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Female *Drosophila melanogaster* suffer reduced defense against infection due to seminal fluid components

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ABSTRACT

Reduced defense against infection is commonly observed as a consequence of reproductive activity, but little is known about how post-mating immunosuppression occurs. In this work, we use *Drosophila melanogaster* as a model to test the role of seminal fluid components and egg production in suppressing post-mating immune defense. We also evaluate whether systemic immune system activity is altered during infection in mated females. We find that post-mating reduction in female defense depends critically on male transfer of sperm and seminal fluid proteins, including the accessory gland protein known as "sex peptide." However, the effect of these male factors is dependent on the presence of the female germline. We find that mated females have lower antimicrobial peptide gene expression than virgin females in response to systemic infection, and that this lower expression correlates with higher systemic bacterial loads. We conclude that, upon receipt of sperm and seminal fluid proteins, females experience a germline-dependent physiological shift that directly or indirectly reduces their overall ability to defend against infection, at least in part through alteration of humoral immune system activity.

1. Introduction

Evidence that immune defense is involved in trade-offs with multiple life-history traits is abundantly apparent in a diversity of organisms ranging from invertebrates such as snails and insects to birds and mammals (reviewed in Schmid-Hempel, 2003; Sheldon and Verhulst, 1996). Defense against systemic infection by many bacterial pathogens (measured as resistance to infection or survival after infection) is reduced by mating in female *Drosophila melanogaster* (Fedorka et al., 2007; Short and Lazzaro, 2010). In the present study, we investigate the immunological and reproductive bases for this post-mating depression in immune defense.

The insect immune system consists of multiple components, including the cellular immune response, the humoral immune response, and melanization. The cellular response functions mainly in the encapsulation or phagocytosis of parasites and pathogens, respectively (reviewed in Lemaitre and Hoffmann, 2007). The humoral immune response is activated upon detection of bacteria and fungi in the hemocoel. It includes production of antimicrobial peptides by the fat body and is stimulated when pattern recognition receptors recognize microbial cell wall compounds and trigger signaling through the Toll and IMD pathways (reviewed in Wang and Ligoxygakis, 2006). Melanization occurs in response to wounding, parasitization or infection and is regulated by the enzyme phenoloxidase (reviewed in Cerenius and Söderhäll, 2004).

These immune system components have been shown to be important for overall defense against infection in insects, which is defined as the ability to tolerate or eliminate infection (Ayres and Schneider, 2008). For this reason, quantitative immune system activity is often measured as a proxy for overall immune defense, under the implicit assumption that increased immune system activity correlates with heightened resistance to infection. This may or may not be the case (Fedorka et al., 2007), and this uncertainty can complicate the interpretation of immunity studies, an issue that has specifically been raised in the context of interactions between mating and immune defense (Lawniczak et al., 2007). Regardless, much of the evidence for trade-offs between immune defense and reproductive success comes from studies demonstrating that mating and/or reproduction reduces proximal measures of systemic immune system activity or capability. In damselflies, the ability to encapsulate a foreign object inserted into the hemocoel decreases with increasing oviposition in females (Siva-Jothy et al., 1998), and sperm storage is negatively correlated with encapsulation ability in leaf-cutting ant queens (Baer et al., 2006). In the beetle *Tenebrio molitor*, mating results in a decrease in phenoloxidase activity in both males and females (Rolff and Siva-Jothy, 2002). Mating has mixed effects on the immune system of the cricket *Allonemobius socius*, reducing hemocyte number, encapsulation ability and lytic activity in both males and females,

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but increasing phenoloxidase activity in females (Fedorka et al., 2004).

While measurements of immune system activity certainly are informative, increases in immune activity do not always correlate with improved tolerance of infection or with heightened ability to eliminate pathogens (Adamo, 2004; Lawniczak et al., 2007; Viney et al., 2005). For this reason, it is informative to also assess the efficacy of immune defense, which we measure in this study as the ability to fight and/or survive systemic infection. In *D. melanogaster*, multiple studies have investigated how mating affects both immune system activity and organism-level defense against infection. Females have been shown to demonstrate a short-term increase in the expression of at least one and often many antimicrobial peptide (AMP) genes after mating, at least in the reproductive tract and possibly in other tissues (Fedorka et al., 2007; Innocenti and Morrow, 2009; Kapelnikov et al., 2008; Lawniczak and Begun, 2004; Mack et al., 2006; McGraw et al., 2004; Peng et al., 2005b; Wigby et al., 2008). These data would seem to predict higher immunocompetence after mating. In fact, however, female *D. melanogaster* suffer reduced ability to defend against infection by pathogenic bacteria after mating (Fedorka et al., 2007; Short and Lazzaro, 2010), although the ability to eliminate non-pathogenic bacteria injected into the body cavity is not compromised (McKean and Nunney, 2005; Wigby et al., 2008). As of now, no mechanism has been demonstrated for the observed reductions in defense against infection after mating. Notably, all previous studies documenting the increase in AMP expression after mating have been performed using uninfected females. Whether mating affects AMP expression in flies suffering from pathogenic infection remains an important but untested question.

