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Animal Biology 69 (2019) 117–136

Does mating negatively affect female immune defences in insects?

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Submitted: July 26, 2018. Final revision received: January 25, 2019. Accepted: February 4, 2019

Abstract

Immunity is an important mechanism of protection against pathogens and parasites. One factor that can influence immunity is mating. During mating, male-derived materials are transferred to females, and the physical contact also involves the potential risk of sexually transmitted infections, and wounding. Thus, mating can challenge a female's immune system. This review focuses on exploring how immunity and mating interact in female insects. Although mating has been shown to cause female immune responses in several species, the responses do not always match the observed resistance to pathogens/parasites. Mating up-regulates female immune responses while female resistance is reduced compared to virgin females in some species, and vice versa in other taxa. We discuss why mismatches occur and why post-mating female resistance differs among species, and suggest that measured immune responses may not correlate with female resistance. Also, the mating system will play a major role. Polyandrous mating systems can generate intense post-mating sexual conflict, which can impose high costs of mating on females. Reduced female post-mating resistance may be due to direct suppression of female immunity by males. Alternatively, polyandry may increase the risk of sexually transmitted infections. If this is the major factor driving female post-mating resistance, females of polyandrous species should have higher post-mating immunity. To date, there are insufficient numbers of studies to fully answer the question '*does mating negatively affect female immune defences in insects?*' To elucidate the links between immunity and mating in females, we need more studies in more species with varied mating systems.

Keywords

Immune function; immune response; mating; sexual conflict; timing of measurement

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Introduction

Immunity is an important mechanism for organisms to protect themselves against pathogens and parasites. Hence, immunity is an important determinant of an individual's fitness (Zuk & Stoehr, 2002; Lazzaro & Little, 2009). However, immunity is costly (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000; Zuk & Stoehr, 2002). Because resources are limited, trade-offs between immunity and other life-history traits will invariably occur (Sheldon & Verhulst, 1996; Zuk & Stoehr, 2002). In addition, life history traits often increase or decrease the likelihood of exposure to pathogens, thereby altering the relative importance of immunity. One factor that can influence immune defences is mating. This review focuses on understanding how immunity and mating interact in females.

Mating is a situation where self and non-self agents interact. During mating, foreign materials (e.g., sperm and other ejaculate components) are transferred to females, and male genitalia can also directly wound female genitalia and the reproductive tract (e.g., Crudgington & Siva-Jothy, 2000; Blanckenhorn et al., 2002; Kamimura, 2007). Moreover, the physical contact of mating involves the potential risk of sexually transmitted infections or parasites (Smith & Dobson, 1992; Hurst et al., 1995; Knell & Webberley, 2004; Whitlow, 2004). Thus, mating frequently challenges the female's immune system. If the act of mating up-regulates the females' immune system, other life-history traits such as fecundity or longevity may be compromised due to trade-offs. On the other hand, mating also changes females' physiology, for example by stimulating egg maturation (South & Lewis, 2011). These physiological changes are often under endocrine control (e.g., juvenile hormone) (Schwenke et al., 2016), and these hormones are also involved in immunity (e.g., Rolff & Siva-Jothy, 2002; Chen et al., 2016; Schwenke & Lazzaro, 2017). For example, mating induces the release of juvenile hormone, which down-regulates enzyme activity involved in the humoral immune system of the mealworm beetle *Tenebrio molitor* Linnaeus, 1758 (Rolff & Siva-Jothy, 2002). Furthermore, juvenile hormone promotes oocyte maturation of mated females of the fruit fly *Drosophila melanogaster* Meigen, 1830 (Soller et al., 1999), whereas it suppresses their resistance to bacterial infection (Schwenke & Lazzaro, 2017). It is therefore possible that females' immune systems are down-regulated by mating if both reproduction and the immune system are mediated by the same hormone that acts antagonistically. This in turn may make mated females more vulnerable to pathogens and parasites. So how does the females' immune system respond to mating?

When considering the immune system, it is important to discriminate between a response to being infected *per se* and an effective immune response to a pathogen or parasite (Zuk & Stoehr, 2002). Although mating has been shown to elicit a female immune response in several invertebrates (Lawniczak et al., 2007; Morrow & Innocenti, 2012), the female immune response does not tell us whether female immunity confers resistance or makes them susceptible to pathogens and/or parasites after mating. As the immune system consists of multiple mechanisms and pathways

