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Reproduction–Immunity Trade-Offs in Insects

Robin A. Schwenke,[1,2] Brian P. Lazzaro,[1,2,*] and Mariana F. Wolfner[1,3,*,†]

[1]Field of Genetics, Genomics, and Development, [2]Department of Entomology, [3]Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853; email: ras599@cornell.edu, bplazzaro@cornell.edu, mfw5@cornell.edu

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*Corresponding authors

†Invited author

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Abstract

Immune defense and reproduction are physiologically and energetically demanding processes and have been observed to trade off in a diversity of female insects. Increased reproductive effort results in reduced immunity, and reciprocally, infection and activation of the immune system reduce reproductive output. This trade-off can manifest at the physiological level (within an individual) and at the evolutionary level (genetic distinction among individuals in a population). The resource allocation model posits that the trade-off arises because of competition for one or more limiting resources, and we hypothesize that pleiotropic signaling mechanisms regulate allocation of that resource between reproductive and immune processes. We examine the role of juvenile hormone, 20-hydroxyecdysone, and insulin/insulin-like growth factor-like signaling in regulating both oogenesis and immune system activity, and propose a signaling network that may mechanistically regulate the trade-off. Finally, we discuss implications of the trade-off in an ecological and evolutionary context.

INTRODUCTION TO LIFE-HISTORY TRADE-OFFS

Physiological trade-off: the negative impact that one trait has on another biological trait within an individual

Evolutionary trade-off: the negative, genetically determined correlation between two fitness-promoting traits among individuals within a population

At its core, life-history evolution is a matter of optimization rather than maximization. Many traits that influence fitness are genetically and physiologically interrelated. Thus, increases in the fitness value of one trait may result in a corresponding decrease in the fitness value of another (127, 128). Reproduction and immune defense can be mutually constraining, with increased reproductive activity limiting immune performance and activation of the immune system resulting in decreased reproductive output. Both reproduction and immune responses are energetically costly, and the trade-off between them is likely due to alternative allocation of limiting energetic resources.

Trade-offs can occur at two discrete scales: physiological and evolutionary. At the level of an individual organism, trade-offs may arise as a consequence of direct physiological conflict between two traits or processes. For example, if both processes require the same limiting resource, allocation of the resource to one process inherently reduces the amount of that resource available to the other. We refer to these as physiological trade-offs. In an example that we discuss in more detail below, immunity and reproduction may trade off physiologically if, for example, both processes rely on dietary protein and protein nutrition is limiting. Physiological trade-offs are often plastic, meaning that they are responsive to environmental conditions, and an individual may shift allocations from one process to another as needed. Following the example above, a reproductively inactive insect may be able to devote fully sufficient protein resources to the immune response, but once the same individual becomes reproductively active, she may preferentially allocate that protein to egg provisioning and immune performance can become compromised.

At the population level, evolutionary trade-offs can occur if there is genetic variation among individuals for allocation between traits. In order for an evolutionary trade-off to exist in the example discussed above, there must be some individuals who are genetically predisposed to allocate protein preferentially to reproduction and others who are genetically programmed for preferential allocation to immunity. If we were to examine the correlation between reproductive output and immune performance across individuals in the population, we might expect to find that individuals with better-than-average immunity tend to show reduced fecundity, and vice versa. We refer to these trade-offs as evolutionary because natural selection can effectively act on the underlying genetic variation. Genetic variation for evolutionary trade-offs is likely to be maintained as a consequence of fluctuating selection in spatially or temporally heterogeneous environments (85). Specifically, when pathogen prevalence is low, natural selection may favor increased allocation toward reproductive output. When infection pressure is high, however, selection may favor heightened immunity. It is important to appreciate that plastic physiological trade-offs can exist within individuals without a corresponding evolutionary trade-off at the population level (50). Whether physiological and evolutionary trade-offs share their mechanistic bases remains an open question in life-history biology.

