Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction

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Synopsis  Successful reproduction requires contributions from both the male and the female. In *Drosophila*, contributions from the male include accessory gland proteins (Acps) that are components of the seminal fluid. Upon their transfer to the female, Acps affect the female’s physiology and behavior. Although primary sequences of Acp genes exhibit variation among species and genera, the conservation of protein biochemical classes in the seminal fluid suggests a conservation of functions. Bioinformatics coupled with molecular and genetic tools available for *Drosophila melanogaster* has expanded the functional analysis of Acps in recent years to the genomic/proteomic scale. Molecular interplay between Acps and the female enhances her egg production, reduces her receptivity to remating, alters her immune response and feeding behavior, facilitates storage and utilization of sperm in the female and affects her longevity. Here, we provide an overview of the *D. melanogaster* Acps and integrate the results from several studies that bring the current number of known *D. melanogaster* Acps to 112. We then discuss several examples of how the female’s physiological processes and behaviors are mediated by interactions between Acps and the female. Understanding how Acps elicit particular female responses will provide insights into reproductive biology and chemical communication, tools for analyzing models of sexual cooperation and/or sexual conflict, and information potentially useful for strategies for managing insect pests.

Introduction

Reproduction is critical for the maintenance of life on earth. Successful reproduction requires interactions between male and female cells, molecules, and genomes. In animals with internal fertilization, males transfer to females not only sperm but also seminal fluid that contains a cocktail of molecules important for reproductive success. In this article, we discuss such molecules of the seminal fluid and how they interact with the female. We focus on *Drosophila*, since researchers have been able to utilize its powerful genetic tools to dissect the molecular contributions and interplay of the male and female that affects the female’s reproductive physiology and behavior.

Seminal fluid in *Drosophila* is composed of secretions from several tissues of the male reproductive tract including the accessory gland, the seminal vesicle, and the ejaculatory duct/bulb (see Chapman 2001; Gillott 2003; Wolfner et al. 2005 for reviews). In particular, molecules from the male accessory gland profoundly influence the female’s reproductive physiology and behavior in *Drosophila* (reviewed by Chen 1996; Wolfner 1997, 2002; Gillott 2003; Ravi Ram and Ramesh 2003; Chapman and Davies 2004; Wolfner et al. 2005; Wong and Wolfner 2006). Recent advances in bioinformatics, genomics and proteomics have greatly expanded our knowledge of these molecules, and genetic analyses have shed light on their functions and interactions.

Here, we review the male’s accessory gland proteins (Acps), the major components of the seminal fluid. First, we discuss the general nature of the Acps that are known thus and the interesting evolutionary dynamics of Acp genes. We then discuss the functions and mechanisms of action of specific Acps. We highlight several recent examples of the interactions between Acps and the female at the molecular level. We conclude by suggesting future directions for research on molecules derived from the male accessory gland.

Identification and evolutionary dynamics of accessory gland proteins (Acps)

Identification of Acps

The accessory gland is a secretory tissue of the *Drosophila* male’s reproductive tract (Fig. 1). Each of the accessory gland’s two lobes comprises a single layer of secretory cells surrounding a lumen; the secretory cells are in turn surrounded by a muscular...
The two types of secretory cells ("main cells" and "secondary cells") (Bairati 1968) express different but partially overlapping sets of Acp genes (Bertram et al. 1992). The importance of products of the accessory gland in reproduction by *D. melanogaster* was first seen in transplantation studies: male accessory glands transplanted into virgin females caused increased egg production and decreased receptivity (Garcia-Bellido 1964; Merle 1968). Since normal females show these same responses after they have mated, the transplantation results indicated that molecules manufactured in the accessory gland and transferred into the females in the seminal fluid are primarily responsible for the alterations in the behavior of mated females.

The obvious importance of products of the accessory gland prompted investigators to identify particular Acps. One Acp, the sex peptide (SP, also called Acp70A) was identified by testing fractionated protein/peptide extracts of accessory glands for their ability to induce egg laying and to reduce a female's likelihood to remate (Chen et al. 1988). A single peptide was purified, and shown to be encoded by an accessory-gland-specific RNA. An additional 17 Acps were identified by screening for RNAs expressed specifically (or predominantly) in the male's accessory gland (Schäfer 1986; DiBenedetto et al. 1987; Monsma and Wolfner 1988; Simmerl et al. 1995; Wolfner et al. 1997; see Supplementary Table 1). Because Acps must be secreted from the accessory gland in order to be transferred to females, the latter study applied an additional criterion to focus attention on proteins with functions in reproductive interaction between males and females: Acp genes must encode proteins or peptides with a predicted signal sequence that permits extracellular secretion.

With the release of the *D. melanogaster* genome sequence (Adams et al. 2000), differential expression-screening was extended via a comprehensive EST screen (Swanson et al. 2001a) that identified 34 additional Acp genes satisfying the stringent criteria noted above (Mueller et al. 2005; see Supplementary Table 1). Statistical tests based on the frequency of multiple hits in the EST screen suggested that the number of stringently defined Acps was 72−106 (Mueller et al. 2005). By isolating RNA from whole flies or from male accessory glands and using gene-specific primers, we identified three more Acp genes that fit the stringent criteria (Ravi Ram K and Wolfner MF, unpublished data) (Supplementary Table 1). Additional methods have brought the number of known Acps closer to the predicted total. Because 16 Acps were reported to have gene duplicates (Mueller et al. 2005), Holloway and Begun (2004) searched for additional Acp gene duplicates and identified three such genes expressed in the accessory gland, and thus likely to encode Acps (Supplementary Table 1).

In an alternative way to identify Acp genes via differential gene expression, Julian Dow and his group (at University of Glasgow, UK) used microarrays to identify transcripts expressed in the accessory gland (Chintapalli et al. 2007; for their gene expression data, please see http://flyatlas.org/). Their microarrays detected 52 of the 58 previously identified Acps and identified other genes with high expression in accessory glands. In particular, we note that 46 new potential Acp genes in their data set meet the stringent criteria of highly enriched expression in the accessory gland, and of encoding proteins with predicted signal sequences. Although these 46 genes are expressed in the accessory gland, it is possible that they may be also be expressed in other tissues not analyzed by Chintapalli et al. (2007). Thus, the total number of Acps identified so far by differential or RNA-based screening, or by biological assay is 104 (Supplementary Table 1).
That 104 genes are identified by the stringent criteria likely underestimates the total synthetic complexity of the male's accessory gland, since proteins that are not accessory-gland-specific but are nevertheless expressed in accessory glands and transferred to females could also exert important reproductive functions. Several methods can be used to identify secreted proteins expressed in, but not necessarily specific to, the accessory gland. For example, in a proteomics analysis Walker et al. (2006) used mass spectrometry to find 13 proteins in the secretions of the accessory gland. Of these, five corresponded to the products of Acp genes previously identified by stringent-expression criteria (Wolfner et al. 1997; Swanson et al. 2001a; Mueller et al. 2005). The remaining eight proteins are predicted to contain signal sequences and their accessory-gland-expression is confirmed by microarrays (see http://flyatlas.org/). These results suggest that further proteomic analysis and further analysis of microarray data (e.g. http://flyatlas.org/) will be rich sources for new Acp genes, adding to the 112 Acp genes known so far (Supplementary Table 1).