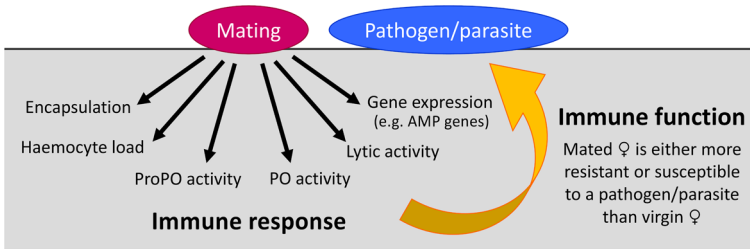


Figure 1. A schematic outline of post-mating responses, showing the distinction between immune response and immune function. Immune responses are examples listed in table 1. The grey background indicates the inside of an organism's body. Abbreviations: AMP genes, antimicrobial peptide genes; PO, phenoloxidase; ProPO, prophenoloxidase.

which respond to specific foreign materials (Gillespie et al., 1997; Lemaitre & Hoffmann, 2007), it is possible that an observed post-mating female immune response is not related to actual resistance to a particular pathogen or a parasite. It is therefore necessary to consider how the female immune response contributes to their immune defence. To distinguish female immune response to mating or infection/wounding involved in mating from female actual resistance to pathogens/parasites, we will describe the former as the 'post-mating immune response' and the latter as the 'post-mating immune function'. That is, post-mating immune response indicates observed phenomena caused by mating or infection/wounding involved in mating, whereas post-mating immune function represents effects of mating on subsequent female resistance to pathogens/parasites (fig. 1).

To examine the links between immunity and mating, we need model systems in which both immunity and mating are relatively well understood, and ideally can be manipulated experimentally. Insects are suitable organisms to study the links between immunity and mating because a large body of research on immunity (Schmid-Hempel, 2005; Rolff & Reynolds, 2009) and mating (Thornhill & Alcock, 1983; Shuker & Simmons, 2014) has been carried out in this group. Here, we review studies investigating the effects of mating on female immunity in insects and show that measured immune responses to mating do not always match female immune function. We then discuss whether mating tends to have a negative effect on female immune defences or not.

Post-mating immune response

In terms of gene expression, mating is well known to change female transcription levels (Lawniczak & Begun, 2004; McGraw et al., 2004; Mack et al., 2006; Innocenti & Morrow, 2009; Rogers et al., 2008; Gomulski et al., 2012). However, the evidence of how mating alters expression of immune genes in females is mixed (table 1). Mating activates expression of immune genes in some species (e.g., fruit fly *D. melanogaster*: Lawniczak & Begun, 2004; McGraw et al., 2004; Kapelnikov et al., 2008; Innocenti & Morrow, 2009; bumblebee *Bombus terrestris* Linnaeus,

Table 1.

A list of studies investigating female post-mating immune responses in insects.

Insect species	Measurement(s) after mating	Immune indicator(s)	Response	Reference(s)	
Ant	<i>Formica paralugubris</i>	2, 36 h & 7 d	Phenoloxidase activity	↓	Castella et al. (2009)
	<i>Lasius niger</i>	24 h, 1, 2 w & 4 m	Prophenoloxidase activity Phenoloxidase activity	↓, =, ↑, ↓ =	Dávila et al. (2015)
	<i>Acromyrmex echinator</i>	ca 4 m ^a	Prophenoloxidase activity Phenoloxidase activity	↓ =	Dávila et al. (2015)
	<i>Atta colombica</i>	24 h	Prophenoloxidase activity Phenoloxidase activity	↑ ↓	Dávila et al. (2015)
Beetle	<i>Tenebrio molitor</i>	24 h	Phenoloxidase activity Haemocyte load	↓ =	Rolf & Siva-Jothy (2002)
	<i>Carabus lefebvrei</i>	2 d	Phenoloxidase activity Lytic activity	↓ ↓	Giglio et al. (2016)
Bumblebee	<i>Bombus terrestris</i>	2, 6 & 24 h	Gene expression	↑	Barribeau & Schmid-Hempel (2017)
Cricket	<i>Acheta domesticus</i>	3 w ^b	Encapsulation ability	↓	Bascuñán-García et al. (2010)
	<i>Allonemobius socius</i>	24 h	Haemocyte load Phenoloxidase activity Lytic activity Encapsulation ability	↓ ↑ ↓ ↓	Fedorka et al. (2004)
	<i>Gryllus texensis</i>	>3 d ^b	Phenoloxidase activity	=	Shoemaker et al. (2006)
Dung fly	<i>Scathophaga stercoraria</i>	24 h	Phenoloxidase activity	=	Schwarzenbach et al. (2005)
Earwig	<i>Forficula auricularia</i>	7 d & 4 m ^b	Haemocyte load Phenoloxidase activity	= =, ↓ ^c	Vogelweith et al. (2017)
Fruit fly	<i>Drosophila melanogaster</i>	2 h 1-3 h 1-16 h & 1-5 d 3 & 27 h 3 h 4 h 6 h	Gene expression	↑	Lawniczak & Begun (2004) McGraw et al. (2004) Peng et al. (2005) Fedorka et al. (2007) Kapelnikov et al. (2008) Wigby et al. (2008) Innocenti & Morrow (2009)

Table 1.