Reproduction and immune defense are intricately linked with other life-history traits (reviewed in 113, 117), highlighting the complexity of life-history evolution. In this article, we review the literature on reproduction–immunity trade-offs in female insects. Although we focus on female insects, reflecting the preponderance of data, accumulating evidence suggests that male reproduction also has immunological costs (e.g., 44, 92, but see 58) and that explicit differences in life-history strategies between the sexes can result in a sexually dimorphic immune system (111). For instance, premating sexual signals (e.g., horn length as in beetles, or pigmentation) can directly influence immune function and the evolution of host defense via sexual selection (reviewed in 82). Thus, many factors contribute to observed differences in life-history strategies. Here, we focus our discussion on the interactions between postmating processes and immunity in female insects with special attention given to mechanisms governing the trade-offs.

The search for mechanisms underlying life-history trade-offs is challenging. It is comparatively easy to observe that two fitness-related traits are negatively correlated at the level of the whole organism. For example, we can readily observe that reproductively active insects have reduced resistance to infection (**Table 1**), and we may hypothesize that a resource reallocation is the basis for the observed trade-off. But it is much more difficult to determine the identity of the limiting resource or the cellular mechanism that specifies and regulates differential allocation. Yet the identification of these mechanisms is critical for understanding how traits trade off and how trade-offs evolve. In this review, we show how condition-dependence of physiological and evolutionary trade-offs can reveal the identity of limiting resources (e.g., 94), and we discuss how pleiotropic hormones and signaling pathways may regulate resource allocation (e.g., 47, 51). We incorporate varied insect taxa that exhibit a diversity of reproductive strategies and experience distinct selective pressures into our discussion of organism-level traits, but the underlying molecular mechanisms have been ascertained primarily in genetically manipulable organisms such as *Drosophila melanogaster*. Not all specific pathways and mechanisms established in *D. melanogaster* may operate the same way in all taxa. With recent advances in molecular techniques, such as CRISPR/Cas9 genome editing (reviewed in 115), however, we are optimistic that mechanistic questions can soon be efficiently addressed in nonmodel organisms.

Resource allocation: the shunting of a nutrient or resource toward one trait instead of another

Antimicrobial peptides: immune effector molecules that lyse bacterial cells or inhibit their growth

Constitutive immunity: expression of immune system molecules in the absence of infection

Phenoloxidase: enzyme required for defensive melanization and production of oxidative free radicals

REPRODUCTIVE ACTIVITY INHIBITS IMMUNITY

There is widespread empirical support across multiple orders of insects for mutual constraint between immunity and reproduction (**Tables 1** and **2**), with a much smaller number of observed increases in female immunity as consequence of mating and reproduction (**Table 1**). Experiments to assess these trade-offs often involve genetic or physiological manipulation of reproductive capacity paired with assays of immunological capacity or, alternatively, manipulation of immune status followed by measurement of reproductive output. Immune traits that are commonly measured include survivorship of pathogenic infection, pathogen load sustained at various time points after infection, count or activity of circulating defensive blood cells (hemocytes), expression levels of genes encoding antimicrobial peptides, and phenoloxidase activity, because phenoloxidase is involved in defensive melanization and production of oxidative free radicals (56, 129). The background levels of these traits can be measured in the absence of infection (constitutive immunity). Alternatively, the traits can be quantified after presenting a noninfectious immune elicitor or nonpathogenic microbe (induced immunity), or after infection with a bona fide pathogen (fighting infection). These distinct but complementary approaches can give different results, providing further depth to our understanding of trade-offs. The cost of induced immunity may be higher than the cost of constitutive immunity because of the additional deployment of immune effector molecules, so trade-offs may be more readily observed under infection conditions. However, it may be impossible to distinguish costs of the immune response from consequences of pathogen virulence after a bona fide pathogenic infection. As in any experimental context, the data collected must be interpreted in terms of the design and assumptions of the experiment that was performed.

