminergic and glutamatergic. J Cellular Physiol. 2006; 209:183–198. [PubMed: 16826564]

Samakovlis C, Kylsten P, Kimbrell DA, Engstrom A, Hultmark D. The andropin gene and its product, a male-specific antibacterial peptide in *Drosophila melanogaster*. EMBO J. 1991; 10:163–169. [PubMed: 1899226]

Schully SD, Hellberg ME. Positive selection on nucleotide substitutions and indels in accessory gland proteins of the Drosophila pseudoobscura subgroup. J Mol Evol. 2006; 62:793–802. [PubMed: 16752217]

Scott D. Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. Proc Natl Acad Sci USA. 1986; 83:8429–8433. [PubMed: 3095835]

Shirangi TR, McKeown M. Sex in flies: What ‘body-mind’ dichotomy? Dev Biol. 2007; 306:10–19. [PubMed: 17475234]

Simmons, LW. Sperm competition and its evolutionary consequences in the insects. Princeton University Press; Princeton: 2001.

Singh SR, Singh BN. Female remating in Drosophila: Comparison of duration of copulation between first and second matings in six species. Curr Sci. 2004; 86:465–470.

Sirot LK, Buehner NA, Fiumera AC, Wolfner MF. Seminal fluid protein depletion and replenishment in the fruit fly, *Drosophila melanogaster*: an ELISA-based method for tracking individual ejaculates. Behav Ecol Sociobiol. 2009; 63:1505–1513.

Smith AA, Holldobler B, Liebig J. Hydrocarbon signals explain the pattern of worker and egg policing in the ant *Aphaenogaster cockerelli*. Journal of Chemical Ecology. 2008; 34:1275–1282. [PubMed: 18709507]

Soller M, Bownes M, Kubli E. Control of oocyte maturation in sexually mature Drosophila females. Dev Biol. 1999; 208:337–351. [PubMed: 10191049]

Soller M, Haussmann IU, Hollmann M, Choffat Y, White K, Kubli E, Schafer MA. Sex-peptide-regulated female sexual behavior requires a subset of ascending ventral nerve cord neurons. Curr Biol. 2006; 16:1771–1782. [PubMed: 16979554]

Spieth, HT.; Ringo, JM. Mating behavior and sexual isolation in Drosophila. In: Ashburner, MM.; Carson, HL.; Thompson, JN., editors. The Genetics and Biology of Drosophila. Vol. 3c. Academic Press; New York: 1983. p. 223-284.

Swanson WJ, Aquadro CF, Vacquier VD. Polymorphism in abalone fertilization proteins is consistent with the neutral evolution of the egg's receptor for lysin (VERL) and positive darwinian selection of sperm lysin. Mol Biol Evol. 2001; 18:376–83. [PubMed: 11230538]

Swanson WJ, Vacquier VD. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. Science. 1998; 281:710–2. [PubMed: 9685267]

Swanson WJ, Vacquier VD. The rapid evolution of reproductive proteins. Nature Rev Genet. 2002; 3:137–144. [PubMed: 11836507]

Swanson WJ, Wong A, Wolfner MF, Aquadro CF. Evolutionary expressed sequence tag analysis of Drosophila female reproductive tracts identifies genes subjected to positive selection. Genetics. 2004; 168:1457–1465. [PubMed: 15579698]

Takemori N, Yamamoto MT. Proteome mapping of the *Drosophila melanogaster* male reproductive system. Proteomics. 2009; 9:2484–2493. [PubMed: 19343724]

Tamura K, Subramanian S, Kumar S. Temporal patterns of fruit fly (Drosophila) evolution revealed by mutation clocks. Mol Biol Evol. 2004; 21:36–44. [PubMed: 12949132]

Tompkins L, Siegel RW, Gailey DA, Hall JC. Conditioned courtship in Drosophila and its mediation by association of chemical cues. Behav Genet. 1983; 13:565–578. [PubMed: 6422921]

Tram U, Wolfner MF. Seminal fluid regulation of female sexual attractiveness in *Drosophila melanogaster*. Proc Natl Acad Sci USA. 1998; 95:4051–4054. [PubMed: 9520491]