(Continued.)

Insect species		Measurement(s) after mating	Immune indicator(s)	Response	Reference(s)
Medfly	<i>Ceratitis</i>	1 d 2 & 4 h	Gene expression	↑ = ↓ ^d ↓, ↑	Gomulski et al. (2012) Msaad Guerfali et al. (2018)
Mosquito	<i>Anopheles gambiae</i>	2, 6 & 24 h	Gene expression	=	Rogers et al. (2008)

Symbols and letters: =, means that the degree of immune responses does not differ between mated and virgin females (i.e., no effects of mating); ^a, after their mating flight; ^b, unclear when and how many times females had mated before measurements; ^c, age-dependent; phenoloxidase activity significantly increased with age in virgin females but not in mated females; ^d, the response varied among six immune genes.

1758; Barribeau & Schmid-Hempel, 2017; medfly *Ceratitis capitata* (Wiedemann, 1824): Gomulski et al., 2012; Msaad Guerfali et al., 2018) but not in other species (e.g., mosquito *Anopheles gambiae* Giles, 1902; Rogers et al., 2008). Whole bodies, abdomens, reproductive tracts, and/or heads have been used for immune gene expression analyses. Thus, it is not clear to what extent these differences are due to tissue-specific variation in gene expression. Antimicrobial peptide (AMP) genes in particular, are activated by mating in *D. melanogaster* (whole bodies: Lawniczak & Begun, 2004; McGraw et al., 2004; Peng et al., 2005; Fedorka et al., 2007; Innocenti & Morrow, 2009; abdomens: Wigby et al., 2008; reproductive tract: Kapelnikov et al., 2008). AMPs are produced as a first line of the immune defence against viruses, bacteria, fungi and parasites (Zhang & Gallo, 2016).

In addition to quantifying the level of immune gene expression, several factors have been measured to assay post-mating immune response in a variety of insect species. These include encapsulation ability, haemocyte load, and/or activity of enzymes, such as prophenoloxidase (proPO), phenoloxidase (PO) and lysozyme (table 1, fig. 1). Encapsulation is a reaction in which multiple layers of haemocytes and/or a coat of melanin encapsulate the invading pathogens (Gillespie et al., 1997), such as fungi (Butt et al., 1988) and parasites (Eslin & Prévost, 1996, 1998). The degree of melanisation is normally used as a measure of encapsulation ability (e.g., Bascañán-García et al., 2010). The amount of haemocytes, which represents the number of immunocytes in the haemolymph (Rolff & Siva-Jothy, 2002), is also used to quantify encapsulation (e.g., Fedorka et al., 2004). Melanotic encapsulation is associated with ProPO and PO activities (Gillespie et al., 1997; Söderhäll & Cerenius, 1998). In invertebrates, PO exists in the blood in an inactive form ProPO, which is activated by pathogens and wounds (Söderhäll & Cerenius, 1998). Lysozyme defends against bacterial infection (Gillespie et al., 1997) by hydrolysing the bacterial cell walls and causing bacterial lysis (Ragan et al., 2009). The enzyme activity is expressed as lytic activity (e.g., Fedorka et al., 2004). These immune factors are frequently compared between mated and virgin females (table 1). Relatively higher

values in mated compared to virgin females indicate that mating up-regulates these aspects of the female immune response. In contrast, when they are lower in mated females, it suggests that mating down-regulates females' immune response.

Examination of these post-mating immune factors also reveals mixed results similar to those shown for gene expression patterns (table 1). Mating down-regulates PO activity in the ant *Formica paralugubris* Seifert, 1996 (Castella et al., 2009) and the mealworm beetle *Tenebrio molitor* (Rolff & Siva-Jothy, 2002), while mating does not affect PO activity in the field cricket *Gryllus texensis* Cade & Otte, 2000 (Shoemaker et al., 2006) and the yellow dung fly *Scathophaga stercoraria* (Linnaeus, 1758) (Schwarzenbach et al., 2005). In the ant *Atta colombica* Guérin-Meneville, 1844, mating up-regulates ProPO activity, but down-regulates PO activity (Dávila et al., 2015). In the ant *Lasius niger* (Linnaeus, 1758), time after mating affects ProPO activity, while mating has no effect on PO activity (Dávila et al., 2015). In the ant *Acromyrmex echinator* (Forel, 1899), mating down-regulates ProPo activity, but does not affect PO activity (Dávila et al., 2015). In the ground cricket *Allonemobius socius* (Scudder, 1877), mating up-regulates PO activity, but down-regulates haemocyte load, lytic activity and encapsulation ability (Fedorka et al., 2004). In the beetle *Carabus lefebvrei* Dejean, 1826, mating down-regulates both PO and lytic activities (Giglio et al., 2016). In the earwig *Forficula auricularia* Linnaeus, 1758, mating down-regulates PO activity when females become older, while having no influence on haemocyte load (Vogelweith et al., 2017). Mating clearly causes an immune responses in females of several insect species. However, the direction of post-mating immune responses varies among measured factors and species. Mating up-regulates female immune responses, measured as PO activity, in some species, whereas it down-regulates female immune responses in others, and in some insects there is no difference in immune responses between mated and virgin females.

