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Mating and immunity in invertebrates

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Mating and immunity are intimately linked to fitness. In both vertebrates and invertebrates, recent investigations into mate choice for immunity, tradeoffs between reproduction and immunity, and the relationships between post-mating processes and immune function have revealed that mating and immunity are also intimately linked to each other. Here, we focus on invertebrates and critically examine the evidence that immunity is under sexual selection, both pre- and post-mating, and explore other hypotheses linking mating and immunity. We find little evidence for a consensus regarding which theories best account for the accumulating empirical data. However, we suggest that progress can quickly be made by exploiting the intrinsic strengths of invertebrate model systems.

Introduction

In this review, we discuss the theory and concepts that are central to the many relationships between pre- and post-mating processes and immunity in invertebrates. Both immunity, or the variety of physiological responses that counter pathogen infection in an individual [1], and the ability to reproduce successfully are important components of the fitness of an individual. There is accumulating evidence across diverse taxa that the two processes are linked, indicated by investigations of mate choice for immunity, tradeoffs between reproduction and immunity, and the relationships between post-mating processes and immune function.

Testing theories relating mating and immunity require large-scale experiments that measure heritability, genetic correlations and fitness. Invertebrates are ideal for this kind of work, and have been assumed to provide a simple, experimentally tractable model for investigating the innate immune system because they show conservation of innate immunity genes with vertebrates [2,3] but lack adaptive immunity. However, recent discoveries of, for example, specificity against pathogens [4] reveal that the complexity of invertebrate immune systems could rival that of vertebrates (Box 1). Such complexity provides further potential parallels with vertebrates, where connections between mate choice and the immune system are well established (reviewed in Ref. [5]).

The emerging data linking a range of both pre- and post-mating reproductive processes in invertebrates currently come from studies of arthropods, and so we focus on this group here. We discuss the theory and concepts central to the observed relationships between pre- and post-mating processes and immunity and examine the specific predictions of theories relating the two. We also highlight the degree to which these empirical data address or support these predictions. Understanding the relationships between traits involved in mating and immunity will ultimately provide a deeper understanding of the forces shaping the evolution of these two important fitness components.

Pre-mating sexual selection and immunity

Theories linking pre-mating sexual selection and immunity have focused on good genes indicator models, in which secondary sexual traits, such as the dark wingspots of calopterygid damselflies [6], reflect heritable aspects of the immunity of their bearer [7,8]. 'Good genes' are difficult to define because so much of the genome of an organism contributes to health and viability. However,

Glossary

The following immune parameters are typically measured in the studies in Table 1. See Refs [62,63] for comprehensive reviews of insect immunity.

Encapsulation: the ability to form compact capsules of haemocytes around foreign material. Capsules are formed in response to multicellular pathogens or artificial immune challenges, such as nylon filaments. Encapsulated pathogens are thought to be killed by a combination of isolation from nutrients and the active release of cytotoxic molecules into the capsule by host cells. Assays of encapsulation usually measure the optical density of capsules, which is a combined property of their thickness and degree of melanisation. Occasionally, capsule volume alone is measured (denoted as EN_v in Table 1). Larger or more optically dense capsules are assumed to be more efficient at killing pathogens.

Haemocytes: the cellular component of arthropod blood. A subset of haemocytes are involved in immune responses, either by transporting molecules such as PO to the location of immune challenges, or by physically contributing to the formation of capsules around foreign material. Assays of haemocytes involve counting their number, either in absolute terms or per unit of blood. It is assumed that larger numbers indicate an ability to mount larger or more efficient immune responses.

Lytic activity: the ability to mount an induced anti-pathogen response by the expression of soluble molecules that disrupt the structural integrity of invading pathogens. Bacteria and viruses are the chief targets of lysis. Molecules with lytic activity include the diverse antimicrobial peptides (AMPs), which are synthesised in massive numbers in response to infection, are small in size and specific in their action [64]. Lytic activity is measured by assessing the rate at which fractions of blood destroy bacterial cell suspensions *in vitro*, a measure assumed to reflect *in vivo* lytic activity.