As a general rule, increased reproductive activity reduces constitutive and induced immunity across a diversity of female insects. Fedorka et al. (44) showed that female ground crickets (*Allonemobius socius*) sustained progressively fewer circulating hemocytes with increasing copulation frequency. Mealworm beetles (*Tenebrio molitor*) and wood ants (*Formica paralugubris*) show a reduction in phenoloxidase activity after mating (24, 112), although Fedorka et al. (44) saw the opposite pattern with *A. socius*. Nevertheless, hemolymph samples from mated *A. socius* females were less bacteriolytic than hemolymph samples from virgin females (44), demonstrating reduced constitutive immunological effectiveness. Mating also reduces cellular encapsulation and melanization

Table 1 Immunological consequences of mating and reproduction

Immune elicitor	Order	Species	Phenotype	Effect on immunity	Reference(s)
Unchallenged	Diptera	*Drosophila melanogaster*	Mating induces AMP expression in reproductive tissues	+	39, 83, 90, 103
	Coleoptera	*Tenebrio molitor*	Mating reduces PO activity	–	112
	Orthoptera	*Allonemobius socius*	Mating reduces hemocyte number and bacterial lytic ability	–	44
			Mating increases PO activity	+	
	Hymenoptera	*Formica paralugubris*	Mating reduces PO activity	–	24
Nonpathogenic	Diptera	*Drosophila melanogaster*	No difference in bacterial load between mated and virgin females (*Escherichia coli*)	=	16, 94
	Orthoptera	*Allonemobius socius*	Mating reduces encapsulation response (nylon filament)	–	44
		Acheta domesticus	Mating reduces encapsulation response (nylon filament)	–	17
	Odonata	*Matrona basilaris* subsp. *japonica*	Mating reduces encapsulation response (nylon filament)	–	123
	Hymenoptera	*Atta colombica*	Mating and sperm storage reduce encapsulation response (nylon filament)	–	14
Pathogenic	Diptera	*Drosophila melanogaster*	Mating reduces survivorship after infection and phenotype persists in absence of egg maturation (*Pseudomonas aeruginosa*)	–	43
			Mating reduces resistance to infection; effect is dependent on the presence of a germline and the receipt of Sex Peptide (*Providencia rettgeri*, *Providencia alcalifaciens*)	–	120, 121
			Mating has no effect on survivorship and bacterial load (*Enterococcus faecalis*, *Pseudomonas aeruginosa*)	=	120
	Coleoptera	*Tenebrio molitor*	Mating enhances resistance (*Beauveria bassiana*)	+	136
	Orthoptera	*Gryllus texensis*	Mating enhances resistance to infection (*Serratia marcescens*)	+	119
	Hemiptera	*Acyrthosiphon pisum*	Positive relationship between fecundity and susceptibility to parasitoid attack	–	59

Abbreviations: AMP, antimicrobial peptide; PO, phenoloxidase.

Table 2 Reproductive consequences of immunity and infection

Immune elicitor	Order	Species	Phenotype	Effect on reproduction	Reference(s)
Nonpathogenic	Diptera	*Drosophila melanogaster*	Reduced fecundity with increasing concentrations of immune elicitor (LPS)	–	101
			Reduced fecundity (HK bacteria species)	–	18, 94, 145
		Anopheles gambiae	Reduced ovarian protein and number of eggs laid (LPS)	–	4
			Increased number of apoptotic follicles (LPS or Sephadex beads)	–	5
	Coleoptera	*Euoniticellus intermedius*	Fewer brood balls (LPS)	–	106
	Orthoptera	*Acheta domesticus*	Fewer eggs later in life (nylon filament)	–	17
		Gryllus texensis	Reduced oviposition rate (wounding; HK *Serratia marcescens*)	–	126
			Reduced protein in egg (HK *Serratia marcescens*)		
		Hemideina crassidens	Repeated challenges reduced number of eggs laid and protein content per egg (LPS)	–	73
Pathogenic	Diptera	*Drosophila melanogaster*	Reduced fecundity during acute phase of infection (*Providencia rettgeri*)	–	68, 84, 94
			Reduced fecundity during fungal infection (*Beauveria bassiana*)	–	18
			Lower fecundity after surviving parasitoid wasp infection (*Asobara tabida*)	–	45
		Drosophila nigrospiracula	Negative relationship between ectoparasite load and egg number (*Macrocheles subbadius*)	–	104
		Anopheles gambiae	Increased number of apoptotic follicles (*Plasmodium yoelii nigeriensis*)	–	5
		Anopheles stephensi	Reduced fecundity during infection (*Plasmodium yoelii nigeriensis*)	–	64
		Armigeres subalbatus	Reduced ovarian protein and increased time to oviposition (*Brugia malayi*)	–	46
	Orthoptera	*Teleogryllus oceanicus*	Reduced viability of stored sperm (*Serratia marcescens*)	–	95

