Toxic love

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Summary

Living organisms compete for survival and reproduction, whereupon the fittest live and thrive and the weakest fail and some cases even die. This battle for life acts on different levels, causing individuals of distinct species as well as individuals of the same species to compete over a variety of limiting resources such as food, breeding sites and mates. An important form of competition is driven by sexual conflict and often occurs when reproductive strategies between males and female diverge. These occur because there are differences in the evolutionary interests of the sexes over, for example, optimal reproductive rate, gamete size and parental investments. This has led to the evolution of different strategies to alter or overcome the manipulation of one sex by other, while maintaining a base line level of cooperation sufficient to ensure successful reproduction. This sexual conflict is an important evolutionary process as it can drive rapid evolutionary change.

The manipulation of one sex by the other through molecular interactions has been illuminated in studies using the fruit fly *Drosophila melanogaster.* Males tend to maximize their chances at fatherhood by releasing both sperm and semen inside the female's body during mating. The effects of semen proteins can benefit both sperm and eggs, but intriguingly they can also favour the interests of males whilst generating costs in females, resulting in sexual conflict. In *Drosophila melanogaster*, the female body has been the battlefield of sexual conflict, as semen proteins exert their effects in females after mating. This manipulation by males through molecular interactions can inflict substantial physical and physiological costs of mating in females. One enigmatic seminal fluid protein the 'Sex Peptide', generates strikingly diverse changes in female physiological and reproductive behaviour. Sex Peptide triggers remarkable female post mating responses including altered fertility, immunity, libido, eating and sleep patterns, by the activation of diverse sets of genes.

In many studies of the molecular mechanisms of female manipulation via the effects of Sex Peptide, genetic variation is minimised in order to clearly delineate biological functions. However, to understand the evolutionary processes and dynamics that characterise Sex Peptide mediated interactions between males and females, it is important to study this genetic variation. With high-throughput sequencing technologies that have provided resources such as >200 fully sequenced DGRP lines (*Drosophila* Genome Reference Panel), we traced the impact of the enigmatic Sex Peptide on the fruitfly genome.

In this thesis I performed an in-depth investigation of the phenotypic and genomic differences among 30-32 DGRP lines, with respect to male release of, and female responses to, Sex Peptide. I measured phenotypic variation for Sex Peptide release in males; and in females the phenotypic variation in immune responses, egg laying, receptivity and

longevity in response to Sex Peptide receipt. I compared these phenotypic post-mating responses to those of females that mated to males with a null-allele for Sex Peptide, to distinguish the specific response to Sex Pepetide. I mapped these phenotypes to genomic variation using Genome Wide Association Studies and conducted functional characterizations on the genomic variation identified.

In chapter 2, we developed and successfully employed a novel quantification method, the immuno-Q-PCR. Using this, we detected significant variation among 31 DGRP lines in Sex Peptide release in males to wild type Dahomey females during mating. Our study showed no significant variation in mating latency or mating duration between males from 31 DGRP lines, indicating this variation in Sex Peptide transfer was not mediated by differences in mating behaviour among the lines. To search for genetic variations that were associated with variation in Sex Peptide release, we conducted a GWAS. This analysis yielded significant associations between Sex Peptide release and a set of 54 candidate genes. An extensive gene ontology search revealed that these top candidate genes clustered within the following functional categories: development, membrane, protein and RNA processing and reproduction. A literature search on the reproductive gene cluster showed that four of these genes were seminal fluid proteins. Some have yet unidentified functions; two are cyclic nucleotide phosphodiesterase that seem to be involved in male fertility and female mating behaviour; some are involved in germ cell development in males and/or in females; others are uniquely expressed in male testis and/or accessory gland protein but have unknown molecular and biological functions. We presume that the significant variation detected in Sex Peptide transfer might relate to Sex Peptide's role in mediating sexual conflict. This is consistent with the idea that sexual conflict can maintain genetic variation in reproductive traits. Our study highlighted new candidate genes not detected by any other methods and that might show novel associations with Sex Peptide in determining reproduction and post-mating gene expression in females.

Chapter 3, revealed that mating and the transfer of Sex Peptide can induce the expression of several AMP genes in females, and that there was significant phenotypic variation in these responses among lines. The induction of, and variation in, AMP gene expression was recorded in isogenic lines of two different *D. melanogaster* populations (French and DGRP). The lines differed both in whether or not they induced the expression of AMPs after mating, and the extent to which they did so after receipt of Sex Peptide. Immune gene expression was not always upregulated in response to Sex Peptide. For some lines it was even down-regulated in females mated to SP^+ compared to virgin and/or female mated to SP^0 males. In other lines Sex Peptide had no effect at all, or none in addition to the response to mating itself. Furthermore, there were also differences among the three immune genes

tested in detail, with those being regulated by the Imd pathway (*Dpt-B, Mtk*) being more responsive to Sex Peptide than the gene (*IM1*) under the regulatory control of the Toll pathway. The GWAS performed on the variation in expression of the antimicrobial AMPs in response to Sex Peptide in the DGRP population identified 13 candidate genes for *Mtk* (Toll and Imd pathway), 51 candidate genes for *Dpt-b* (Imd pathway) and 38 candidate genes for *IM1* (Toll pathway). The network analysis indicated that the majority of these genes are part of different networks, which suggests that most have several different functions in the organism, one role of which could be direct or indirect modulation of the immune response. For all these candidate genes, genetic variation was significantly associated with variation in the expression of AMPs after mating or Sex Peptide receipt. The functional annotation revealed that 8 of these candidate genes code for immunoglobulin superfamily proteins, and 8 modulate the Imd immune pathway, with 6 of these showing negative regulation.

Chapter 4 showed that across the tested DGRP lines, the transfer of Sex Peptide had a clear overall effect to significantly reduce of re-mating, increase egg laying and increase in lifespan. However, the extent of these effects varied significantly across lines. This phenotypic variation in response to Sex Peptide was tracked through GWAS, revealing a set of genes involved for each of these phenotypes. For receptivity, 2 candidate genes were identified by the GWAS, of which one regulates the Jak/Stat pathway. There is, however, no clear link to how these genes may interfere in reducing the receptivity. For egg laying a total of 104 candidate were identified by the GWAS, where by 13 of these genes show direct involvement in the development and the regulation of egg laying, and half of the rest are highly expressed in early embryonic stages. Finally, the GWAS performed on the starvation survival hazard ratio revealed 2 candidate genes, of which *daw* is known to determine adult lifespan. These results confirm the pleiotropic effect of Sex Peptide in influencing female post mating responses. However, contrary to expectation and earlier findings, Sex Peptide receipt in general extended rather than shortened lifespan.