Phenoloxidase: an active enzyme resulting from a proteolytic cascade initiated when non-self material is detected, or when a wound is received. PO converts tyrosine-based precursor molecules to melanin, which is formed around pathogens, usually as part of haemocyte capsules; melanisation results in a reduction in the permeability of capsules, thereby suffocating the pathogen. In addition, the cascade by which PO is activated produces cytotoxic byproducts, which might also contribute to the death of pathogens. Assays measure the enzymatic activity of PO, and assume that higher activity is equivalent to a greater immune response.

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Box 1. The diversity and complexity of invertebrate immunity

Molecular diversity and specificity are key to immune responses in vertebrates [65,66], but their roles in invertebrate immunity are only now beginning to be uncovered. Mechanisms for generating diversity within individuals, such as alternative splicing, somatic mutation, gene conversion and rearrangement [65–67] are now being shown to affect all the basic components of the arthropod immune response (Figure 1, main text).

There is great variety in the types of molecules and cells involved in immunity. For example, in insects, large classes of AMPs appear to be absent in whole orders, or are only identified in single species [64]. Different classes of haemocytes, distinguishable using antibody or genetic markers, can be confined to specific taxa or life stages, and haemocyte types shared among taxa can be functionally diverse [67]. Mechanisms of encapsulation also vary. Life stages that have few haemocytes, such as some fruit fly *Drosophila melanogaster* larval stages and mosquito *Anopheles gambiae* adults, encapsulate pathogens by depositing melanin alone, and do not form cellular capsules [69]. Consequently, the relationship between an immune parameter and resistance to a particular pathogen is likely to be taxon specific.

Recent research has also revealed memory-like processes and specificity in invertebrate immune systems [4,65,67,68]. Although invertebrates lack the immunoglobulins responsible for immunological memory in vertebrates, initial exposure to a pathogen can 'prime' the immune system, making subsequent immune responses more effective (reviewed in Ref. [70]). Larvae of the mealworm beetle *Tenebrio molitor* injected with bacterial peptides showed a long-lasting general antimicrobial response [71]. Priming can also confer resistance to specific pathogen types. For example, *D. melanogaster* can prime specifically for fungal infection via the selective activation of antifungal pathways [72]. Bumblebee *Bombus terrestris* workers show specificity against infection with a previously encountered bacterial species [4] and also show increased resistance to bacteria if their mother (the queen) has previously been exposed [73], indicating that priming can also be trans-generational. The mechanisms by which priming and specificity work are unclear, but if they prove to be widespread, the conceptual boundary between vertebrate and invertebrate immune responses is reduced [70].

The discovery of considerable immunological diversity and memory-like processes are exciting developments, possibly enabling specific interactions between, for example, sperm and the female immune system. However, these discoveries also caution against oversimplifying invertebrate immune systems and the assays designed to measure them (Box 2).

given the broad threat posed by parasites and pathogens, genes affecting immunity and pathogen resistance are excellent candidates for such 'good genes'. This idea, originally proposed by Hamilton and Zuk [9], has spawned nearly 25 years of research examining whether sexually selected traits signal pathogen resistance to potential mating partners.

Models of pre-mating sexual selection for immunity

Indicator models of sexual selection propose that sexual signals convey information about male quality, which is thought to be at least partially dependent on underlying good genes. For sexual signals to reflect male quality honestly, they must be costly and condition dependent, such that only individuals in good condition can expend resources on them. Immunity is also likely to be costly and condition dependent [1]. A general criticism of good genes indicator models is that, under strong directional selection, genetic variation is difficult to maintain. However, parasite-mediated models of sexual selection offer a solution because changes in both the species and genotypes of pathogens can maintain genetic variation for immune function and, hence, some aspect of fitness in the host [9,10].