Post-mating immune function

In species where mating down-regulates indicators of the immune response such as PO activity, it is concluded that mating 'corrupts' immunity (e.g., Rolff & Siva-Jothy, 2002). However, the negative post-mating immune response does not necessarily mean that mated females become more susceptible to pathogens and/or parasites. It is important to examine whether the change caused by mating actually reduces female immune function or not. Microbial/parasitic challenge is a good method to quantify the direct effects of mating on female immune function. Here females are infected with bacteria, fungi or parasites after mating, and their survival or ability to clear the foreign pathogen is compared with that of same-aged virgin females. If the survival or clearance ability in mated females is better than in virgin females, it suggests that mating makes mated females more resistant against the specific microbe/parasite it was tested against. If the survival or clearance ability in

mated females is worse than in virgin females, it indicates that mating makes females more susceptible to the examined pathogens and parasites. To the best of our knowledge, the effects of mating on female immune function when challenged with microbes/parasites has to date only been examined in six insect species (table 2). In five of the six species, both post-mating immune response and immune function have been tested.

An intensively studied species is the fruit fly *D. melanogaster* where mating increases immune gene expression. Two studies using non-pathogenic *Escherichia coli* (Migula, 1895) Castellani & Chalmers, 1919 bacteria showed that there were no differences in bacterial load between mated and virgin females (McKean & Nunney, 2005; Wigby et al., 2008; table 2). On the other hand, when infected with the pathogenic bacterium *Pseudomonas aeruginosa* (Schroeter, 1872) Migula, 1900, female survival varied with timing of infection after mating; females infected 3 and 9 h after mating had lower survival than virgin females, whereas there was no difference in survival between mated and virgin females when infected 21 and 27 h after mating (Fedorka et al., 2007). Since AMP immune genes are more highly expressed in females 3 h after mating than in virgin females in the absence of infection (table 1), Fedorka et al. (2007) suggested that female post-mating gene expression does not reflect their post-mating immune defence to bacteria.

Short & Lazzaro (2010) infected *D. melanogaster* females with four different pathogenic bacteria, *Providencia rettgeri* (Hadley et al., 1918) Brenner et al., 1978, *Pr. alcalifaciens* (de Salles Gomes, 1944) Ewing, 1962, *Ps. aeruginosa* and *Enterococcus faecalis* (Andrewes & Horder, 1906) Schleifer & Kilpper-Bälz, 1984 at 2–3 h after mating, and compared the survival and ability to clear the bacterial infection between mated and virgin females for each bacterial infection. Mated females infected with *Pr. rettgeri* or *Pr. alcalifaciens* had lower survival than virgin females, and the bacterial load of mated females was higher than that of virgin females. On the other hand, when infected with *Ps. aeruginosa* or *En. faecalis*, there was no difference in survival or bacterial load between mated and virgin females (Short & Lazzaro, 2010). The result of *Ps. aeruginosa* is inconsistent with the findings of Fedorka et al. (2007) who also used *Ps. aeruginosa* as a pathogen. The effect of mating on female immune function is likely to depend on the pathogenic strain or species used (Short & Lazzaro, 2010) and genetic variation in host resistance (e.g., Tinsley et al., 2006; Kutzer et al., 2018). Short et al. (2012) only used *Pr. rettgeri* and quantified the effects of mating on survival and bacterial load. To determine how long mating impacted on subsequent immune function, they infected *D. melanogaster* females with *Pr. rettgeri* at different time points after mating (table 2). Moreover, they simultaneously measured bacterial load and transcriptional levels of several AMP genes at different time points after infection to examine any potential association. The survival of mated females was lower than that of virgin females even when infected 26.5 h after mating, and the bacterial load of mated females was significantly higher than that of virgin females when infected within

Table 2.
A list of studies investigating effects of mating on female immune function with microbial/parasitological challenge in insects.