Tsaur SC, Ting CT, Wu CI. Positive selection driving the evolution of a gene of male reproduction, Acp26Aa, of Drosophila: II. Divergence versus polymorphism. Mol Biol Evol. 1998; 15:1040–6. [PubMed: 9718731]

Tsaur SC, Wu CI. Positive selection and the molecular evolution of a gene of male reproduction, Acp26Aa of Drosophila. Mol Biol Evol. 1997; 14:544–9. [PubMed: 9159932]

Villella A, Hall JC. Neurogenetics of courtship and mating in Drosophila. Adv Genet. 2008; 62:67–184. [PubMed: 19010254]

Walker MJ, Rylett CM, Keen JN, Audsley N, Sajid M, Shirras AD, Isaac RE. Proteomic identification of *Drosophila melanogaster* male accessory gland proteins, including a pro-cathepsin and a soluble gamma-glutamyl transpeptidase. Proteome Sci. 2006:4. [PubMed: 16571109]

Watnick TJ, Jim Y, Matunis E, Kernan MJ, Montell C. A flagellar polycystin-2 homolog required for male fertility in Drosophila. Curr Biol. 2003; 13:2179–2184. [PubMed: 14680634]

Wedell N, Gage MJG, Parker GA. Sperm competition, male prudence and sperm-limited females. Trends Ecol Evol. 2002; 17:313–320.

Wigby S, Chapman T. Sex peptide causes mating costs in female *Drosophila melanogaster*. Curr Biol. 2005; 15:316–321. [PubMed: 15723791]

Wigby S, Sirot LK, Linklater JR, Buehner N, Calboli FCF, Bretman A, Wolfner MF, Chapman T. Seminal Fluid Protein Allocation and Male Reproductive Success. Curr Biol. 2009; 19:751–757. [PubMed: 19361995]

Wolfner MF. Battle and ballet: molecular interactions between the sexes in Drosophila. J Heredity. 2009; 100:399–410.

Wong A, Albright SN, Wolfner MF. Evidence for structural constraint on ovulin, a rapidly evolving *Drosophila melanogaster* seminal protein. Proc Natl Acad Sci USA. 2006; 103:18644–9. [PubMed: 17130459]

Wong A, Albright SN, Giebel JD, Ram KR, Ji SQ, Fiumera AC, Wolfner MF. A role for Acp29AB, a predicted seminal fluid lectin, in female sperm storage in *Drosophila melanogaster*. Genetics. 2008a; 180:921–931. [PubMed: 18757944]

Wong A, Turchin MC, Wolfner MF, Aquadro C. Evidence for positive selection on *Drosophila melanogaster* seminal fluid protease homologs. Mol Biol Evol. 2008b; 25:497–506. [PubMed: 18056920]

Wong, A.; Christopher, AB.; Buehner, NA.; Wolfner, MF. Immortal coils: Conserved dimerization motifs of the Drosophila ovulation prohormone ovulin. Submitted

Xue L, Noll M. Drosophila female sexual behavior induced by sterile males showing copulation complementation. Proc Natl Acad Sci USA. 2000; 97:3272–3275. [PubMed: 10725377]

Xue L, Noll M. Dual role of the Pax gene paired in accessory gland development of Drosophila. Development. 2002; 129:339–346. [PubMed: 11807027]

Yamamoto D. The neural and genetic substrates of sexual behavior in Drosophila. Adv Genet. 2007; 59:39–66. [PubMed: 17888794]

Yang CH, Belawat P, Hafen E, Jan LY, Jan YN. Drosophila egg-laying site selection as a system to study simple decision-making processes. Science. 2008; 319:1679–1683. [PubMed: 18356529]

Yang CH, Rumpf S, Xiang Y, Gordon MD, Song W, Jan LY, Jan YN. Control of the postmating behavioral switch in Drosophila females by internal sensory neurons. Neuron. 2009; 61:519–526. [PubMed: 19249273]

Yapici N, Kim YJ, Ribeiro C, Dickson BJ. A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature. 2008; 451:33–37. [PubMed: 18066048]

Yew JY, Dreisewerd K, Luftmann H, Muthing J, Pohlentz G, Kravitz EA. A new male sex pheromone and novel cuticular cues for chemical communication in Drosophila. Curr Biol. 2009; 19:1245–1254. [PubMed: 19615904]