Predicted and observed phenotypic correlations between indicators and immunity If only high-quality males can maximally invest in the sexual signal and the trait being signalled, then good genes indicator models predict a positive correlation between these traits [7]. Positive correlations between sexual signals and immunity have been found in several studies (Table 1; [6,11–18]). However, positive correlations are not necessarily predicted when the trait being signalled is immunity. For example, condition might be a balance between minimising costs of pathogenesis and costs of immunity; balancing these costs might result in a positive or negative relationship between the magnitude of an immune parameter and condition (and, thus, the sexual signal) [19]. Additionally, it is possible that individuals investing more in signals can maintain higher fitness despite lower immunological parameters [20], again making both positive or negative relationships between signals and immune traits possible. Furthermore, different components of the immune system can trade off with each other, leading to both positive and negative correlations between sexual signals and immune measures in the same model system (e.g. Ref. [15], Table 1; Box 2). Positive correlations between sexual signals and immunity are often interpreted as support for good genes condition-dependent models, but, as we show here, positive correlations are not exclusive predictions of such models.

If sexual signals and immunity are both costly and resource limited, negative relationships between a sexual signal and immunity will be revealed by experimental manipulations that force over- or underinvestment in either trait [20]. For example, if resources are used to enhance sexual signals, males can suffer increased pathogen susceptibility because resources are diverted away from the immune system. There is some evidence for this (Table 1; [21–24]): wolf spider males presented with females increased their drumming rate at the expense of lytic activity ([21] see Glossary and Figure 1 for all immunological terms). However, even under this scenario, negative relationships are not necessarily expected, especially if individuals can control their investment in immunity versus sexual signals. For example, upon immune insult, males of the *Tenebrio* mealworm beetle were more attractive to females and had higher levels of phenoloxidase, contradicting the predictions of a resource-based tradeoff model [25]. This was interpreted as dishonest signalling, but without quantifying the relationship between immunity and fitness, it is difficult to judge.

In general, the predictions of phenotypic correlations under good genes indicator models are somewhat confusing, not least because they involve costs and condition dependence and these are difficult to measure. Most studies have not measured costs, and not all studies have found sexual signals or immunity to be condition dependent (e.g. Ref. [23]). Thus, it seems prudent to examine predictions of good genes indicator models that

Table 1. Phenotypic and genetic correlations between secondary sexual signals and immune parameters