Insect species	Microbe/Parasite species	Effects of mating	Infection after mating	Assay after infection		Reference		
				Survival	Microbial/parasitic load			
Beetle	<i>Tenebrio molitor</i>	Fungus	<i>Beauveria bassiana</i>	+	3 h	1-15 d	Valtonen et al. (2010)	
	<i>Anoplophora glabripennis</i>	Fungus	<i>Metarhizium brunneum</i>	? ^a	? ^b	1-28 d	5 & 9 d	Fisher & Hájek (2016)
Bumblebee	<i>Bombus terrestris</i>	Parasite	<i>Crithidia bombi</i>	+	6 h	7 d	Barribeau & Schmid-Hempel (2017)	
	<i>Gryllus texensis</i>	Gram-negative bacteria	<i>Serratia marcescens</i>	+	5-12 d ^b	1-8 d	Shoemaker et al. (2006)	
Fruit fly	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Serratia marcescens</i>	+	3 d	0.5-5 d	Worthington & Kelly (2016b)	
	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Escherichia coli</i>	=	? ^b	3 d	McKean & Nunney (2005)	
Fruit fly	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Pseudomonas aeruginosa</i>	-	3 & 9 h	3 d	Fedorka et al. (2007)	
	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Escherichia coli</i>	=	21 & 27 h	3 d	Wigby et al. (2008)	
Fruit fly	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Providencia rettgeri</i>	-	2-3 h	1-7 d	24 ± 0.5 h	Short & Lazzaro (2010)
	<i>Drosophila melanogaster</i>	Gram-negative bacteria	<i>Providencia alcalifaciens</i>	-				
Fruit fly	<i>Drosophila melanogaster</i>	Gram-positive bacteria	<i>Pseudomonas aeruginosa</i>	=				
	<i>Drosophila melanogaster</i>	Gram-positive bacteria	<i>Enterococcus faecalis</i>	=				

Table 2.
(Continued.)

Insect species	Microbe/Parasite species	Effects of mating	Infection after mating	Assay after infection		Reference
				Survival	Microbial/parasitic load	
Medfly	Gram-negative <i>Providencia rettgeri</i>	=	2-3 h		4 h	Short et al. (2012)
		-	2-3 h		12 & 24 h	
		-	2.5, 6, 12 & 24 h		24 h	
<i>Ceratitis capitata</i>	Gram-negative bacteria	+	2.5 & 26.5 h	1-5 d	24 h	Msaad Guerfali et al. (2018)

Symbols and letters: =, means that immune function does not differ between mated and virgin females (i.e., no effects of mating); ?, refers to: ^a, This study showed combined data of males and females, so we cannot see how immune function differs between mated and virgin females; ^b, unclear when and how many times females had mated before infection.

24 h after mating (Short et al., 2012). These results indicate that the effect of mating on immune function continued for at least 24 h after mating in *D. melanogaster*. The transcriptional levels of AMP genes were lower in mated than in virgin females at 4 and 12 h after infection, but they were higher in mated females than in virgin females at 24 h after infection. While the bacterial load did not differ 4 h after infection, it was higher in mated females 12 and 24 h after infection compared to virgin females (Short et al., 2012). The authors suggested that the lower early AMP gene expression in mated females may have contributed to the observed increase in bacterial load. Short et al. (2012) also demonstrated that male seminal fluids reduced female immune function and that female egg production also contributed towards a reduction in their immune function. They therefore concluded that the reduction in immune function represents a cost of mating in females.

In contrast, mated females of the medfly *C. capitata* infected with *Pr. rettgeri* 2 h after mating survived better than virgin females (Msaad Guerfali et al., 2018). Similarly, female *G. texensis* crickets had higher survival than virgin females when infected with the bacterium *Serratia marcescens* Bizio, 1823 at more than three days after mating (Shoemaker et al., 2006; Worthington & Kelly, 2016a). Mated females of the mealworm beetle *T. molitor* exposed to *Beauveria bassiana* (Bals.-Criv.) Vuill. fungus spores 3 h after mating also survived better than virgin females (Valtonen et al., 2010). Female *B. terrestris* bumblebees orally infected with the intestinal gut parasite *Crithidia bombi* Gorbunov, 1987 at 6 h after mating had lower number of parasite cells than virgin females at seven days after infection (Barribeau & Schmid-Hempel, 2017). Thus, mated females are more resistant to the pathogens or parasites than virgin females in these four species: *C. capitata* medflies, *G. texensis* crickets, *T. molitor* beetles and *B. terrestris* bumblebees.

There is one interesting study in addition to those listed in table 2. The antibacterial activity of the haemolymph of uninfected *F. paralugubris* ant females was measured at 36 h and seven days after mating (Castella et al., 2009). In this assay, instead of infecting females directly, the haemolymph solution was dropped on a culture medium containing live bacteria. The zone where bacterial growth had been inhibited was compared between mated and virgin females. While there was no difference at 36 h after mating, the haemolymph of mated females had higher antibacterial inhibition ability than that of virgin females at seven days after mating (Castella et al., 2009). This implies that mated females of *F. paralugubris* may become more resistant to bacteria at a later time point after mating.