Acps fall into conserved protein classes

The 112 Acp genes encode peptides and proteins that can be categorized into a range of predicted molecular classes. These classes include (1) small novel peptides or larger prohormone-like molecules that could be cleaved to release biologically active peptides, (2) predicted glycoproteins, and (3) proteins with sequence motifs predictive of either enzymatic functions (proteases and protease inhibitors, lipases), cellular or organismal protection (antimicrobial proteins, thioredoxins, and oxidoreductases) or sperm binding (lectins and cysteine rich secretory proteins (CRISPs)) (Table 1). The peptide, proteolysis regulator, CRISP, or lectin classes are found in the same proportion in the 58 stringently selected Acps as in the additional 54 Acps identified in microarrays or by proteomics. Interestingly, Mueller et al. (2004) reported that these classes of seminal proteins are conserved between Drosophila and mammals, even though the primary amino-acid sequences of individual proteins have not been conserved. Subsequent studies have reported a similar phenomenon in additional taxa; functional classes of seminal-fluid proteins are conserved, although most particular seminal-fluid proteins are taxon-specific [primates (Clark and Swanson 2005), crickets (Andrés et al. 2006; Braswell et al. 2006), medflies (Davies and Chapman 2006), honeybees (Collins et al. 2006); see Poiani 2006 for review].

The conservation of seminal-fluid protein classes across a wide range of organisms with quite different reproductive physiologies is intriguing, given that several proteins in each of these conserved classes are known to have important reproductive functions in higher vertebrates (Okamura et al. 1999; Malm et al. 2000; Murer et al. 2001; Chen et al. 2002; Fouchecourt et al. 2002; Suarez 2002; Busso et al. 2005; Kraus et al. 2005; Ellerman et al. 2006; Nixon et al. 2006; O’Rand et al. 2006) and, as we shall discuss, in Drosophila. The evolutionary conservation of seminal-fluid protein classes suggests that exploitation of Drosophila molecular genetics may identify seminal-fluid functions that apply generally across taxa.

Evolutionary dynamics of Acps

Many studies in a wide range of taxa have shown that certain traits and molecules involved in sexual reproduction evolve rapidly (for example, see Civetta and Singh 1999; Swanson and Vacquier 2002; Clark et al. 2006; Panhuis et al. 2006). In the case of Drosophila, induction of post-mating responses in the female involves interactions between Acps and molecules in the female (in the reproductive tract or elsewhere; see below). Rapid evolution of Acps could result from sexually antagonistic coevolution between Acps and Acp-receptors in the female due to sexual conflict arising from the differing reproductive interests of males and females (Rice and Holland 1997; Rice 2000). Alternatively, rapid evolution of

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**Table 1** Number of Acps in a given protein class (For subclasses under each protein class and for CG numbers of Acps in each class, please see the Supplementary Table 1).

<table>
<thead>
<tr>
<th>Accessory gland protein class (Number of molecules)</th>
<th>Peptides/prohormones (44)</th>
<th>Proteases (18)</th>
<th>Protease inhibitors (7)</th>
<th>Acid lipases (7)</th>
<th>Lectins (8)</th>
<th>CRISPs (8)</th>
<th>Defensin (1)</th>
<th>RNase (1)</th>
<th>Macroglobulin (1)</th>
<th>Thioredoxin (1) and Oxido reductases (2)</th>
<th>Fasicilin (1)</th>
<th>Alkaline phosphatase (1)</th>
<th>Collagen like (3)</th>
<th>Hydrolase (1)</th>
<th>Chaperones (4)</th>
<th>Protein folding (3)</th>
<th>Peroxidase (1)</th>
<th>Total (112)</th>
</tr>
</thead>
</table>

| Accessory gland protein class (Number of molecules) | Peptides/prohormones (44) | Proteases (18) | Protease inhibitors (7) | Acid lipases (7) | Lectins (8) | CRISPs (8) | Defensin (1) | RNase (1) | Macroglobulin (1) | Thioredoxin (1) and Oxido reductases (2) | Fasicilin (1) | Alkaline phosphatase (1) | Collagen like (3) | Hydrolase (1) | Chaperones (4) | Protein folding (3) | Peroxidase (1) | Total (112) |
Acps might result from selection due to forces such as cryptic female choice (Eberhard 1996) or sperm competition (Harshman and Prout 1994; Clark et al. 1995, 1999). In either case, Acps are expected to be under selection pressure. Indeed, although functional classes of seminal proteins appear to be conserved across organisms, a surprisingly high number of Acps show rapid evolutionary change in their sequences of primary amino acids (Swanson et al. 2001a; Mueller et al. 2005).

The recent release of the genome sequences of 12 Drosophila species has allowed a broad examination of the evolutionary dynamics of Acps. Approximately 36% of Acps shared among members of the D. melanogaster subgroup (D. melanogaster, D. simulans, D. sechellia, D. erecta, and D. yakuba) appear to have experienced positive selection (sequence regions with an elevated relative level of non-synonymous amino-acid substitutions (dn)/synonymous amino-acid substitutions (ds); dn/ds >1) (Swanson et al. 2001a; Mueller et al. 2005; Haerty et al., submitted for publication). This proportion is significantly higher than the proportion of non-reproductive genes that show such rapid evolution (Haerty et al. submitted). The trend of positive selection is not limited to Acps of any particular protein class; it occurs for Acps across several different protein classes. Positive selection on Acp genes is not limited to Drosophila, since Acp genes in crickets and prostate proteins in humans also show signs of positive selection (Clark and Swanson 2005; André’s et al. 2006; Braswell et al. 2006).

The genomic comparative data also provide evidence for rapid loss/gain of Acp genes (Mueller et al. 2005). For example, D. simulans contains apparent Acp genes that lack homologs in its close relative D. melanogaster (Swanson et al. 2001a; Begun and Lindfors 2005; Mueller et al. 2005). Comprehensive analysis of genome-sequence data from the 12 Drosophila species showed that species increasingly distant from D. melanogaster have fewer detectable homologs to D. melanogaster Acps: less than 50% of the D. melanogaster Acps have clear homologs outside of the melanogaster/obscura species groups (Haerty et al., submitted for publication). Interestingly, the majority of Acp genes that encode predicted peptide/prohormones lack homologs outside the D. melanogaster subgroup, suggesting that these proteins might mediate reproductive events only in species close to D. melanogaster. Similarly, homologs (detectable by BLAST searches) of Drosophila Acps are rare in the honeybee Apis mellifera (Collins et al. 2006) and in the mosquito Aedes aegypti (Sirot LK et al., manuscript in preparation). Consistent with rapid between-species evolution, there is also evidence that Acps are subjected to positive selection within species (Begun et al. 2000; Kern et al. 2004; Begun and Lindfors 2005; Schully and Hellberg 2006).