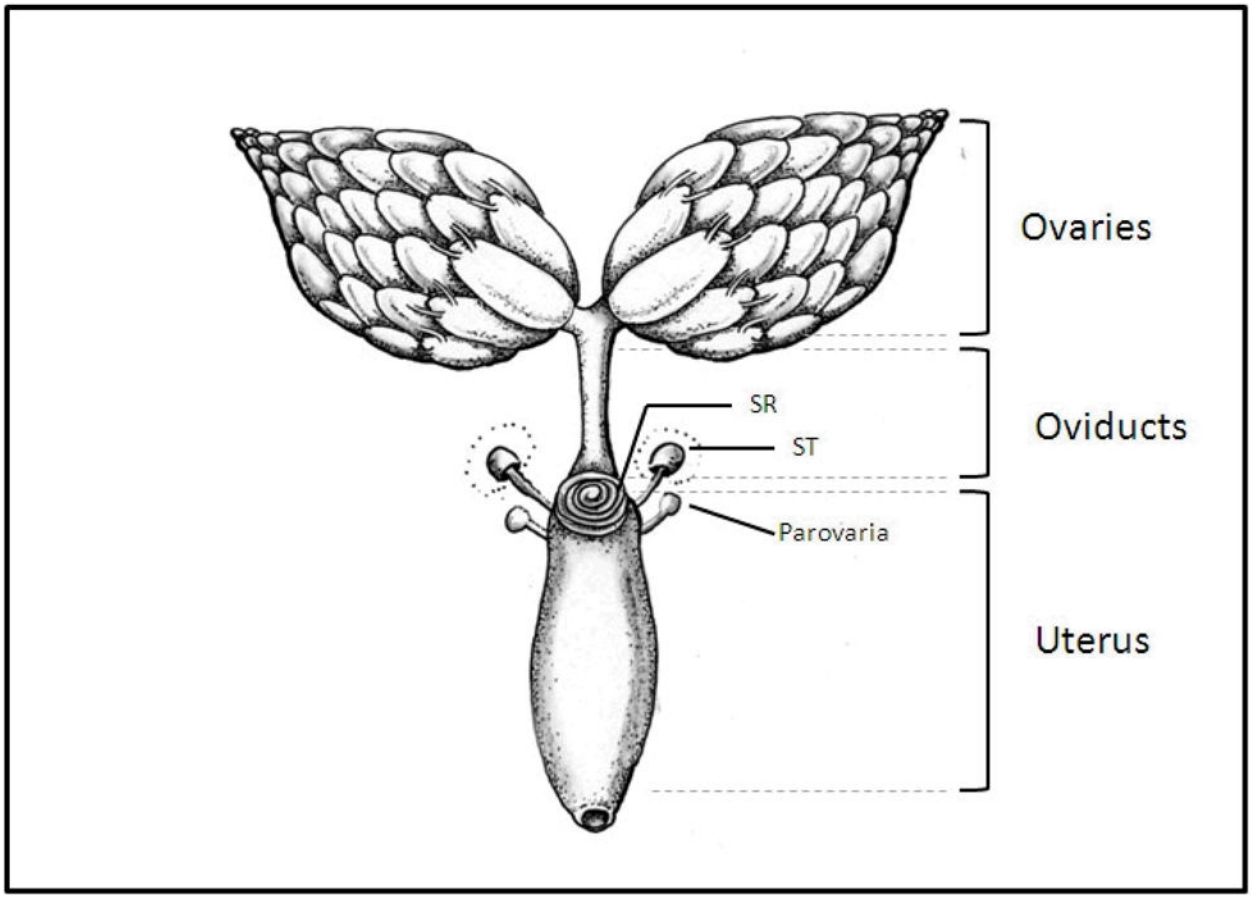

Figure 1.
Female *Drosophila melanogaster* reproductive tract. During mating, the ejaculate is transferred into the uterus. From there, different components of the ejaculate move to different locations within the female reproductive tract. Some seminal fluid proteins also move out of the reproductive tract and into circulation. SR: seminal receptacle; ST: spermatheca. Drawing by J. Sitnik; for clarity, some parts have been simplified in the figure.