Microbial/parasitic challenges show either that mating reduces female immune function against some, but not all, bacteria (*D. melanogaster* flies) or that mating enhances female immune function to foreign substances (*C. capitata* medflies, *G. texensis* crickets, *T. molitor* beetles and *B. terrestris* bumblebees and perhaps *F. paralugubris* ants). Below, we discuss why female post-mating immune function does not always match measured immune response and why there are differences in female post-mating immune function among species.

When and what do we measure for immunity?

As shown above, female post-mating immune responses do not always match the consequences of immune function. Mating induces the expression of AMP genes in *D. melanogaster* flies, whereas mated females are sometimes more susceptible to pathogens and survived worse than virgin females. While mating reduces PO activity in *T. molitor* beetles, mated females survived better than virgin females. In *G. texensis* crickets, although PO activity is not changed following mating, mated females survived better than virgin females. In *C. capitata* medflies, mating only modestly activates the expression of immune genes, but mated females survived better than virgin females. Only in *B. terrestris* bumblebees, female post-mating immune response and the consequence of immune function positively correlated. In terms of immunity, mating appears to be costly for *D. melanogaster* females but not for other insects. Why are there such differences among species? Below, we discuss some possibilities for these differences focusing on potential disparities in the methodology used.

In microbial/parasitic challenges, there are two different time frames: the time between mating and experimental infection and the time between infection and measuring female survival or resistance. A negative effect of mating on female immune function was still observed in female *D. melanogaster* infected with *Pr. rettgeri* 24 and 26.5 h after mating (Short et al., 2012) and infected with *Ps. aeruginosa* 9 h after mating (Fedorka et al., 2007). In contrast, there were no observed negative effects of mating on female survival when infected with *Ps. aeruginosa* at 21 and 27 h after mating (Fedorka et al., 2007). In *G. texensis* crickets where mating enhances female survival, females were infected with bacteria more than three days after mating (Shoemaker et al., 2006; Worthington & Kelly, 2016a). If females are infected later after mating in *D. melanogaster*, the results may be different. Furthermore, even when the timing of infection is similar, the use of different microbial/parasite species may generate different results. In *T. molitor* beetles, for example, the timing of infection was 3 h after mating (Valtonen et al., 2010), which is similar to that in *D. melanogaster* flies injected with bacteria (Short & Lazzaro, 2010; Short et al., 2012). However, the beetle females were exposed to fungus spores. Normally, fungal spores attached on the surface of insects germinate, penetrate the cuticle, and then reach the haemolymph (Pedrini, 2017). Thus, it is likely to take more time for fungi to affect host fitness than for bacteria injected directly into the haemolymph. McKean & Nunney (2005) and Wigby et al. (2008) did not detect any effects of mating on female *D. melanogaster* bacterial load at three days after infection. This finding may be explained by the use of a non-pathogenic *E. coli* bacterium, but also because of the timing of measuring bacterial load. It is possible that the peak of increase in *E. coli*'s bacterial load might have been missed. As bacteria, fungi and parasites activate different immune pathways of the hosts (Gillespie et al., 1997; Lemaitre & Hoffmann, 2007), the time required for immune function can also vary among pathways.

In *D. melanogaster*, moreover, the time points of infecting females with bacteria often overlap the period when the expression of immune genes is being induced by mating *per se*. Infecting flies with bacteria is normally achieved by stabbing the thorax/abdomen with a thin needle (Neyen et al., 2014). However, this piercing can itself induce expression of AMP genes, without any need for pathogen transfer (Wigby et al., 2008). This may consequently also influence female immune function, but we do not know how piercing affects and potentially interacts with the expression of immune genes already activated by mating. To exclude the piercing effect from immune challenges, other methods need to be considered for inducing infection. In the case of *B. terrestris*, although females were infected with parasites 6 h after mating, there was no significant effect of the parasites on immune gene expression 18 h after infection (Barribeau & Schmid-Hempel, 2017). In this case they were orally infected. Although microbial/parasitic injection is a useful method to quantify the direct effects of mating on female immune function, an appropriate method that in itself does not contribute to the observed immune response should ideally be used.

In *T. molitor* beetles, mating down-regulates PO activity (Rolff & Siva-Jothy, 2002), while another study found mating enhances female survival (Valtonen et al., 2010) (tables 1 and 2). In *G. texensis* crickets, PO activity and female survival are not correlated (Shoemaker et al., 2006). It is possible that PO activity is not a good indicator of immune response against the tested microbial infections. Indeed, PO activity is shown to be unrelated to microbial infections in some insect species including *G. texensis* (reviewed in González-Santoyo & Córdoba-Aguilar, 2012). PO is an enzyme involved in the melanisation reaction toward wounds or pathogens in invertebrates (Söderhäll & Cerenius, 1998; González-Santoyo & Córdoba-Aguilar, 2012; fig. 1). PO should therefore be related to immune function against pathogens other than microbes (e.g., Miller & Cotter, 2017). In other species listed in table 1, PO and lytic activities, haemocyte load and encapsulation ability were measured as indicators of immune response. In order to investigate effects of mating on female immune function, it is important to know which immune response is relevant to the target immune function to be quantified (Adamo, 2004).