Not all Acps, however, are rapidly evolving: some show signs of evolutionary conservation. For example, the sex peptide (SP), which elicits several postmating responses in Drosophila females (see below), is well conserved at the primary-sequence level in 12 species of Drosophila whose genomes have been sequenced (Haerty et al., submitted for publication), although D. subobscura has duplicate SP genes that show signs of adaptive divergence (Cirera and Aguadé 1997). Homologs of SP can even be found in insects outside of the Drosophila lineage. For example, an SP homolog is detectable by BLAST search of the honeybee genome (Honeybee Genome Sequencing Consortium 2006; Ravi Ram K and Wolfner MF, unpublished data), although the pattern of expression of this gene is unknown. In the moth Helicoverpa armigera, male accessory glands contain molecules with immunoreactivity to Drosophila SP (Nagalakshmi et al. 2004), and injection of Drosophila SP into virgin Helicoverpa armigera females decreases their production of sex pheromone and stimulates their synthesis of juvenile hormone, suggesting that effects of SP may be conserved (Fan et al. 1999, 2000).

Acps with important roles in reproduction can, surprisingly, exhibit conservation and rapid evolution within the same protein. A striking example is provided by ovulin, a prohormone Acp that stimulates the release of eggs from the ovary (Herndon and Wolfner 1995; Heifetz et al. 2000). Ovulin is a rapidly evolving Acp and is among the most rapidly evolving proteins in Drosophila (Aguadé et al. 1992; Tsaur and Wu 1997; Tsaur et al. 1998, 2001). Yet ovulin also contains three predicted coiled-coil domains, whose sequences or predicted conformations are conserved among Drosophila species (Wong et al. 2006). Wong et al. (2006) suggested that these domains, which appear to be involved in ovulin’s self-interaction, may allow the protein to maintain its overall 3D conformation so that it can tolerate high rates of evolution at other sites. Similar explanations have previously been proposed for other proteins with both rapidly evolving and conserved regions, e.g., abalone sperm lysin (Yang et al. 2000), mammalian ZP3 (Swanson et al. 2001b; Jansa et al. 2003), and MHC (Hughes and Nei 1988).

Finally, it is interesting to note that the chromosomal locations of Acp genes are biased to autosomes.
in *D. melanogaster*. Only seven of the 112 known Acp genes are located on the X chromosome. The remaining 105 Acp genes are evenly distributed across chromosomal arms: 2L (30 Acps), 2R (24 Acps), 3L (25 Acps), and 3R (26 Acps) (Fig. 2). Since the X-chromosome constitutes about 19% of the *D. melanogaster* genome (*D. melanogaster* genome release 5.1), 21 Acp genes would be expected to be X-linked if their distribution in the genome were random. The presence of only seven X-linked Acps is significantly different from this expectation (*P* < 0.01; Chi-Square test *x*² = 8), supporting prior reports that the *D. melanogaster* X chromosome is deficient in genes with male-biased expression (Wolfrner et al. 1997; Swanson et al. 2001a; Parisi et al. 2003; Ranz et al. 2003; Mueller et al. 2005; Haerty et al., submitted for publication).

Within the autosomes, about 30% of the Acp genes are found in tandem or in clusters. Most of these clusters are on the second chromosome (Table 2). Fifteen Acp genes are duplicated; in 13 of these cases, the duplicates are found in the same cluster (Holloway and Begun 2004; Mueller et al. 2005; flyatlas data: http://flyatlas.org/; Ravi Ram K and Wolfner MF, unpublished data). Acps that are tightly linked sometimes have similar evolutionary dynamics (e.g., CG8622, CG15616 and CG8626, or CG9997 and CG14061) (Haerty et al., submitted for publication), but sometimes do not (for example, ovulin appears to have evolved rapidly whereas its neighbor Acp26Ab has not (Aguadé et al. 1992; Tsaur and Wu 1997; Aguadé 1998; Tsaur et al. 1998; Depaulis et al. 2003; Zurovcova et al. 2006). Although some Acps appear to have been subject to positive selection, it is still not possible to determine which of several selective pressures noted earlier has driven this. Understanding the functions of Acps and mechanisms through which they induce post-mating responses in females will help to determine these forces.

**Acps affect several processes in mated females**

Injections of *D. melanogaster* accessory glands into unmated females showed that the accessory gland molecules can elicit increased egg laying and can reduce receptivity to mating (Garcia-Bellido 1964; Merle 1968). Those experiments, however, could not assess sperm-related functions of Acps since the injections were done on unmated females. Transgenic flies that fail to make proteins in the main cells of their accessory glands (DTA-E) (Kalb et al. 1993) or that have no/reduced accessory glands (Prd-rescue) (Xue and Noll 2000) were instead used to further dissect the role of Acps in postmating physiological and behavioral responses of females (Table 3).

Postmating responses in females appear to occur in two phases. For the first 24 h, there is a short-term increase in egg production, ovulation, and egg deposition, as well as a decrease in receptivity to remating. This “short-term response” depends on Acps (Kalb et al. 1993), and is mostly independent of sperm. Persistence of these changes for more than 24 h, however, requires the presence of stored sperm in the female (“long-term response”) (Manning 1962, 1967; Gromko et al. 1984). Some Acps might only act in the short term but at least one Acp, the SP, is essential for the long-term response (Kubli 2003; Liu and Kubli 2003; Peng et al. 2005a; see below). Acps also facilitate storage of the sperm transferred into the mated female (Tram and Wolfner 1999), play roles in sperm utilization (Hihara 1981; Fuyama 1983; Xue and Noll 2000) and sperm competition (Harshman and Prout, 1994), and also affect feeding behavior of females (Carvalho et al. 2006). Acps also contribute to the decreased longevity observed in mated females (Chapman et al. 1995). Furthermore, Acps include proteins with antimicrobial activity (Lung et al. 2001) and some that regulate the expression of antimicrobial genes in mated females (McGraw et al. 2004; Peng et al. 2005b), suggesting that accessory-gland products help protect sperm, females and/or eggs from microbial infection.
Knowledge of the tissues in mated females that are targeted by individual Acps is useful for predicting functions and mechanisms by which these molecules work. Acps may act to induce postmating changes in females by binding to receptors in the reproductive tract. For example, octopamine participates in the neuronal control of muscle contraction in the reproductive tracts of cockroaches, grasshoppers and Drosophila (Bamji and Orchard 1995; Lee et al. 2003; Monastirioti 2003; Cole et al. 2005; Middleton et al. 2006; Rodriguez-Valentin et al. 2006). The female reproductive tract of D. melanogaster is highly innervated and is rich in vesicles that likely contain neuro-modulators such as octopamine. Receipt of Acps regulates the release of the contents of some of these vesicles (Heifetz and Wolfner 2004), potentially triggering muscle contractions that facilitate ovulation or movement of the egg through the reproductive tract to the outside or release/entry of sperm from/into storage (Heifetz and Wolfner 2004). Alternatively, an Acp could potentially affect egg production, ovulation and egg laying, feeding, receptivity, sperm storage and longevity of the female through neuroendocrine pathways in mated females. For this, an ACP might have to enter the circulatory system (hemolymph) to reach its targets and/or the muscles of the reproductive tract from the outside.

