Sex peptide and the sperm effect in *Drosophila melanogaster*

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On mating, *Drosophila* females undergo dramatic alterations in their reproductive physiology and behavior (Fig. 1) (1). Egg production and egg laying are significantly increased, and the female's propensity to remate is drastically reduced. These changes in the female have been shown to be initially induced by seminal fluids transferred from the male to the female during mating and to persist because of the presence of stored sperm in the female (referred to as the "sperm effect"). The male seminal fluid proteins are synthesized in paired secretory organs of the male reproductive tract called accessory glands, the products of which are referred to as accessory gland proteins (Acps). Acps have been studied because of their importance for reproduction as well as their interesting evolutionary dynamics. Two articles in this issue of PNAS provide additional insights into Acp function and the sperm effect by characterizing a null mutant (2) and RNA interference knockdowns (3) for one of the most interesting Acps, the Sex Peptide (SP) [Acp70A (4)].

Mating-induced changes in female *Drosophila* have been described as occurring in two phases, short- and long-term stages. The short-term effect is attributed largely to the rapid action of several Acps, some acting before and during the storage of sperm. Through the use of males lacking Acps, sperm, or both, it was determined that the actions of Acps on their own last no longer than 1 day after mating (5, 6). The functions of the Acps have been studied by several methods, the two most robust using genetics to either ectopically express the Acp in females or generate null mutants (knockouts) in which the males lack specific Acps (1). These two genetic approaches have determined the functions of four Acps. By injection or ectopic expression, SP was found to increase egg laying and reduce female receptivity (4, 7). Ectopic expression of the protease inhibitor Acp62F showed it is toxic to *Drosophila*, although the exact reproductive function of Acp62F remains unclear (8). The toxicity of Acp6F is consistent with previous studies demonstrating that the receipt of accessory gland proteins reduces the female's life span (9, 10). Through knockouts, it has been demonstrated that Acp36DE is important for sperm storage (11, 12) and Acp26Aa (ovulin) functions to stimulate ovulation (13, 14). The long-lasting alterations of the female's physiology require the presence of sperm, and the mechanism by which the presence of sperm acts remains mysterious (15). Speculations have included that the sperm induce the release of female substances, stimulate stretch receptors in the sperm storage organs, or release male-derived factors bound to sperm (16).

Screening for Acp knockouts has proven extremely difficult, because no *a priori* phenotype could be predicted that would allow the design of a mutant screen. Indeed, the Acp knockout phenotypes discovered to date cause only partial fertility reduction and thus do not allow straightforward genetic selection screens. However, recent advances in targeting specific loci in *Drosophila* by homologous recombination (17) or RNA interference (18) show promise for efficiently targeting specific genes for analysis. It is through these two methods that molecular insights into the function of SP and the sperm effect are reported in this issue of PNAS (2, 3). These studies indicate that the sperm effect is due to sperm acting as a carrier and reservoir for SP (and perhaps other Acps).

Fig. 1. Image of *Drosophila* mating. The female decreases her remating rate and increases her egg-laying rate after mating due, in part, to the transfer of SP from the male to the female. (Photograph courtesy of Avis C. James and Gary Wolsieffer.)

The generation of SP-deficient flies has revised our understanding of the sperm effect in *Drosophila*. Chapman *et al.* (3) generated flies deficient in SP by expressing a SP sense–antisense construct specifically in male accessory glands, producing knockdown males with no detectable SP. Liu and Kubli (2) generated males lacking SP by directly disrupting the SP gene through homologous recombination. Despite the use of completely different methods and *Drosophila* strains, the two studies produced strikingly similar results. Females mated to SP-deficient males were initially more receptive to remating and produced fewer eggs than females mated to control males. These results confirm previous findings of SP's short-term effects (4). Females mated to SP-deficient males still showed some reduction in receptivity and stimulation of egg laying, confirming that other Acps also affect these processes, such as Acp26Aa, which increases ovulation (13, 14). The main surprise of the findings in both articles was the lack of a sperm effect in females mated to SP-deficient males: the mating-induced changes did not persist past 1 day in these females, despite normal sperm storage and usage. Previously, it had been hypothesized that the presence of sperm produced a signal to the female that maintained the mated status of reduced receptivity and increased egg production. The results from these two studies are consistent with the idea that the sperm effect is in fact an Acp effect, and that sperm act as a carrier and reservoir for at least SP and maybe other Acps (16). In this model, SP bound to the sperm is continuously released from the sperm stored in the mated female to maintain the female's elevated egg-laying rate and reduced propensity to remate, and the molecular basis of the sperm effect is SP.