During copulation, males transfer sperm and seminal fluid proteins in their ejaculates. Seminal fluid proteins, especially those made in the male accessory glands (accessory gland proteins, or Acps), have dramatic effects on female behavior and physiology. For example, Acp36DE causes conformational changes of the uterus (Avila and Wolfner, 2009) and is required for proper sperm storage after mating (Neubaum and Wolfner, 1999). Acp26Aa (ovulin) stimulates ovulation in mated females for approximately one day post-mating (Heifetz et al., 2000; Herndon and Wolfner, 1995). The Acp known as sex peptide (SP, also called Acp70A) has many effects on mated females, including reducing their receptivity to subsequent mating (Chapman et al., 2003; Chen et al., 1988; Liu and Kubli, 2003), promoting proper release of sperm from female storage organs (Avila et al., 2010), increasing intake of food (Carvalho et al., 2006) and decreasing siesta sleep (Isaac et al., 2010). SP has also been shown to be at least in part responsible for increased AMP gene expression in females after mating (Domanitskaya et al., 2007; Peng et al., 2005b). Interestingly, however, SP induces increases in juvenile hormone III-bisepoxide production in corpora allata incubated *in vitro* (Moshitzky et al., 1996), and juvenile hormone (JH) has been shown to suppress immune system activity (Flatt et al., 2008; Rolff and Siva-Jothy, 2002). Furthermore, seminal fluid, particularly SP, stimulates long-term increases in egg production (Chen et al., 1988; Soller et al., 1997), and egg production has been shown to trade-off physiologically (Fellowes et al., 1999) and evolutionarily (McKean et al., 2008) with immune defense. It is therefore possible that, despite inducing short-term modest increases in AMP expression, SP and other ejaculate components might cause overall reductions in systemic defense against infection. To begin to elucidate the mechanism by which females suffer reduced defense against infection after mating, we tested the effect of mating on expression of immune genes during infection and used genetic manipulations to identify critical steps in copulation and reproduction that depress immune defense.

2. Methods

2.1. Fly stocks and maintenance

Wild type flies are Canton S (CS) in all cases. "Spermless" males and "eggless" females are *tud*^{1} *bw sp*/CS and are generated from a cross between *tud*^{1} *bw sp* females and CS males. *tud*^{1} is a recessive maternal effect mutation, and offspring of *tud*^{1} mothers fail to form a germline (Boswell and Mahowald, 1985). Sons of *tudor* females do transfer accessory gland proteins during mating (Kalb et al., 1993). Egg-producing control females, which serve as a genotype control for eggless females, are also *tud*^{1} *bw sp*/CS. However, they are generated from a cross between *tud*^{1} *bw sp*/CyO females and CS males, and therefore produce eggs normally. "Spermless/Acpless" (DTA-E) males have ablated accessory glands due to expression of diphtheria toxin subunit A in their accessory gland main cells (Kalb et al., 1993). They fail to produce sperm and main cell accessory gland proteins (Kalb et al., 1993). Sex peptide null males are SP^{0}/Δ^{130} and were generated from a cross between SP^{0}/TM3, *Sb ry* and Δ^{130}/TM3, *Sb ser* (Liu and Kubli, 2003). Sex peptide null flies were donated by Eric Kubli.

All flies were reared on standard Cornell media (8.3% glucose, 8.3% Brewer's yeast, and 1% agar, plus 0.04% phosphoric acid and 0.4% propionic acid added to inhibit microbial growth in the food). Flies were kept at 24 °C on a 12 h light–dark cycle.

2.2. Mating setup

Male and female virgins were collected within 8 h of eclosion, separated by sex, and aged in groups of ~25 with *ad libitum* access to food. All flies were three days post-eclosion at the time of mating. The day before each experiment, females were anaesthetized on CO_2, put into individual glass mating vials, randomly allocated to a mating treatment and allowed to recover overnight. Females that were to remain virgins were anaesthetized and also put into individual vials. The following day, single, unanaesthetized males were aspirated into vials containing females within three hours of incubator "dawn." Mating pairs that copulated for less than 15 min were discarded before infection in order increase our confidence that the male had adequate time to transfer the full complement and amount of ejaculate (where appropriate) and to ensure that females mated to mutant males mated for similar lengths of time as females mated to wild type males. More than 95% of all copulations lasted for longer than 15 min, so the number discarded from our experiment represents a small fraction of the total number of copulating pairs. After mating, males were removed, and females that ceased mating within roughly 10 min of each other were combined into vials of ~10 flies per vial. Virgin females were combined in similarly sized groups to control for possible housing effects.

2.3. Bacterial infection

Mated females were infected 2–3 h after mating unless otherwise noted. In all cases, control virgin females were infected in parallel with their mated counterparts. Females were anaesthetized on CO_2 and pierced in the thorax with a 0.15 mm anodized steel needle (FST) dipped in a dilute overnight culture of *Providencia rettgeri*. The strain of *P. retteri* used in this experiment is a natural bacterial pathogen of *D. melanogaster*, and resistance to it has been shown to be reduced by mating (Short and Lazzaro, 2010). *P. rettgeri* is a moderate bacterial pathogen, causing ~40% mortality over 3–7 days in virgin *D. melanogaster* infected under our procedures. Overnight cultures were started from a single bacterial colony, grown overnight at 37 °C to saturation in liquid Luria Broth (LB), then diluted in additional LB to A_{600} = 1.0 (±0.05).