Tissue-targets for 22 Acps have been determined by western blotting of proteins from dissected tissues, by immunostaining or through GFP/mRFP tagging of Acps (Monsma et al. 1990; Bertram et al. 1996; Lung and Wolfner 1999; Heifetz et al. 2000; Ravi Ram et al. 2005; mRFP: Ravi Ram and Wolfner, manuscript in preparation). Interestingly, each Acp targets its own characteristic pattern of tissues in mated females, suggesting great diversity in the mechanisms and sites through which the Acps act. Nine Acps localize to sperm-storage organs (Bertram et al. 1996; Bloch Qazi et al. 2003; Peng et al. 2005a; Ravi Ram et al. 2005), four to the ovary base (Heifetz et al. 2000; Ravi Ram et al. 2005) and at

Table 2 Chromosomal locations of clusters of some Acps (~30% of the total).

<table>
<thead>
<tr>
<th>Acp cluster</th>
<th>chromosome</th>
<th>position</th>
<th>size (in kb)</th>
<th>Acps</th>
<th>Note</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2L 5891–5894K</td>
<td>3 kb</td>
<td>Ovulin, Acp26AB</td>
<td>Only ovulin is rapidly evolving</td>
<td>Monsma and Wolfner 1988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8392–8395K</td>
<td>3 kb</td>
<td>Acp29AB, CG17799</td>
<td>Acp gene duplicates</td>
<td>Holloway and Begun 2004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10640–10648K</td>
<td>8 kb</td>
<td>CG1872, CG18284, CG17977</td>
<td>Acp gene duplicates</td>
<td>Mueller et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11015–11019K</td>
<td>4 kb</td>
<td>Acp32CD, CG14913</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19960–19962K</td>
<td>2 kb</td>
<td>CG34051, CG13965</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3288–3291K</td>
<td>3 kb</td>
<td>CG11112, CG11113</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2R 5703–5706K</td>
<td>3 kb</td>
<td>CG1652, CG1656</td>
<td>Acp gene duplicates</td>
<td>Mueller et al. 2005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8733–8736K</td>
<td>3 kb</td>
<td>CG17575, CG30488, CG30486</td>
<td>Acp gene duplicates</td>
<td>Mueller et al. 2005; Ravi Ram K and Wolfner MF, unpublished data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12635–12638K</td>
<td>3 kb</td>
<td>CG8622, CG15616, CG8626</td>
<td>Acp gene duplicates (All three are rapidly evolving)</td>
<td>Holloway and Begun 2004</td>
<td></td>
<td></td>
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<tr>
<td>3L 7431–7434K</td>
<td>3 kb</td>
<td>CG32383, CG32382</td>
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<tr>
<td>18596–18600K</td>
<td>4 kb</td>
<td>CG18234, CG18233</td>
<td></td>
<td></td>
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<tr>
<td>20953–20956K</td>
<td>3 kb</td>
<td>CG11037, CG10587</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3R 21655–21658K</td>
<td>3 kb</td>
<td>CG9074, CG5016, CG4986</td>
<td></td>
<td>Simmerl et al. 1995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24581–24584K</td>
<td>3 kb</td>
<td>CG9997, CG14061</td>
<td>Both are rapidly evolving</td>
<td>Mueller et al. 2005; Ravi Ram K and Wolfner MF, unpublished data</td>
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at least 10 exit the reproductive tract to enter the circulation of the mated female (Monsma et al. 1990; Lung and Wolfner 1999; Ravi Ram et al. 2005). At least one ultimate target of SP is likely to be the brain, since in vitro experiments have shown this peptide to be capable of binding to brain tissue (Ottiger et al. 2000). The targeting patterns of Acps appear to be independent of protein class or the rate of an Acp’s evolution (Ravi Ram et al. 2005).

Acps increase mated females’ egg production, ovulation and egg deposition

Egg-laying in Drosophila is an outcome of a cascade of events that include progression of eggs through oogenesis (egg production), release of mature oocytes from the ovary (ovulation), passage of eggs through the oviducts to reach the uterus where they are fertilized, and finally the deposition of eggs on the substratum (oviposition) (Soller et al. 1999; Heifetz et al. 2000; Bloch Qazi et al. 2003). To date, two Acps, SP (Chen et al. 1988; Chapman et al. 2003b; Liu and Kubli 2003) and ovulin (Herndon and Wolfner 1995; Heifetz et al. 2000, 2005), have been demonstrated to stimulate the egg-laying process in the mated female. These two Acps are not redundant in function but instead act at different stages of the egg-laying process. SP acts at the level of oogenesis and perhaps oviposition whereas ovulin is needed to stimulate ovulation.

SP is a 36-amino-acid novel peptide. In vitro incubation of corpora allata with SP increases the production of juvenile hormone BIII (JHBIII) from this gland (Moshitzky et al. 1996). JHBIII is known to play roles in the maturation of ovaries in dipterans (reviewed by Kelly et al. 1987) and in the production and uptake of yolk proteins into D. melanogaster oocytes (Riddiford 1993; Spradling 1993). Kubli (2003) thus suggested that SP increases JHBIII levels in mated females, leading to an accumulation of yolk in the oocytes that would release oogenesis from a previtellogenic block prior to mating (Soller et al. 1997, 1999). Although the N-terminal region of SP is required for its effects on JHBIII production (Fan et al. 2000), the C-terminal portion of SP also affects egg production or egg deposition, possibly by binding to specific targets (suboesophageal ganglion, the cervical connective, and in parts of the thoracal-abdominal ganglion) in the central nervous system (Ottiger et al. 2000).

Ovulin is a prohormone-like polypeptide of 264 amino acids (Monsma and Wolfner 1988) that stimulates the release of oocytes from the ovary (Heifetz et al. 2000). In mated females, ovulin localizes mostly to the base of the ovary (Heifetz et al. 2000), suggesting local action, perhaps on the musculature. Some ovulin also enters the circulation of mated females, suggesting the possibility of acting through neuroendocrinal targets (Monsma et al. 1990; Lung and Wolfner 1999). The effect of ovulin is evident as early as 90 min postmating (Heifetz et al. 2000) and is only detected during the first 24 h after mating (Herndon and Wolfner 1995). The brief time course of its action has led to the model that ovulin stimulates the release of mature oocytes that had previously accumulated in a virgin female’s ovary, thereby facilitating a subsequent increase in the rate of oogenesis triggered by other Acps like SP (Heifetz et al. 2000; reviewed by Wolfner 2002).