Species	Secondary sexual trait	Immune measure[a] and secondary sexual trait correlation			Comments	Refs
		+	0	−		
Phenotypic correlations						
Mealworm beetle *Tenebrio molitor*	Pheromones on filter disks	EN, PO			Females preferred pheromones from males that showed higher EN and PO upon immune insult	[11]
		PO	EN		Nutritional manipulation indicated condition dependence of pheromones and PO, but not of EN	[12]
			LY	EN, PO	JH injection increased male attractiveness, but decreased PO and EN; LY unaffected	[22]
		PO			Males increased PO and were more attractive following immune challenge by nylon monofilament insertion	[25]
Field cricket *Gryllus campestris*	Daily calling rate			Adult LPS injection[b]	Immune activation reduced daily calling rate independent of high or low nutrition treatment	[23]
	Harp size			Nymph LPS injection[b]	Nymphal LPS injected males had smaller, less well melanised harps as adults independent of high or low nutrition treatment	[24]
Field cricket *Teleogryllus commodus*	Song component	EN			Males with longer calling song syllables (unattractive) had lower EN	[13]
House cricket *Acheta domesticus*	Song component	HM	EN_v		Males with more syllables per chirp in calling song (attractive) had higher HM	[14]
Field cricket *Gryllus bimaculatus*	Courtship song	EN		LY	Females preferred courtship songs from males with high EN but low LY. EN and LY also negatively correlated	[15]
	Male dominance	EN, LY			Females preferred dominant males that had higher EN and LY than subordinates	[82]
Wolf spider *Hygrolycosa rubrofasciata*	Mobility	LY	EN		Females preferred males with higher drumming rates and were more likely to encounter more mobile males; in absence of females, males with higher drumming rates had higher EN, but not LY whereas males with higher mobility had higher LY, but not EN	[16]
	Drumming rate	EN	LY			
				LY, EN	In presence of females, males with higher drumming rates had lower LY and a tendency for lower EN	[21]
Banded agrion damselfly *Calopteryx splendens xanthostoma*	WPH[a]	PO	PO, EN_v		In a natural population, males with darker, more homogenous wingspots had lower parasite burdens after controlled infections, but showed no relationship between WPH and PO before immune insult; following insult, only males with lighter, more heterogeneous wingspots showed increased PO. No relationship between EN_V and WPH	[17]
	Wingspot size	EN	HM		Males with larger wingspots had faster EN ability, but no difference in HM although HM and EN were correlated with each other. Males with more symmetrical wingspots had faster EN	[6]
Rubyspot damselfly *Hetaerina americana*	Wing pigmentation	EN			Territorial males had larger wingspots, greater EN ability and higher mating success but females did not preferentially mate with males with larger wingspots. Wing pigment is more likely to give information to other males about fat reserves, suggesting that the pigment relationship with EN is indirect	[83]
Horned beetle *Euoniticellus intermedius*	Horn length	PO	EN		Males with larger horns had higher PO but not EN ability.	[18]
	Pronotum length		EN, PO		No correlation between pronotum length and immune parameters	
Genetic correlations						
House cricket *Acheta domesticus*	Body size (proxy for calling rate)	EN_v, HM			Variation in both immune traits heritable and correlated with sexually selected trait. HM genetically correlated with EN_v	[27]
Fruit fly *Drosophila melanogaster*	Mating success (measure of attractiveness)	HM			Lines selected for increased parasite resistance had higher mating success in competition. Genetic correlation could be explained as a byproduct of artificial selection	[3]
Scorpionfly *Panorpa vulgaris*	Salivary secretions		LY, HM		Significant effect of sires on HM but not LY of sons. However, offspring of males with high expression of salivary secretions did not have higher immune measures, although tendency for sons to have higher LY. Positive correlation between HM and LY	[28]

[a]Abbreviations: EN, encapsulation; HM, haemocytes; LY, lytic activity; PO, phenoloxidase; WPH, wing pigment heterogeneity. See Glossary for description of terms.
[b]LPS (lipopolysaccharide derived from *Serratia marcescens*) injections result in immune system activation but do not cause pathogenic consequences because the parasite itself is not injected.

Box 2. Measuring immunity

There have been few physiological assays or immunity measures used to assess the immunity of an individual organism (e.g. lytic activity or encapsulation). However, given the complexity of the invertebrate immune system (Box 1), it would seem prudent to take multiple measures of immunity [66,74]. This approach is being adopted, but is not without problems. For example, where multiple immune parameters have been measured, they can be uncorrelated or even negatively correlated with each other (Table 1, main text). Tradeoffs between different components of immunity have been demonstrated [75,76], but they have not yet been systematically analysed across invertebrate taxa. However, if, for example, some immune measures are traded off against both sexual signals and other immune measures, drawing conclusions based on phenotypic correlations would be misleading.

Another major issue is whether individual immune parameters accurately reflect the ability of an individual to respond to a pathogenic challenge. There is some evidence for relationships between pathogen resistance and immune measures: for example, *Drosophila* selected for parasitoid resistance have twice as many haemocytes compared with susceptible hosts [77], suggesting that haemocyte count is causal in parasitoid resistance. However, a bigger immune response is not necessarily better [29] and direct tests of host resistance need to be completed for more systems [74]. Additionally, whereas sequence polymorphism in pathogen recognition and intracellular signalling molecules is associated with pathogen resistance in *Drosophila melanogaster* [78], the relationship of immune gene expression to pathogen resistance is not yet known. Hence, variation in the expression of immune genes might not signal variation in immunity *per se*.