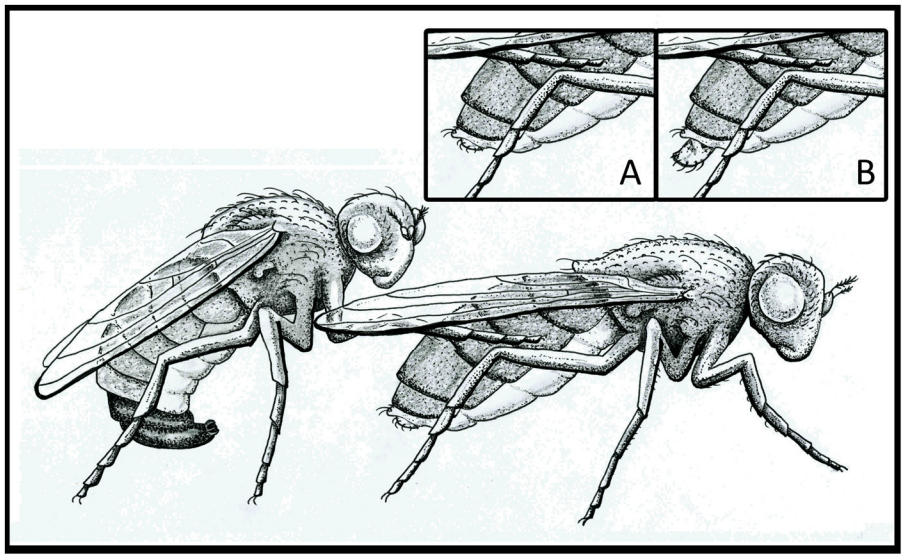

Figure 2.
Mating attempt by male and (insets) position of female abdomen when she is receptive (A) and unreceptive (B; ovipositor extruded) to the mating attempt. Drawing by J. Sitnik.

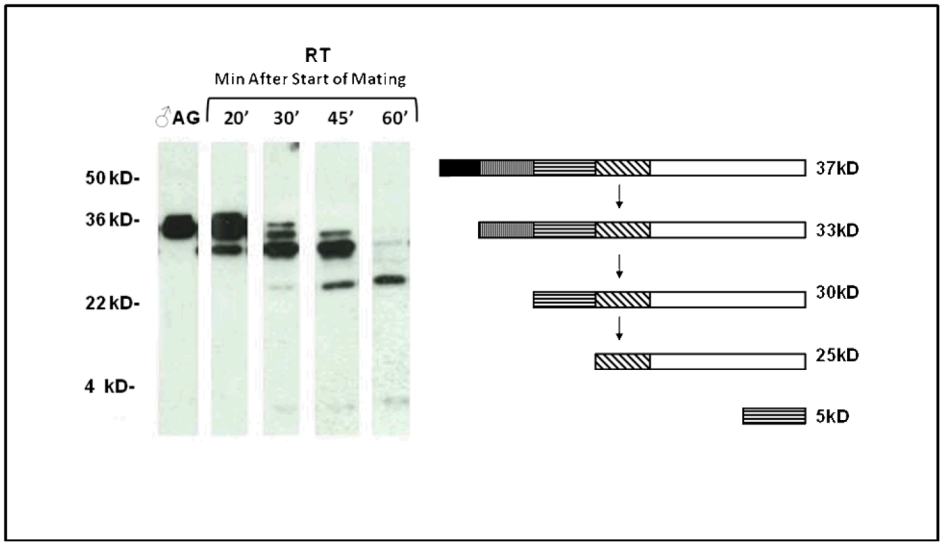

Figure 3.

Ovulin processing. Western blot showing intact ovulin in the male and ovulin processing products over time after the start of mating (ASM) in the female reproductive tract. Schematic of ovulin processing is on right (schematic adapted from Park & Wolfner 1995; 37kDa is the size of intact ovulin; in some places on the blot, bands are broad due to glycosylation variants that are not shown in the schematic).