Functional studies using a null mutant or RNA interference knockdown of Acps are imperative not only to understand the molecular basis of reproductive signaling in *Drosophila* but also to gain insight into their evolution. The evolution of reproductive proteins is fascinating in that their genes tend to include some of the most divergent found within the genomes of several organisms (19). The selective pressure driving their divergence remains unknown, although processes such as sexual conflict (20) and sexual selection (21) have been proposed. Detailed functional characterization of Acps will be invaluable in helping to elucidate the selective pressures driving the divergence of reproduc-

See companion articles on pages 9923 and 9929.

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tive proteins. For example, the results from the RNA interference-induced knockdown of SP (3) suggest that SP is not responsible for the mating-induced cost to female fitness (9, 10). Sexual conflict theory predicts an arms race between the sexes due to genes whose function is beneficial in one sex being detrimental to the opposite sex (20, 22). Even if sexual conflict drives the evolution of some reproductive genes (22), it may not be responsible for the putative selective events in the evolutionary history of SP deduced from polymorphism surveys (23). Consistent with this observation, SP is not extraordinarily divergent among species of *Drosophila* (24), as are other Acps (25), but additional sequencing of SP from more species is needed to gain a clear picture of SP's evolutionary history. The finding that SP apparently does not incur a cost of mating for females is also consistent with this interpretation.

The identification of sperm as a carrier and reservoir of SP raises another interesting evolutionary hypothesis. Some species of *Drosophila* produce sperm with enormous coiled tails, which, if straightened, sometimes exceed the length of the fly itself (26). Could one selective pressure leading to extreme sperm tail length involve the need to store and deliver large quantities of Acps (2)? Acps, in addition to SP, are also known to bind sperm (11), suggesting that the phenomenon of sperm acting as an Acp reservoir may perhaps be more general. Although intriguing, this question requires additional investigation.

The exciting and surprising findings observed from SP knockout flies generated by homologous recombination or from RNA interference knockdowns of SP should prompt the use of similar methods to explore the function of additional Acps. *Drosophila* seminal fluid is relatively complex, with an estimated 83 separate genes contributing to the mixture (27). It will be invaluable to determine the function of the other Acps to gain a detailed understanding of *Drosophila* reproductive biology and the role of reproductive proteins in the process of speciation. Several Acps have the signature of what have recently been referred to as "speciation genes," genes within a genome whose rapid divergence is associated with reproductive isolation and generation of new species (28, 29). The Acps as a class are 2-fold more divergent at both the protein (30) and nucleotide (27) levels as compared with nonreproductive tissues. Generation of null mutants for the Acps will allow transgenic approaches to study functional differences observed within and among species of *Drosophila* by expression of different versions of the Acp in a null background. Such studies could help elucidate the molecular basis for the variation in male mating success (31) and determine the functional basis for the high levels of polymorphisms observed for some Acps (32, 33).

The selective pressure driving the divergence of reproductive genes remains unknown.

The study of the genetics of speciation is progressing rapidly (29), including the discovery of several loci implicated in reproductive isolation (34) and hybrid inviability (28, 35). The key to understanding their role in the speciation process will be an intersection of evolutionary genetics and detailed functional characterization of the gene products. *Drosophila* Acps and their female receptors are an ideal system to study the evolutionary dynamics of genes potentially involved in the speciation process. Detailed functional characterization of specific gene products by knockout or knockdown studies as demonstrated for SP (2, 3) and other Acps (11, 13), theoretical models (22, 36), and evolutionary genetics aimed at understanding the selective forces acting on the Acp genes (25, 32), coupled with laboratory population studies (20), should provide an integrated understanding of the process of reproductive isolation through divergence of genes mediating sexual reproduction.

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