Genotype-dependent host–pathogen interactions can also affect the relationship between immune parameters and pathogen resistance. Artificial immuno-solicitors might measure a general potential response against a wide range of pathogens, but some pathogens can evade the immune system (reviewed in Ref. [79]). In such cases, defence mechanisms concerned with efficient recognition of the invader might be more relevant to resistance than are measures of immune responses elicited once recognition has occurred. Immune function also often differs between males and females (e.g. [28,47]), and immune gene expression is also affected by age [80], circadian rhythms [81] and nutritional status [47]. These variables are therefore both important to control for and interesting to examine further. Many of these ideas, although speculative, are also testable in arthropod models, especially *Drosophila* (see Ref. [8]), where the ability to manipulate immunity directly provides a powerful analytical tool. It will be interesting and relatively straightforward, for example, to eliminate the expression of immune genes in male reproductive tissues, and to study the fitness effects on females mated to these males.

are likely to offer more conclusive evidence of sexual selection for immunity. Here, we discuss predictions that, if upheld, would provide much stronger support for good genes models.

Predicted and observed genetic correlations between indicators and immunity A fundamental prediction of all good genes indicator models is the existence of a genetic correlation between the indicator and the immune trait [7]. An individual choosing a mate on the basis of a secondary sexual trait will therefore also be indirectly exerting choice on the genes underlying immunity. Genetic correlations can be revealed and quantified by measuring the covariance between two traits in formal breeding experiments [26]. This requires large sample sizes and multiple generations, the sort of experiments for which invertebrate models were developed. Despite this, measuring genetic correlations between sexual signals and immune traits has rarely been done, and there is currently no strong evidence in any invertebrate species that such correlations exist, although some researchers have claimed that patterns in their data are indicative of genetic correlations [3,27,28] (Table 1). If sexual signals