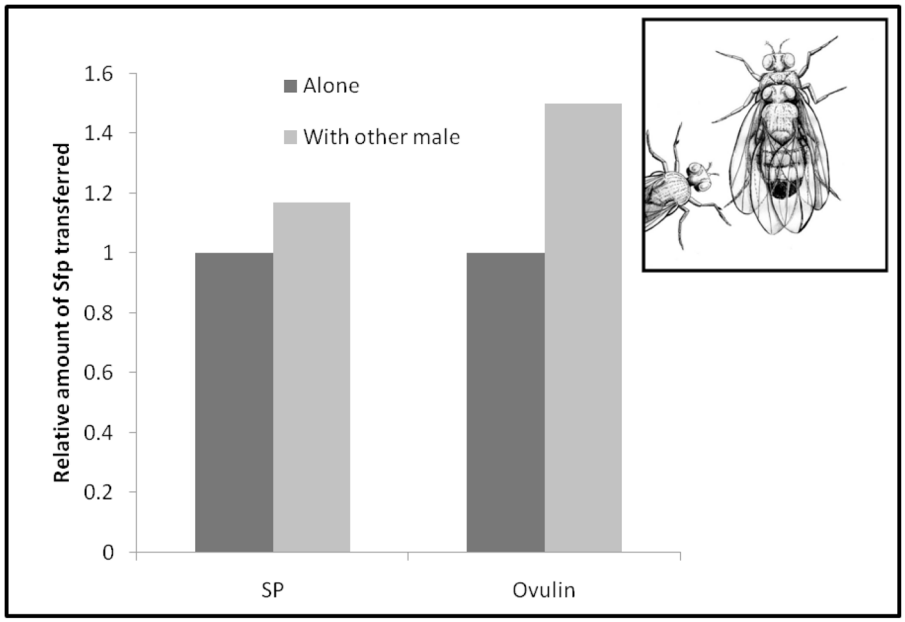

Figure 4.

Male *D. melanogaster* transfer more sex peptide and ovulin when they are in the presence of another male before and during copulation than when they are alone. Data figure adapted from Wigby *et al.* 2009. Drawing by J. Sitnik; for clarity, some body parts have been simplified in the figure.