Abbreviations: HK, heat-killed; LPS, lipopolysaccharide.

of implanted nylon filaments in a variety of insects (14, 17, 44, 123), indicating reduced capacity to defend against macroparasites (25). Mating reduces the probability that *Drosophila melanogaster* females will survive a diverse array of pathogenic, bacterial infections, and mated female flies show higher pathogen loads and reduced inducibility of genes encoding antibacterial peptides after pathogenic infection (43, 120, 121). Interestingly, however, mating does not decrease the ability of female *D. melanogaster* to clear nonpathogenic *Escherichia coli* infections (16, 93). Therefore, the trade-off between mating and immunity is evident only when the infection is pathogenic.

Maintenance cost: the physiological cost of constitutive levels of immunity

The observation that mating reduces female *D. melanogaster* resistance to infection appears to be at odds with the recurrent observation that the expression of genes encoding antimicrobial peptides are modestly induced as a consequence of mating and the transfer of male seminal fluid proteins (71, 83, 90). Upon closer inspection, however, this upregulation of antimicrobial peptide genes may be largely restricted to the reproductive tract (39, 89). This tissue-specific induction may potentially be a local, prophylactic protection against sexually transmitted infection (76, 97) and may have little consequence in fighting a systemic infection.

INFECTION AND IMMUNE ACTIVATION REDUCE REPRODUCTIVE CAPACITY

Data from a diverse array of insects indicate that activation of immune responses decreases reproductive output and capacity (**Table 2**). In *D. melanogaster*, bacterial or fungal infections reduce fecundity (18, 68, 94, 101, 145). Similar effects are seen in Orthoptera, where induction of the immune system with heat-killed bacteria or bacterial cell wall components reduces egg production in the house cricket (*Acheta domesticus*) (17), the Wellington tree weta (*Hemideina crassidens*) (73), and the Texas field cricket (*Gryllus texensis*) (126). In *Anopheles* mosquitoes, challenge with bacterial cell wall components or infection by *Plasmodium* spp. significantly reduces the accumulation of protein in the ovaries, promotes the apoptosis of follicle cells, and reduces the number of eggs laid (4, 5, 64). Immune signaling and/or the presence of pathogens may signal to degrade newly forming follicles, limiting egg production in Diptera (31, 40, 132). This could allow resources to be shunted back to immunity and recovery from infection (70).

Whereas the above examples describe fecundity costs of immune deployment, constitutive maintenance of elevated immune potential can also reduce reproductive capacity (**Table 3**). *D. melanogaster* genotypes with high resistance to bacterial infection show low fecundity even when uninfected. However, this phenomenon is seen only when dietary yeast is limited, suggesting that nutrition is responsible for the constraint (68, 94). *D. melanogaster* that were artificially selected for increased resistance to a bacterial infection also evolved correlated reduction in egg viability (143), as did strains of Indian meal moth (*Plodia interpunctella*) that had been selected for resistance to a granulosis virus (20). In contrast, pigmentation in *T. molitor* females, a trait that can be experimentally selected for and represents higher constitutive phenoloxidase activity (6), did not correlate with fecundity, suggesting that the constitutive expression of some immune modulators need not always have reproductive consequences (7).

As shown by the examples above and in **Tables 2** and **3**, most published studies imply that immune induction and/or infection-responsive processes physiologically trade off with reproductive processes. Additional studies showing an evolutionary trade-off indicate constitutive costs of maintaining greater immunity even in the absence of infection (but see 7). This is largely a consequence of available experimental methodologies. Deployment costs, or the costs of mounting an immune response (79, 91), can be experimentally measured in terms of alterations to physiological allocation before and after infectious challenge (e.g., 15). Immunological maintenance costs (79, 91), however, are experimentally revealed by comparing reproductive potential in the absence of