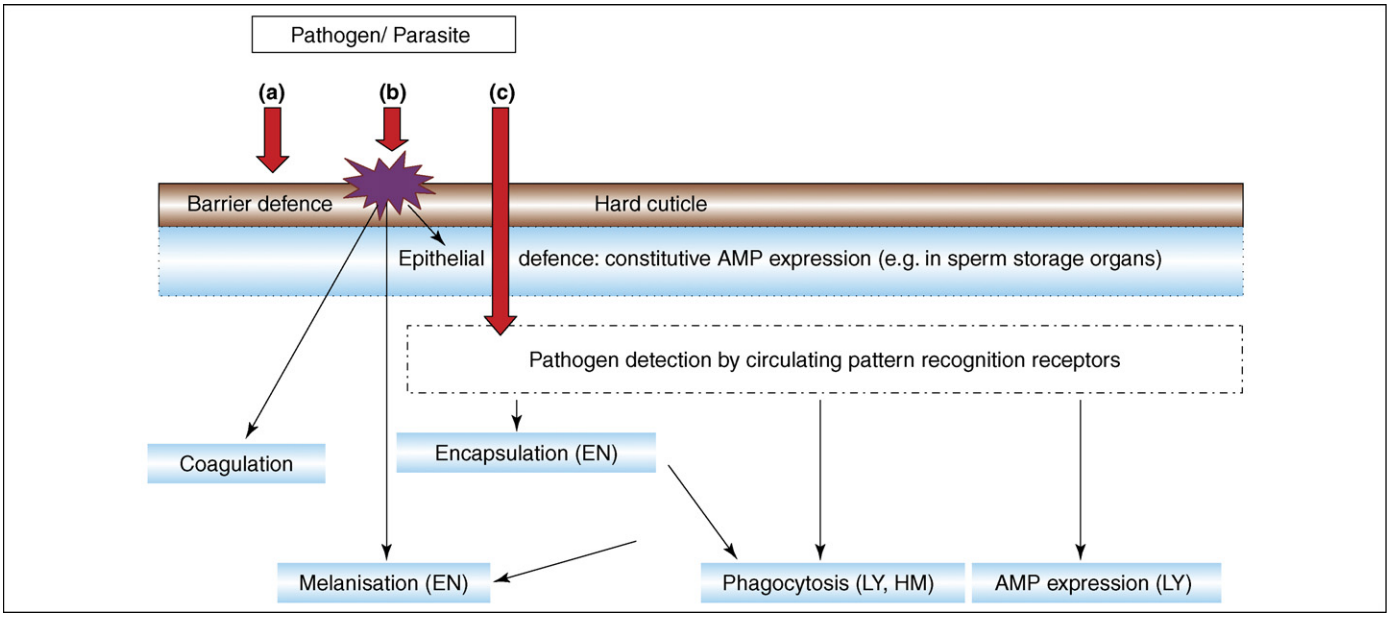

Figure 1. The generalised arthropod immune response. Blue rectangles indicate processes that kill pathogens. Abbreviations of immune processes are given where they correspond to assays of immunity used in Table 1 (main text). Red arrows indicate three potential pathogen paths: **(a)** the hard cuticle is the primary barrier against infection. If the pathogen gets past this point, or attacks at a site where there is no hard exoskeleton protection **(b)** (e.g. the reproductive tract), it can be killed by the epithelial immune response [38,39], which relies on the production of antimicrobial peptides (AMPs) by epidermal cells. If an injury occurs at the pathogen entry point, it can be repaired by coagulation and melanisation. If pathogens get past these barriers and enter the haemolymph **(c)**, haemocytes can respond to infections, especially those that are caused by larger pathogens (e.g. parasitoid wasp eggs), by phagocytosing microorganisms, encapsulating parasitoid eggs, and by melanisation, which depends on the activation of phenoloxidase. Additionally, in the holometabolous insects, pattern recognition receptors can recognize invasion and initiate distinct downstream responses (other insects are thought to rely only on the epithelial production of AMPs) [63].

and immune traits are not genetically correlated within taxa, then immunity-based good genes models of sexual selection are not applicable.

The relationship between immunity and fitness Central to any model describing relationships between sexual selection and immunity is the effect of a particular strategy on the fitness of an individual. Measures of fitness, however, are absent in the studies in Table 1. Instead, measures of immunity have assayed the magnitude of physiological parameters assuming that these reflect efficacy in minimising pathogenesis. However, many studies that measure multiple immunological parameters find that these do not correlate with each other (e.g. Refs [6,12,14,16,22]; Box 2), so the relationship of immunological parameters to 'immunity' and, thus, fitness, is tenuous [29]. Although the measurement of fitness is difficult and time consuming, involving the quantification of lifetime reproduction and survival in appropriate environments, arthropod model systems are ideal for such work [26].

Mechanisms linking pre-mating sexual signals and immunity

Determining the mechanisms underlying relationships between secondary sexual traits and immunity is important for understanding the evolutionary constraints and consequences resulting from such relationships. Tradeoffs can be due to limited resources (including energy) where sexual signals and the immune system compete, for example, for melanin [30]. Melanin is required for the formation of wingspots (secondary sexual traits) and immune reactions in calopterygid damselflies [17] and so could be a common resource needed by both traits. Selection on a melanistic trait in *Tenebrio* mealworm beetles resulted in increased haemocyte levels and phenoloxidase activity, indicating that melanin could also mediate tradeoffs with immunity in this species [31].

Tradeoffs can also be mediated by hormonal or molecular effectors [32]. This is the basis of the immunocompetence handicap hypothesis (ICHH), which proposes that testosterone simultaneously enhances sexual signals and suppresses immunity so that only males with good pathogen resistance genes have attractive signals [32]. Testosterone is absent in invertebrates, but juvenile hormone (JH) might have a similar role. In *Tenebrio* mealworm beetles, JH upregulates mating and/or attractiveness while suppressing the expression of phenoloxidase, potentially lowering pathogen resistance [22,33]. It also has major effects on several aspects of reproduction and suppresses immune gene expression in *Drosophila melanogaster* [34], although the consequences for pathogen resistance (Box 2) and mate choice are not yet known. Effectors such as melanin and JH not only provide a potential proximate link between signals and immune parameters, but also suggest a simple way in which the two share a genetic basis (i.e. via the genes controlling the expression of the effector itself).