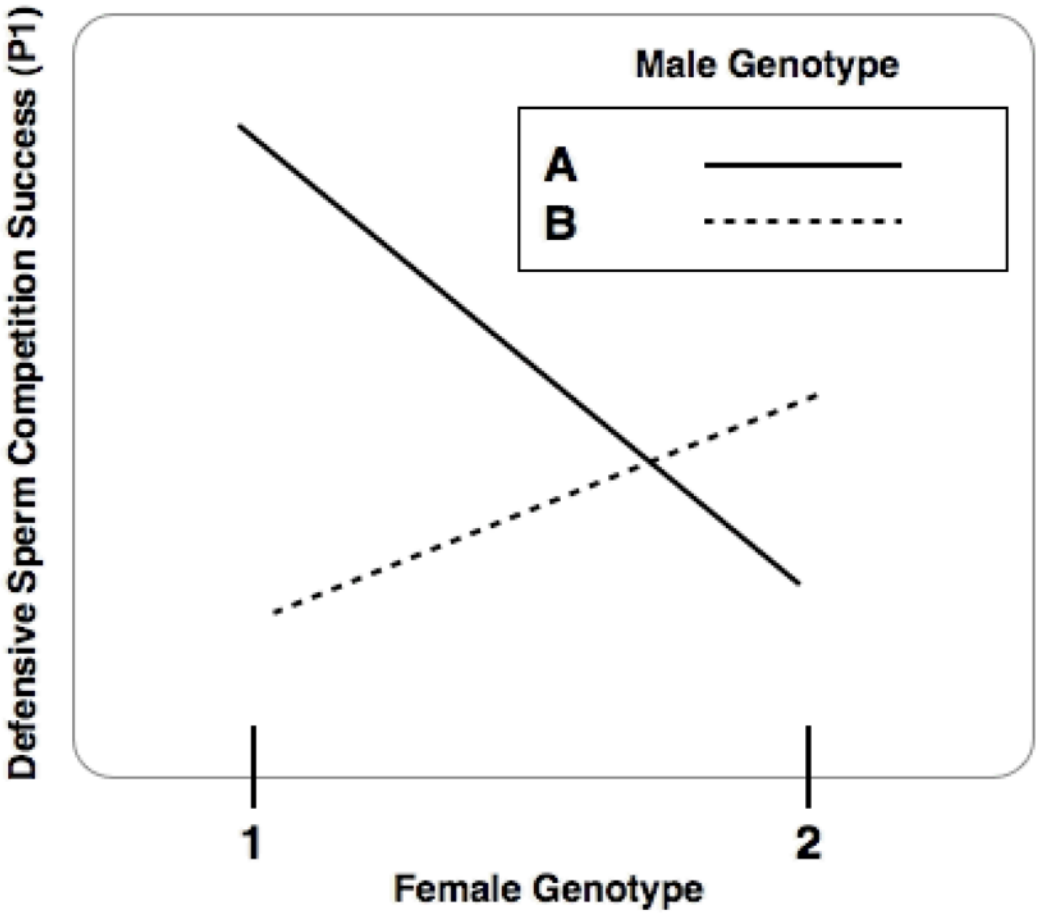

Figure 5.

Male and female genotypes affect outcome of sperm competition experiments. The genotypes of the particular male and female involved in a mating affect the defensive component of sperm defense, P1. P1 measures the proportion of offspring sired by the first male after the female has remated. In this hypothetical example, male A has a much higher P1 than male B when either of them mates with a female of genotype 1. However, when these same males mate a female of genotype 2, male B now has the advantage. Here, the second male to mate in each competition experiment does not vary. These interactions become even more complex when the genotype of the second male to mate is taken into consideration.

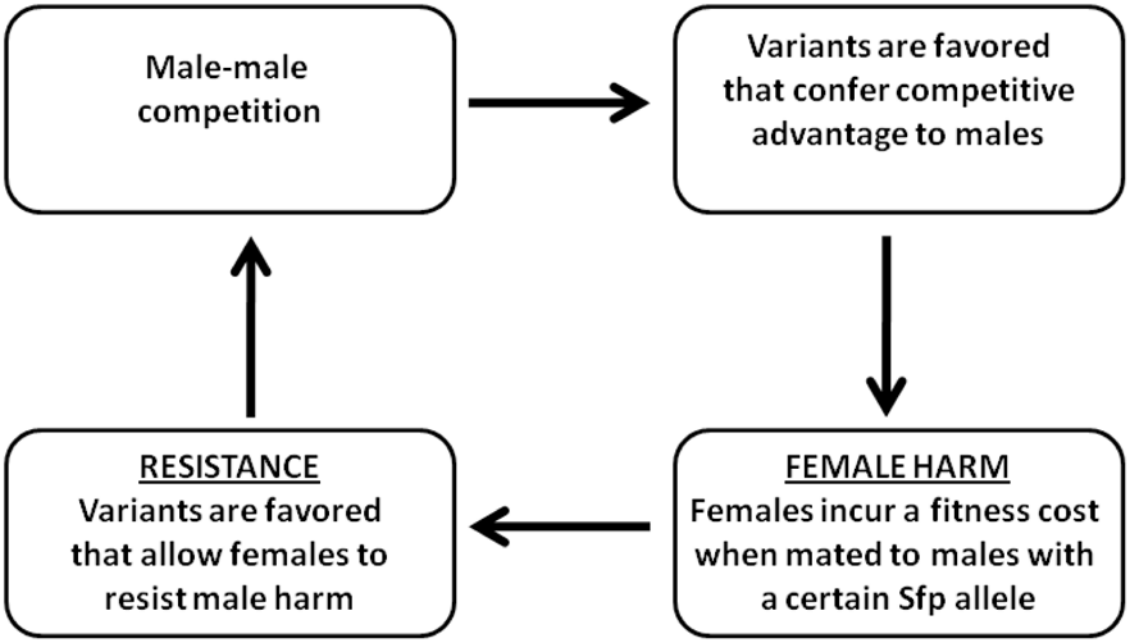

Figure 6.

Sexual conflict may maintain polymorphisms in the population. Competition among males for mates/fertilizations drives selection for new alleles that confer a competitive advantage to males for some reproductive trait. For example, males with this allele may induce a higher rate of ovulation in females by way of a particular Sfp. This higher ovulation rate may be harmful to females due to higher energy costs, driving selection for alleles that allow females to resist the effects of the harmful male allele. This cycle continues, with males developing new competitive strategies, each with a potential female cost associated with them. As a result of this process occurring simultaneously at many loci, polymorphisms are maintained in the population. Adapted from Arnqvist & Rowe 2005.