Ovulin is cleaved into smaller peptides following its transfer to females (Park and Wolfner 1995). A short stretch of amino-acid sequence in one of these peptides is similar in sequence to hormones that stimulate muscular contractions and behavioral changes leading to egg release in the mollusc, Aplysia californica [egg laying hormone (ELH, califins)] (Rothman et al. 1986; Monsma and Wolfner 1988; Newcomb et al. 1988; Bernheim and Mayeri 1995; Wayne et al. 2004; also see DesGroseillers 1990; Wolfner et al. 2005 for review). Ectopic expression of different cleavage products of ovulin (Heifetz et al. 2005) in unmated Drosophila females has shown that ovulin’s two most C-terminal cleavage products are biologically active. Each of the two peptides is independently capable of

<table>
<thead>
<tr>
<th>Acp mediated postmating changes in the female</th>
<th>Acp known to be involved</th>
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<tbody>
<tr>
<td>Increased egg production</td>
<td>Sex peptide (SP, Acp70A)</td>
</tr>
<tr>
<td>Increased ovulation</td>
<td>Ovulin</td>
</tr>
<tr>
<td>Increased egg laying</td>
<td></td>
</tr>
<tr>
<td>Reduced receptivity to re-mating</td>
<td>SP</td>
</tr>
<tr>
<td>Decreased life span (cost of mating) or toxicity</td>
<td>Acp62F, SP, CG8137, CG10433,</td>
</tr>
<tr>
<td>Increased food intake</td>
<td>SP</td>
</tr>
<tr>
<td>Induction of antimicrobial peptide gene expression or of ability to fight infection</td>
<td>SP, CG6168, CG9334, CG10284</td>
</tr>
<tr>
<td>Facilitate sperm storage</td>
<td>Acp36DE</td>
</tr>
<tr>
<td>Sperm maintenance</td>
<td></td>
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<tr>
<td>Sperm utilization</td>
<td></td>
</tr>
<tr>
<td>Sperm competition</td>
<td>Acp36DE</td>
</tr>
<tr>
<td>Processing of reproductive molecules</td>
<td>Ovulin, Acp26Ab, Acp29AB, Acp33A, Acp53Ea; Acp62F, CG8137, CG14560 (based on correlations)</td>
</tr>
</tbody>
</table>
inducing ovulation. Because these experiments involved the expression of ovulin throughout the female’s body, future studies will need to identify the sites at which ovulin and its cleavage products act within the female.

Although SP and ovulin are known to modulate specific steps of the egg-laying process, it seems likely that additional Acps are required for full and concerted stimulation of the many steps in egg laying. Involvement of several Acps in the same biological process provides multilevel regulation to fine-tune the pathway, allowing different Acps to act in concert to give optimal stimulation. Acps such as CG1656, CG8137, CG9334, CG11598, CG14560, and CG17575 might be good candidates to test for roles in stimulating the egg-laying process, since these Acps target either to ovaries or to oviducts or enter the hemolymph (Ravi Ram et al. 2005).

**Acps influence mated females’ feeding behavior**

Egg production is resource intensive (Bradley and Simmons 1997; Drummond-Barbosa and Spradling 2001; Rivero et al. 2001; Partridge et al. 2005; Terashima et al. 2005). Within the female, resources must be allocated between egg production (and other reproductive phenomena) and somatic maintenance (Chippindale et al. 1993; Good and Tatar 2001; Min et al. 2006; see also Zera and Harshman 2001 for review). Since egg production increases dramatically after mating, mated females must allocate more resources towards egg production than must virgin females. This investment in egg production may come at the expense of somatic maintenance in mated females. One means of counterbalancing this cost is nutritional: by ingesting more food after mating, females can obtain additional resources. Interestingly, *D. melanogaster* females do eat more after mating and this feeding is dependent upon Acps (Carvalho et al. 2006). Specifically, SP induces postmating feeding: without SP, females fail to elevate feeding levels after mating (Carvalho et al. 2006). Further, ectopic expression of SP in virgin females demonstrated that SP is sufficient to induce increased feeding (Carvalho et al. 2006).

The effects of SP on feeding raise several new questions (Wong and Wolfner 2006). For example, does its stimulation of feeding underlie SP’s effects on egg production (Soller et al. 1997) since female nutritional status affects the progression of oogenesis (Drummond-Barbosa and Spradling 2001; Terashima et al. 2005)? Might the increased feeding contribute to the decrease in longevity of the mated female (see below) given that longevity has been negatively correlated with nutritional intake (Chippindale et al. 1993; Rauser et al. 2004; also see Piper et al. 2005 for review)? Or is SP’s effect on feeding a consequence of its effects on these processes or of its effect on the hormonal levels within the female (Moshitzky et al. 1996)?

**Acps reduce mated females’ receptivity to remating**

Females mated to wild-type males become less receptive to subsequent males and reject courting attempts from subsequent males (Kalb et al. 1993; Tram and Wolfner 1998; Xue and Noll 2000). Acps are needed for this change in mating-receptivity (courtship-rejection) (Kalb et al. 1993; Tram and Wolfner 1998). In *D. melanogaster*, SP is thus far the only Acp found to lower the receptivity levels of mated females (see Kubli 2003 for review). Injection of synthetic sex peptide into, or ectopic expression of SP in, virgin females reduced their receptivity to mating for 1–2 days (Chen et al. 1988; Aigaki et al. 1991; Nakayama et al. 1997). Furthermore, females mated to males lacking SP re-mated more readily than did mates of normal males (Liu and Kubli 2003; Chapman et al. 2003b; also see Kubli 2003, Swanson 2003 for reviews). Finally, recent association studies by Fiumera et al. (2007) correlated natural alleles of SP (and another Acp, CG6168) with reduction of the female’s receptivity to remating (Fiumera et al. 2007).

Studies of injection and ectopic expression showed the C-terminal portion of SP to be essential for reducing the receptivity of mated females and suggested that SP exerts its action through the hemolymph (Schmidt et al. 1993), perhaps through neural targets identified by *in vivo* studies (Nakayama et al. 1997). However, the mechanism by which SP exerts its effect on receptivity is unknown.