Post-mating processes and immunity

Studies of interactions between post-mating processes and immunity are just beginning to be phrased in terms of life history and there are not yet explicit tests of theories relating the two. Thus, a considerable body of empirical data is accumulating, but the fitness consequences of the relationships remain largely obscure. However, mating clearly has immunological consequences in invertebrates and there are several theories available that could explain the observed patterns.

Observed relationships between immunity and post-mating processes

Immune molecules in reproductive tissues In many invertebrates, the tissues involved in mating harbour a high concentration of immune molecules. *Drosophila melanogaster* males express antimicrobial peptides (AMPs) in their reproductive tract and, during mating, they transfer at least three different AMPs in the seminal fluid [35]. One of these AMPs, andropin, is ejaculatory duct-specific, suggesting a solely sex-related function [36]. In the *D. melanogaster* female reproductive tract, AMPs are constitutively expressed in the oviduct and sperm storage organs [37–39]. Interestingly, AMP expression in these tissues is controlled by different regulatory pathways compared with those governing AMP expression in other tissues, again suggesting a sex-related function [40].

In female bedbugs, the paragenital system, into which males transfer sperm, is rich with haemocytes, suggesting that it evolved partly to minimise the immunological consequences of hypodermic sperm transfer that these insects exhibit [41]. In *Drosophila* species that incorporate ejaculate proteins into the female soma and/or oocytes, the insemination reaction can result in a large, opaque mass (the reaction mass) in the uterus [42]. The function of this mass is unknown, but in *Drosophila nasuta,* an unidentified substance transferred in the ejaculate activates phenoloxidase in the female uterus, resulting in the formation of the reaction mass [43], suggesting a possible immunological function.

Immune modulation following mating Mating has also been shown to suppress or induce aspects of immunity in invertebrates. Microarray analyses indicate that mating changes the expression of many immunity genes, inducing some and downregulating others [44,45]. Receipt of the seminal fluid protein, sex peptide, stimulates expression of several AMPs for a short period after mating in *D. melanogaster* [46]. However, female ability to clear a bacterial infection is reported to be unaffected by mating in *Drosophila* [47] so the function of the short-term AMP expression in this species remains unclear. In other species (e.g. *Gryllus* crickets [48]), mating does increase resistance to bacteria. Hence, a general pattern is not yet clear.

Some immune measures can be downregulated by mating. For example, mated female *Tenebrio* mealworm beetles show decreased phenoloxidase levels [33], mated female damselflies show a decreased encapsulation response [49], and mated crickets of both sexes show downregulation of haemocyte load, lytic activity and encapsulation [50]. *Drosophila melanogaster* males also show a reduced ability to clear a bacterial infection following increased levels of courtship and mating [51]. Hence, it is not currently clear whether there is a general pattern of up- or downregulation of immune measures following

mating in either sex, or whether these changes accurately reflect changes in pathogen resistance (Box 2).

Causes of interactions between post-mating processes and immunity

Sexually transmitted diseases (STDs) Although STDs can cause major fitness costs in many vertebrate species [52], their importance in invertebrates is not well understood [53]. A review of insect STDs suggests that insects are most at risk from the transfer of pathogens onto the cuticle during mating, rather than by genital or seminal fluid transfer [53]. However, it is not yet clear whether this is a case of observational bias, or a real difference between vertebrates and insects. The concentration of immune molecules in reproductive tissues in arthropods suggests that immune challenges during and following mating might be common. However, the only currently known function of reproductive tract AMP expression comes from *Ceratitis capitata* medflies, in which females coat eggs with AMPs as they are laid, protecting them from bacterial attack [54].

Adaptive suppression of immunity If immune responses are costly to maintain and express, then the suppression of immunity following mating might be an adaptive response in females to free resources for use in other reproductive processes [30]. Consistent with this idea, there appears to be an evolutionary tradeoff between egg viability and immunity in *Plodia interpunctella* moths: lines selected in the presence of a virus showed an estimated 15% reduction in fitness [55]. Also consistent with adaptive suppression of immunity for reproductive purposes, short-term reductions in immune parameters have been demonstrated following oviposition in female damselflies [49] and copulation in both sexes of damselflies and *Tenebrio* mealworm beetles [33,49]. In such cases, the transfer of immune-related proteins (or hormones) in the seminal fluid could supplement the downregulated female immune system, even functioning as a 'nuptial gift' that directly increases female fitness.