There are likely to be several potential factors influencing the consequences of microbial/parasitic challenges to female insects. In particular, we need to consider the timing of post-mating infection and the timing of the actual measurements. Deciding on the timing of infection will depend on what we aim to investigate. If we are interested in examining ongoing changes of the effect of mating on female immune function, infection should be done at a point right after mating. In this case, we then need to consider whether the infection methods chosen may affect female post-mating immune response or not. Determining the timing of measurements will rely on which pathogen/parasite is used for the immune challenge. Here, we already need to understand female post-mating immune responses to the pathogen/parasite in the target species. However, it is unlikely that potential disparities in the methodology used will explain all observed differences in female post-mating immunity.

For example, females of the medfly *C. capitata* infected with *Pr. rettgeri* 2 h after mating survived better than virgin females (Msaad Guerfali et al., 2018) unlike what was observed in *D. melanogaster* (Short et al., 2012).

Impact of differences in mating

The biology of mating varies dramatically among species. It is likely that mating imposes costs on female immunity in some insect species but not in others because of underlying differences related to their mating biology. Here, we focus on mating systems and discuss the possibility that different mating systems impose different potential impact of mating on female immunity.

Mating systems differ among species: females of some species mate only once in their life (monandry), so the male they mate with is assured of fathering all offspring and post-mating conflict between male and female is likely to be low (Hosken et al., 2009). In other species, females mate with multiple males (polyandry), potentially generating conflicts between males and the female over which sperm are used to fertilise her eggs and the number of matings by the female (Kokko et al., 2014). Typically, males from polyandrous mating systems are adapted to high levels of sperm competition. In addition to sperm, males transfer seminal fluid proteins to females during copulation, which changes female physiology (Avila et al., 2011). Male seminal fluid proteins that enhance male fertilisation success can be costly to females and can cause sexual conflict over female mating (Edward et al., 2014). In polyandrous *D. melanogaster* flies for example, seminal components including the sex peptide (SP) reduce not only female sexual receptivity (Chen, 1991), stimulate egg maturation, hunger and oviposition, but can also reduce female longevity (Chapman et al., 1995). SP activates immune gene expression in mated *D. melanogaster* females (Peng et al., 2005; Domanitskaya et al., 2007; Wigby et al., 2008), which can reduce female immune function against some bacterial infections (Short et al., 2012; and see discussion above). Male seminal components therefore appear to down-regulate immune function of *D. melanogaster* females. If mated females refrain from remating because of their reduced immune function, fertilisation success of the initial male would be enhanced. In polyandrous species where sexual conflict is intense, males may be selected to reduce female post-mating immune function (Morrow & Innocenti, 2012).

Females of *C. capitata* medflies, *T. molitor* beetles and *G. texensis* crickets mate multiple times (Nakagawa et al., 1971; Drnevich et al., 2001; Bonizzoni et al., 2002; Worthington & Kelly, 2016b). After remating, *C. capitata* females store sperm from different males that are used to fertilize eggs (Scolari et al., 2014), which differs from *Drosophila* flies where sperm displacement occurs (Snook & Hosken, 2004; Manier et al., 2010). Females of *T. molitor* need to remate to ensure they do not run out of sperm, which increases their fecundity (Drnevich et al., 2001). Although *G. texensis* females have higher chances of getting injured during multiple mating, they gain benefits of increased fecundity by replenishing ejaculates, which may

greatly outweigh these mating costs (Worthington & Kelly, 2016b). Worthington & Kelly (2016a) examined whether male courtship, genital contact, accessory fluids, and/or testes-derived components enhance female survival in the presence of bacterial infection and found that genital contact together with accessory fluids and testes-derived components are associated with increased female *G. texensis* survival. Thus, conflict over female mating does not seem to be strong in these species, despite the risk of injury to females, as mating is beneficial overall for both sexes. Females of *B. terrestris* bumblebees mate only once (Schmid-Hempel & Schmid-Hempel, 2000). Although mating induces immune gene expression in *B. terrestris* females, this modification improves female resistance against parasites (Barribeau & Schmid-Hempel, 2017). In monandrous mating systems, post-mating sexual conflict is expected to be rare as males are ensured high paternity assurance (Hosken et al., 2009). Males may be unlikely to impair female immune function after mating in species where sexual conflict is mild or absent, as any cost to female fecundity will also be borne by the mating male. To date there is limited data to examine this possibility, but mosquitos, which lack multiple mating (e.g., *A. gambiae*) and that have been well studied regarding mating and immunity, may be suitable organisms to test this hypothesis.