**Acps and females interact for storing sperm, for subsequent sperm utilization and to influence sperm competition**

Storage of sperm by mated females is important for extending the period of fertility after a mating. Sperm storage also allows for sperm competition and female sperm precedence after instances of multiple mating by the female. As described later, in *Drosophila* stored sperm are essential for the long-term response to Acps. Female *D. melanogaster* store ~20% of the ~4000 sperm that they receive during a mating. Storage can persist for up to ~2 weeks (Kaufman and Demerec 1942; Lefèvre and Jonsson 1962; Fowler 1973; Gilbert 1981; Gromko et al. 1984;

As with the egg-laying process, sperm storage proceeds through a series of stages: progression of sperm through the female reproductive tract after their receipt, entry of sperm into the storage organs, maintenance of viable sperm in storage, and release of sperm from storage for fertilization (reviewed by Bloch Qazi et al. 2003). Both females and males are thought to contribute to the processes involved in sperm storage in D. melanogaster. For example, a female nervous system is necessary for sperm storage (Arthur et al. 1998) and a mated female’s reproductive tract undergoes a characteristic set of changes in shape that appear to facilitate the movement of the sperm mass towards storage and give sperm access to the sperm-storage organs (Adams and Wolfner 2007). Male contributions to sperm storage in D. melanogaster include Acps (Kalb et al. 1993; Neubaum and Wolfner 1999; Tram and Wolfner 1999; Xue and Noll 2000; Adams and Wolfner 2007).

Females that receive sperm from males lacking Acps (Prd-rescue; Xue and Noll 2000) or with depleted quantities of Acps (~1%–DTA-D; Kalb et al. 1993) store 90% fewer sperm than do females mated to wild-type males (Tram and Wolfner 1999). The morphological changes that accompany, and may facilitate, sperm storage do not proceed in such females (Adams and Wolfner 2007). Interestingly, sperm themselves are not needed to trigger the morphological changes in the reproductive tracts of mated females (Adams and Wolfner 2007). It is not clear what role sperm play in the processes necessary for their own storage. At least one individual Acp has been identified as essential for sperm storage and thus is a candidate for triggering these morphological changes: the novel glycoprotein Acp36DE, which enters the sperm-storage organs and binds to sperm (Bertram et al. 1996; Neubaum and Wolfner 1999). Acp36DE does not mediate the entry of first sperm into storage but is needed to enhance the rate at which sperm accumulate into storage (Bloch Qazi and Wolfner 2003). This suggests that Acp36DE might modulate the muscular contractions in the female reproductive tract that move the sperm mass nearer to storage sites. Further studies with Acp36DE mutants will be needed to test this hypothesis.

Apart from facilitating sperm entry into storage, Acps also play an essential role in utilization of stored sperm. For example, females that receive sperm from males lacking Acps are infertile, even though those females do store a few sperm (Xue and Noll 2000). Their fertility can be restored if the females subsequently mate with males that provide Acps (but no sperm) (Xue and Noll 2000; see also Hihara 1981, Fuyama 1983). However, the detailed mechanisms by which Acps promote the utilization or release of sperm from storage are unknown, and specific Acps that cause these effects have not yet been identified.

Drosophila females mate with multiple males and thus receive ejaculate/sperm from different males. The presence of sperm from more than one male within the mated female, made possible by her storage of sperm, provides opportunities for competition between sperm or ejaculates from different males and for sperm preference by the female. Mechanisms may exist that give a particular male’s sperm an advantage after deposition within the female or that lead a female to select his sperm preferentially (Birkhead and Hunter 1990; Eberhard 1996; Birkhead 1998). Given their role in mediating sperm storage, it seemed reasonable that Acps could, perhaps in consequence, affect sperm competition. Harshman and Prout (1994) showed that Acps play a role in sperm competition in D. melanogaster, specifically in “defense”, the ability of previously stored sperm to resist being displaced by the ejaculate of a second male. They observed that females re-mated with males that transferred Acps (but not sperm) had fewer progeny relative to females that had re-mated with males that did not transfer Acps (or sperm) (Harshman and Prout 1994). This reduction in the number of offspring when females re-mated with males that transferred only Acps but not sperm is due to reduced use of a first male’s sperm to fertilize his mate’s eggs (Prout and Clark 2000). Acps are not sole mediators of sperm displacement. The extent of displacement is significantly greater when the second male transfers both sperm and Acps (Gilchrist and Partridge 1995). Further, sperm competition may also be influenced by the act of mating or by the internal anatomy of the female reproductive tract (Scott and Richmond 1990; Joly and Bressac 1994; Eberhard 1996; Miller and Pitnick 2003; Snook and Hosken 2004). Although it has been reported that a male’s sperm can be removed (Civetta 1999), inactivated (Price et al. 1999), or “dumped” (Snook and Hosken 2004) under the influence of the ejaculate of a second male,
mechanisms by which Acps, in particular, participate in sperm competition are still unknown.

Association studies have identified Acps that are suggested to be involved in “offense”, or the displacement of a previous male’s sperm (ovulin, Acp29AB, Acp33A, CG6168, and Acp62F), or in the “defense” of stored sperm against displacement (ovulin or Acp26Ab, Acp29AB, Acp36DE, Acp53Ea, CG14560, CG8137 and Acp33A) (Clark et al. 1995; Fiumera et al. 2005, 2007). These studies present correlations between phenotypes and allelic polymorphisms in the Acp genes; further experiments are necessary to test for direct roles of these Acps in sperm competition. For example, although the association studies suggest a role for ovulin in sperm competition, assays of sperm competition using ovulin-null males revealed no effect on sperm offense or defense (Herndon and Wolfner 1995; Christopher A et al., unpublished data). The disconnect between the results with ovulin-null males and the association studies that surveyed natural alleles of ovulin (Clark et al. 1995; Fiumera et al. 2005) could reflect the effects of particular alleles of ovulin, as opposed to its absence, on sperm competition. It is also possible that the disparity between the results reflects the use of different genotypes of female in the experiments; female genotype is an important determinant in the outcome of sperm competition (Clark et al. 1999). Assays with Acp36DE-null males indicated that the role originally proposed for this Acp in sperm competition appears simply to reflect Acp36DE action in promoting sperm storage (Chapman et al. 2000).

The effects of Acps on sperm storage, sperm utilization and sperm competition are still poorly understood, but there are several candidates for future tests of roles in these processes. These include Acps such as CG1656, CG6289, CG8137, CG9334, or CG14560, which localize to sperm-storage organs (Ravi Ram et al. 2005) and Acps identified in association studies (Clark et al. 1995; Fiumera et al. 2005, 2007). Genetic studies using knockout or knockdown males will allow dissection of the function of these Acps in sperm storage or utilization or in sperm competition.