Sexual conflict Males could also transfer molecules that cause resources to be diverted to egg production at the expense of immunity and at levels beyond those that suit the female, which could then lead to sexual conflict. A recent study in *Allonemobius* crickets showed that polyandrous females showed decreased encapsulation ability relative to monandrous females [56]. Positive correlations detected between immunity and promiscuity in primates have been interpreted as a result of increased STD transfer among more promiscuous species [52]. However, such relationships could also result from sexual conflict in which male ejaculates interfere with female immunity, ultimately leading to more promiscuous females showing increased levels of immunity [56]. The benefits, or otherwise, of post-mating immune suppression will ultimately be manifest in the effects on the fitness of an organism, which, again, has rarely been measured in such studies.

Cryptic female choice Under the theory of cryptic female choice, females use post-mating prezygotic cues to bias the fertilization success of the ejaculate of one male over that of another. Immune cues could provide the basis for female choice. If pathogens are introduced at mating and gametes are susceptible to attack by them, females could indirectly assess male ability to protect sperm from pathogenesis. Alternatively, females could directly assess male quality by the ability of sperm to withstand or escape female-derived immunological attack. Perhaps only sperm that can bypass the non-self recognition process in the female reproductive tract are stored. Two vertebrate AMPs are currently being examined for their contraceptive potential because they cause permanent sperm immobility (reviewed in Ref. [57]). Thus, reproductive tract expression of AMPs might affect fertilization and this is an exciting, although untested, possible mechanism mediating cryptic female choice.

Genetic compatibility Females might also be able to assess genetic compatibility from cellular and molecular interactions between received sperm and seminal fluid and the female reproductive tract, perhaps even via the immune system [58]. Strong male–female genotype interactions detected in sperm competition experiments are consistent with genetic compatibility expectations (e.g. Ref. [59]) and post-mating, prezygotic incompatibilities in *Drosophila* (reviewed in Ref. [60]) could involve the immune system. For example, it is unclear whether the insemination reaction is an immune response, but it has been compared to one and, intriguingly, melanisation of the reaction mass is more dramatic in interspecific crosses between *D. nasuta* and *Drosophila pallidifrons* than in intraspecific crosses [61], suggesting that assessment of genetic compatibility involves the immune system.

Conclusions

Our survey of current theory and data suggest that a cautionary approach is necessary in evaluating the interactions between mating and immunity in invertebrates. We have shown that it is difficult to draw conclusions about the relevance of sexual selection on immunity until predictions of the models, such as genetic correlations between traits and immunity, are tested. It is also clear that, although there are many links between post-mating processes and immunity, the potential causes of these associations (e.g. cryptic female choice) are as yet untested.

However, we have also highlighted areas in which empirical progress can quickly be made and there are many exciting avenues for future work. For example, the relationships between pre- and post-mating immunity (e.g. do females choose the same males using pre- and post-mating criteria as discussed in Refs [5,8]?) remain largely untested. Recent advances in invertebrate immunology, such as the discovery of immunological priming and diversity generating mechanisms (Box 1), suggest the possibility of highly specific immune responses and provide new parallels between vertebrate and invertebrate immunity. It would be interesting to see, for example, if prior successful resistance to pathogens increases male attractiveness or the fertilization success of an ejaculate.

A recurring theme here has been the absence of a fitness-based framework in situations where it is essential. This holds true for both vertebrate and invertebrate studies. However, for most invertebrate model systems, it should be possible to relate both pre-mating and post-mating sexual traits, immune parameters, and

pathogen resistance to fitness [29]. To this end, the intrinsic strengths of invertebrate models are currently underexploited.

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