Mating system can be a driving force for evolutionary changes in both male and female reproductive traits. For example in the yellow dung fly *S. stercoraria*, males kept under polyandrous conditions for several generations evolved to invest more resources in traits that enhanced their sperm competitive success compared to males evolving under monogamous conditions (Hosken, 2001). When male reproductive traits are costly for females (e.g., seminal fluids), females are expected to evolve resistance to such traits to reduce associated costs (Holland & Rice, 1999). In the red flour beetle *Tribolium castaneum* (Herbst, 1797) for example where females are naturally polyandrous, females evolving under monandrous conditions were found to reduce investment in PO activity compared to females evolving under polyandrous conditions (Hangartner et al., 2015). In other words, females appear to need to maintain investment in immunity when evolving under polyandrous conditions. This observation suggests that mating represents an immune challenge for female beetles. It is uncertain whether the immune challenge is due to sexual conflict and relative cost/benefit of multiple mating to females. If variation in the degree of sexual conflict is a major cause for the different effects of mating on female immunity we observe among insect species, there should be corresponding differences in post-mating immune function between individuals experimentally evolved under monandrous and polyandrous conditions. It will be of great interest to explore these potential consequences in evolved populations.

Polyandrous mating systems can also impact on the risk of sexually transmitted infections (Ashby & Gupta, 2013) and parasites (Roberts et al., 2015). In general, the more females mate with multiple males (i.e., polyandry), the higher the risk of potential infections. If costs of multiple mating outweigh the benefits, females will not mate polyandrously. Females of polyandrous species may have evolved

improved post-mating immune function in order to cope with the higher likelihood of sexually transmitted infections and parasites. This possibility gives rise to two predictions: if sexual conflict is key to shaping the effects of mating on female immunity, females of polyandrous species (where sexual conflict is expected to be greater) should show reduced post-mating immune function, whereas if sexually transmitted infections and parasites are key, females of polyandrous species should show higher post-mating immune function than those of monandrous species. To date, these possibilities remain largely untested.

Conclusion and perspectives

Does mating in general negatively affect female immune defences in insects? The answer is partly ‘yes’ and partly ‘no’, because it depends on the species and when immunity is being measured and by what methodology is being used. To date, there are insufficient numbers of studies conducted to conclusively answer this question. In addition, although we did not mention the details on copulatory wounding in this paper, this can influence the females’ immune system (Reinhardt et al., 2015). We also need to consider this point in future studies.

Mating system is likely to be key to clarifying the links between immunity and mating in female insects. It is suggested that mating system has influenced the evolution of some immune genes in primates (Wlasiuk & Nachman, 2010). It is possible that female mating frequency correlates with immune function also in insects. To test this possibility, we need to compare female post-mating immune function between individuals experimentally evolved under monandrous and polyandrous conditions. We can also quantify the impact of mating on female immune function by comparing monandrous and polyandrous species (e.g., Lizé et al., 2014). Furthermore, genetic variation in female mating frequency exists in some insect species (Wedell, 2001; Wedell et al., 2002; Simmons, 2003; Harano & Miyatake, 2005; Price et al., 2008). By using individuals that show divergent genetically determined mating frequencies, we can investigate whether a pre-determined female mating frequency correlates with female immune function within species.

Apart from mating, nutrients can influence female immune defences. In *D. melanogaster* flies, for example, females infected with bacteria survived better when fed a low amount of sugar or an added-yeast diet than when fed a high amount of sugar (Howick & Lazzaro, 2014) or a reduced-yeast diet (Kutzer et al., 2018). In several insect species, males provide a nuptial gift at mating, including prey items, secretions from male glands, and substances transferred in ejaculates (Thornhill & Alcock, 1983; Vahed, 1998; Gwynne, 2008). As nuptial gifts provide additional nutrients to females, nuptial gifts may also affect female immune defences. In *G. texensis* crickets, genital contact together with accessory fluids and testes-derived components are associated with increased female survival in the presence of bacterial infection (Worthington & Kelly, 2016a). The function of ejaculates influencing female immunity has been investigated well in the fruit fly *D. melanogaster*, but

not in other species of insects. It may be a good start to use those insect species where males transfer nutrients to females during mating in order to clarify the links between female immunity and mating. To improve our understanding of the links between female immunity and mating, we need to expand our studies to include a broader range of insect taxa with varying biology.

Acknowledgements

We thank Dr. Ben Longdon for his comments on an earlier version of the manuscript and two anonymous referees for their valuable comments to improve the manuscript. KO has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 746169.

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