**Acps and sperm contribute to the persistence of postmating changes in females**

Increased egg laying and reduced receptivity of a mated *D. melanogaster* female last for about 7–10 days postmating. Stored sperm are necessary for the persistence of these particular postmating changes (Manning 1962, 1967; Gromko et al. 1984; Xue and Noll 2000). Matings without sperm result in changes in egg laying and receptivity for only one day after mating (short-term response). In theory, stored sperm could promote the persistence of these changes through neural triggers that respond to filled or distended storage organs, e.g., as in cabbage white butterflies (Obara et al. 1975) or in *Anopheles* mosquitoes (Klowden 2006), but recent studies have indicated a different role for sperm, in conjunction with at least one Acp, the SP, in the long-term response (Chapman et al. 2003b; Liu and Kubli 2003; Peng et al. 2005a). SP is seen bound to sperm in the mated female (Peng et al. 2005a). Peng et al. (2005a) showed that the whole sperm is initially coated with SP, but that as time elapses after mating, sperm tails appear to lose their bound SP (sperm heads still appear to contain bound SP even 5 days after mating). Peng et al. (2005a) proposed that SP is bound to sperm by virtue of SP’s N-terminus, and that proteolytic cleavage of SP at a predicted cleavage site after the 7th or 8th amino-acid (Arginine or Lysine), releases the C-terminal portion of SP from sperm. The released C-terminal portion of SP is then proposed to enter the female’s circulation, enabling it to reach neural targets (Nakayama et al. 1997; Ottiger et al. 2000; Soller et al. 2006). Consistent with this model, females mated to males that transferred a modified form of SP that cannot be cleaved failed to show a long-term response (Peng et al. 2005a).

**Acps contribute to females’ costs of mating**

In *D. melanogaster*, multiple matings appear to reduce females’ life spans (Fowler and Partridge 1989; Harshman and Zera 2007). Although egg production is known to contribute to this cost of mating (Partridge et al. 1987; Sgro and Partridge 1989), perhaps due to its energetic requirements and consequent resource allocation, Acps also appear to be important contributors to the longevity cost of mating as well. Females that receive Acps during multiple matings show reduced longevity relative to multiply mated females that receive little or no Acps (Chapman et al. 1995). This effect of Acps is separable from costs originating from egg production, although it is formally possible that Acps’ elevation of egg production could additionally affect the cost of mating (Chapman et al. 1995; Ueyama and Fuyama 2003). Reduced longevity would appear to contrast with apparently-beneficial effects of Acps on reproduction, such as promoting sperm storage and increasing egg production. It is possible that it is advantageous to males to harm their mates, since
that may make females less likely to mate with subsequent males (Johnstone and Keller 2000). Alternatively, it is possible that the effects of Acps in decreasing longevity are simply negative side effects of Acps that have other, advantageous, effects. Distinguishing between these options requires knowing which Acps contribute to the cost of mating. Thus far, one Acp, the SP, has been shown by RNAi analyses to contribute to the Acp-mediated mating costs in females. Wigby and Chapman (2005) found that females mated to SP-deficient males lived longer, with higher life time reproductive success, compared to females that received SP. Whether SP has a direct effect that causes a cost of mating, or whether its effect on longevity reflects its modification of the female hormonal milieu (Moshitzky et al. 1996) or its effect in increasing food intake by the female (Carvalho et al. 2006), is unknown.

A potential approach to identify additional Acps that might decrease the longevity of mated females is to determine whether any Acp, on its own, has negative effects on Drosophila. Interestingly, ectopic expression of SP in a way that allowed high levels to enter the hemolymph is toxic to preadult D. melanogaster (Mueller et al. 2007). Given SP’s association with the cost of mating (Wigby and Chapman 2005), this suggests that a toxicity assay may provide a promising way to identify candidate Acps that are deleterious, and could potentially contribute to the longevity cost of mating. This idea is further supported by the observations that ectopic expression of another Acp (Acp62F) is also toxic (Lung et al. 2002) and the chromosomal region containing Acp62F is detected among quantitative trait loci (QTL) that affect the postmating mortality of female in D. simulans, a sibling species of D. melanogaster (Givetta et al. 2005). In addition to SP and Acp62F, two other Acps (of 29 tested) are also toxic upon ectopic expression (Mueller et al. 2007). These two Acps(CG8137 and CG10433) are thus candidates to test for involvement in the cost of mating. As suggested for SP (Chen et al. 1988; Aigaki et al. 1991), both Acp62F and CG8137 are known to enter the circulation of the mated female; the latter two proteins are known or predicted protease inhibitors, respectively, and have almost identical tissue targets in the mated female (Lung and Wolfner 1999; Lung et al. 2002; Mueller et al. 2004; Ravi Ram et al. 2005). It is not possible at present to rule out that the high levels of Acp expression that occur in the toxicity assays could disrupt or misregulate fundamental biochemical processes causing deleterious effects unrelated to the cost of mating (Lung et al. 2002; Mueller et al. 2007). Further studies directly measuring the longevity of females mated to Acp knockout or knockdown males are required to determine whether Acp62F, CG8137, and CG10433 contribute to the cost of mating to females.

**Molecular interplay between Acps and the female**

Recent experiments have begun to uncover a rich molecular interplay between males and females that has implications for reproductive and evolutionary questions. In some ways, reproduction can be viewed as a cooperative joint-venture between two individuals to pass on their gene pools to the next generation, but this view is complicated by the diverse and sometimes conflicting “interests” of males and females as illustrated, for example, by the cost of mating discussed earlier (Parker 1979; Rice 1992; Chapman and Partridge 1996; Rice and Holland 1997; Partridge and Hurst 1998; Rice 2000; Chippindale et al. 2001; Chapman et al. 2003a; Lessells 2006; Pishedda and Chippindale 2006). Indeed, molecular interactions between males and females are more nuanced than purely “cooperative” or “conflicting”, as illustrated below.

**Males and females together regulate the cleavage of Acps**

Acps in D. melanogaster are rich in predicted regulators of proteolysis (Swanson et al. 2001a; Mueller et al. 2004). Almost a quarter of known Acps (25 out of 112) are either predicted proteases or protease inhibitors (see earlier). Such proteolysis regulators could control cleavage of other seminal-fluid proteins. This role has been demonstrated for primate prostate-specific antigen (PSA, a protease) and protein C inhibitor (PCI, a protease inhibitor), which regulate the cleavage of semenogelins and thus semen viscosity (Lilja 1985; Christensson and Lilja 1990; Malm et al. 2000). Proteolysis regulators in seminal fluid could also regulate the exposure of proteins on the sperm surface or, alternatively, protect sperm from degradation of essential proteins, as has been proposed for PSA or PCI in mammals (Kise et al. 1996) and protease inhibitor(s) in the seminal fluids of fishes (Wojtczak et al. 2005) and Drosophila (Lung et al. 2002). Another possible role for proteolysis regulators in the seminal fluid could be to regulate the processing of reproductive molecules with essential physiological functions, either releasing biologically active peptides or, alternatively, restricting the time that an intact reproductive modulator is available to act (Wolfner 2002; Heifetz et al. 2005).
In *D. melanogaster*, three Acps with known essential reproductive functions (ovulin, Acp36DE, and SP) are proteolytically cleaved into smaller forms after their transfer to the female (Park and Wolfner 1995; Bertram et al. 1996; Peng et al. 2005a; Ravi Ram et al. 2006). Studies of ovulin and Acp36DE have begun to tease apart the contribution of the sexes to their cleavages. Park and Wolfner (1995) found that male-derived molecule(s) are necessary for the normal processing of ovulin. They suggested that complete processing of ovulin requires contributions from both males and females. Recently, the processing of ovulin and Acp36DE was shown to require an Acp that is a predicted protease, CG11864 (Ravi Ram et al. 2006). This protease is a predicted member of the astacin metalloprotease family (Mueller et al. 2004). It is synthesized in a 32kDa form in the male accessory gland. As it passes through the male reproductive tract on its way to the female, CG11864 is processed to a smaller form. This processing is reminiscent of the cleavage that converts inactive crayfish astacin to an active, smaller form (Zwilling and Stöcker 1997). It suggests a model in which CG11864 is kept inactive until shortly before entering the female and is activated during transit through the male (Ravi Ram et al. 2006).