Table 3 Evolutionary trade-offs between reproduction and immunity

Experiment	Order	Species	Findings	Relationship between reproduction and immunity	Reference(s)
Selection	Diptera	*Drosophila melanogaster*	Selection for resistance to *Pseudomonas aeruginosa* resulted in decreased egg viability	–	143
		Drosophila nigrospiracula	Selection for behavioral ectoparasite resistance resulted in a correlated reduction in fecundity	–	88
		Scathophaga stercoraria	Populations evolving in polyandry developed larger reproductive tissues and reduced PO activity	–	65, 66
	Lepidoptera	*Plodia interpunctella*	Selection for resistance to the *Plodia interpunctella* granulovirus reduced egg viability	–	20
Genetic and phenotypic correlations	Diptera	*Drosophila melanogaster*	Negative correlation between uninfected fecundity and resistance to *Providencia rettgeri*	–	94
		Aedes aegypti	Negative correlation between uninfected fecundity/hatchability and resistance to *Plasmodium gallinaceum*	–	142
		Tenebrio molitor	No correlation between pigmentation (PO activity) and fecundity	=	7

Abbreviation: PO, phenoloxidase.

infection among genetic strains with high resistance to infection with those with low resistance (e.g., 142, 143). Because of the experimental methodology employed, maintenance trade-offs are almost always revealed as evolutionary, although they must certainly have physiological basis. Correspondingly, the magnitude of physiological trade-offs and deployment costs can vary genetically within populations (e.g., 120) and therefore can be evolutionarily subject to natural selection.

EGG PRODUCTION AND IMMUNITY DEMAND ALLOCATION OF RESOURCES

Deployment cost: the physiological cost of producing an immune response

Both egg production and immunity are energetically costly processes, so a physiological trade-off between them could be mediated by the allocation of a mutually limiting resource. Although egg production is not the only energetic investment associated with reproduction, [other postmating

changes include heightened activity and foraging (reviewed in 10)], we hypothesize that it is likely to be the largest cost endured and therefore it is our focus here.

Fat body: the central organ for metabolic control and energy storage, also the central organ for systemic immunity

Yolk protein and vitellogenin: glycolipoproteins that are incorporated into the developing oocyte

To evaluate the resource allocation hypothesis, it is necessary to have some insight into what the limiting resource(s) might be and to have a mechanistic sense of how that resource(s) is allocated. Fisher (48) articulated this need well as early as 1930, when he wrote

> "It would be instructive to know not only by what physiological mechanism a just apportionment is made between the nutriment devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction."

Since then, the resource allocation model has become a central dogma in life-history theory (55, 81, 110, 127, 140). However, there are precious few examples in which the identity and the management of the resource are well understood. In the next three sections, we compile evidence from the published literature to support the hypothesis that both egg production and immunity in insects are nutritionally limited, and that the physiological trade-off between them may arise through resource allocation mediated by endocrine and metabolic signaling and joint reliance on critical and common tissues such as the fat body.

Although there are differences in the details among taxa [see (22) for a comprehensive review], insect egg production generally begins in the stem cell niche (germarium) of an ovariole, where a cytoblast arises from the asymmetric division of a germline stem cell. The developing cyst undergoes a species-specific number of mitotic divisions and grows in size as it transits posteriorly. Critically, copious quantities of proteins (e.g., yolk proteins and vitellogenins), lipids, RNAs, ribosomes, and organelles are deposited into the growing oocyte to provide nutrients and patterning information for the future zygote (27).

Provisioning of developing oocytes is energetically demanding (11, 138, 139). Thus, the efficiency of egg production depends on the quality of dietary nutrition and a female's metabolic status (38, 41, 132). Oogenesis can be arrested and, at least in *D. melanogaster*, partially developed oocytes can be resorbed during starvation conditions (31, 40, 132). The onset of reproduction drives females of many insects to ingest more food, frequently preferring protein-rich food sources (23, 108, 134). Notably, sanguivorous dipterans require a protein-rich blood meal to complete oogenesis (reviewed in 9).