Although ovulin and Acp36DE are synthesized in the male’s accessory gland, their cleaved forms are detected only after they have entered the female reproductive tract. Thus, in contrast to CG11864, ovulin and Acp36DE are not subject to male-specific proteolysis while in transit through the male. The observation that cleaved forms of ovulin and Acp36DE are detected only after their transfer to the female suggests that processing of these Acps requires a contribution from the female. Yet this processing also requires the Acp CG11864. Thus, Acp proteolysis appears to be step-wise, and regulated by both males and females. At present, the molecular nature of the female’s contribution to this processing is unknown. The female might contribute enzymatic co-factors, or members of a proteolytic cascade that facilitates or mediates the action of CG11864. Alternatively, the female reproductive tract could provide an ionic environment or a pH environment that is optimal for the activity of CG11864. Identifying the female contribution(s) to this proteolysis pathway and how they interact with CG11864 and its targets will help to elucidate how molecular interplay can occur between male and female. This can provide information of use in addressing interesting questions related to sexual cooperation and conflict at the molecular level.

**Male-derived Acps regulate the expression of mated females’ immune genes**

One way in which male-derived Acps could trigger the reproductive changes seen in mated female flies would be by large-scale changes to the female’s proteome or transcriptome. To test whether this is the case, proteome and transcriptome analyses have been carried out on whole mated females or their tissues, at several intervals after mating. These analyses (Lawniczak and Begun 2004; McGraw et al. 2004; Mack et al. 2006) showed that changes in the transcriptome or proteome are very small in magnitude during the first 1–3 h after mating, even though Acp-induced major physiological and behavioral changes have begun during that time. Only a small fraction of the transcriptome changes were due to receipt of Acps (McGraw et al. 2004). The role of Acps in proteome changes has not yet been tested.

An exception to the rule that only minimal changes in RNA abundance occur at 1–3 h postmating involves genes that function in immune response. Seventeen such genes were observed to increase >2-fold in RNA level even in this early postmating time period; nine of the 17 showed this change in response to receipt of Acps (McGraw et al. 2004). Consistent with this finding, females mated to Acp knockout males showed altered levels of postmating expression of genes encoding antimicrobial peptides (Peng et al. 2005b, McGraw LA et al., unpublished data). For example, SP was shown to induce the expression of three antimicrobial peptide genes (Peng et al. 2005b). Ectopic expression experiments have shown independently that three additional Acps can affect a female’s immune response (Mueller et al. 2007), although the mechanism whereby this occurs has not been identified. Ectopic expression of these Acps (CG10284, CG6168, CG9334 out of 21 tested) increases a female’s ability to resist bacterial infection (Mueller et al. 2007). Given the possibility that microbial pathogens could be introduced into the female at the time of mating, induction of antimicrobial peptide genes by mating and Acps may protect the female and/or introduced sperm from microbial infection. Alternatively, the induction of an immune response (or antimicrobial peptides) could be a response to nonself proteins and cells (sperm) introduced by the male. Thus, in the immune response of mated females, there also appears to be interplay between the sexes: in addition to introducing antimicrobial peptides/proteins (Lung et al. 2001), the male appears to stimulate the mated female’s synthesis of antimicrobial peptides.
Interestingly, at later post-mating times (≥6 h postmating) larger changes are seen in the female’s transcriptome, both in the lower reproductive tract and in the whole body (Mack et al. 2006; McGraw LA et al., manuscript in preparation). The dependence of these changes upon receipt of Acps is unknown. These results, together with findings that Acps can modulate vesicle release in the female reproductive tract (Heifetz and Wolfner 2004; noted above), suggest that mature virgin females already possess the molecular machinery to carry out the initial postmating changes and only require Acps (or, for some changes, other triggers) to activate this machinery. Long-term postmating changes, however, require synthesis of new macromolecules in the female. Interestingly, among Acp-responsive genes at 1–3 h postmating are three transcription factors that could, in theory, underlie some of the subsequent Acp-dependent changes (McGraw et al. 2004).

Future directions
In order to understand the role of Acps in reproduction, it is essential to identify the full suite of Drosophila Acps. Here, we have integrated results from multiple laboratories using several approaches to generate an updated list of 112 Acps reported to date (Supplementary Table 1; Schäfer 1986; Chen et al. 1988; DiBenedetto et al. 1987; Monsma and Wolfner 1988; Simmerl et al. 1995; Wolfner et al. 1997, Swanson et al. 2001a; Holloway and Begun 2004; Mueller et al. 2005; Walker et al. 2006; Chintapalli et al. 2007). Acp discovery is occurring rapidly in Drosophila. Therefore, the list of Drosophila Acps is likely to expand further in the near future. As Acps are identified, mutant, knockdown, and ectopic-expression approaches (e.g., Aigaki et al. 1991; Herndon and Wolfner 1995; Neubaum and Wolfner 1999; Lung et al. 2002; Chapman et al. 2003b; Liu and Kubli 2003; Wigby and Chapman 2005; Carvalho et al. 2006; Ravi Ram et al. 2006; Mueller et al. 2007) will continue to identify functions of individual Acps. Future molecular and genetic studies will delineate molecular mechanisms, receptors and pathways through which Acps act, and will tease apart the interplay between male and female to the level of specific molecules and their domains. Unravelling these aspects will provide useful information and tools for further tests of models of sexual cooperation and sexual conflict, and for understanding the mechanisms and consequences of chemical communications between individuals. At present, Acps have been identified in only a few insects other than Drosophila, e.g., honeybees (Collins et al. 2006), crickets (Andrés et al. 2006; Braswell et al. 2006), mosquitoes (Sirot LK et al., manuscript in preparation). Identification of Acps in other insects could provide insights into the diversity of sexual interactions. Understanding the molecular mechanisms of reproductive interactions and the degree to which they use conserved pathways (despite the rapid evolution of sequence of some effectors, such as some Acps) also has the potential to assist in designing potential strategies of management of insect pests. Finally, the conservation of biochemical classes of proteins from Drosophila through mammals (Mueller et al. 2004) suggests that Drosophila genetics can provide a rapid way of assessing the reproductive roles of conserved classes of seminal-fluid proteins.

Supplementary data are available at ICB online.

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