Immune defense is also energetically and metabolically costly (15, 53, 122). Bumble bee (*Bombus terrestris*) workers are less resistant to starvation after immune system activation (99), and studies in multiple insects have established that resistance to infection is enhanced upon ingesting a protein-rich diet (86, 93, 94, 105, but see 75). Upon immune stimulation, *T. molitor* and *Spodoptera exempta* larvae shift their feeding preference toward protein-rich food sources (26, 105) and, at least in *Spodoptera* spp., ingestion of a high-protein diet permits increased production of antimicrobial molecules in the hemolymph (86, 105). In *D. melanogaster*, dietary L-arginine helps improve resistance to parasitoid wasps via lamellocyte proliferation and nitric oxide production (78). Although a high-quality diet can enhance the host immune response, the net effect of diet on infection is complicated because infecting pathogens may also be able to access nutrients ingested by the host (34). Indeed, one hypothesis to explain illness-induced anorexia is that cessation of host feeding deprives infecting pathogens of nutrients (3, 12, 105), albeit with possible negative collateral consequences for reproduction (18). Finally, because different branches of the immune response may have different micronutritional requirements, it may be impossible to maximize all components of the immune system simultaneously (33), giving rise to trade-offs between immune system components (e.g., 32).

The energetic demands imposed by reproduction and immunity suggest that competition for nutritional resources could be at the center of the trade-off between them. In searching for the control center for the reproduction–immunity trade-off, our attention was drawn to the fat body. This tissue is the central metabolic control organ (reviewed in 8). The fat body is critically important for oogenesis because it is a major site for yolk protein and vitellogenin production for oocytes (72, 77). It is also the primary organ of systemic immunity (reviewed in 63).

Short et al. (121) observed that *D. melanogaster* females lacking germ cells (and therefore cannot form eggs) do not suffer from reduced immunity after mating, in contrast to females that produce eggs. These findings support the hypothesis that reproduction and immunity compete for nutritional resources. However, the cost of reproduction still persists in *D. melanogaster* females that arrest egg production at stage 4 of oogenesis, even though these females never produce mature oocytes (43). In corroboration of the persistence of the trade-off in the sterile females, evidence from a locust (*Locusta migratoria*) system suggests yolk protein is still produced in the absence of egg production (29). Therefore, the *D. melanogaster* stage 4 mutants may still invest resources into yolk protein production rather than into immunity. This could explain why postmating immunosuppression occurs in these females despite the absence of fully formed eggs.

Further support for nutritional mediation of the trade-off between reproduction and immunity comes from studies that showed that providing *D. melanogaster* females with dietary yeast ad libitum improved fecundity and resistance to infection and without any evolutionary trade-off between these phenomena (94). Yet access to food ad libitum did not fully rescue fecundity in chronically immune-stimulated *G. texensis* (126). This disparity highlights the importance of future studies to identify the resource shared between the two processes, the required intake of the nutrient, and how ratios of dietary components can influence the two traits.

Signaling pleiotropy: a signaling cascade that regulates two distinct traits

20E: 20-hydroxyecdysone

IIS: insulin/insulin-like growth factor-like signaling

JH: juvenile hormone

SIGNALING PLEIOTROPY MAY UNDERLIE RESOURCE ALLOCATION

If egg provisioning and immune defense rely on a common resource pool, the host organism should have a signaling mechanism to shunt those resources toward one process or the other. We propose that the physiological trade-off between reproduction and immunity in insects is regulated by endocrine signals, specifically the balance between juvenile hormone (JH) and 20-hydroxyecdysone (20E) and by altered metabolic status mediated by insulin/insulin-like growth factor-like signaling (IIS). Although we are specifically concerned here with the trade-off between reproduction and immunity, as a general principle, pleiotropic signaling pathways are likely to be common switches for regulating life-history trade-offs (see also 51, 61, 144).

Juvenile Hormone and 20-Hydroxyecdysone

JH and 20E have been characterized in insects primarily for their role in regulating metamorphosis (100). However, the balance between JH and 20E is important for activation of egg maturation and provisioning in a variety of phylogenetically diverse insects. Additionally, JH can be a direct or indirect antagonist of immune function in insects, and 20E is a known potentiator of insect immunity. We propose a model in which JH/20E signaling mediates the trade-off between reproduction and immunity, at least in part, by regulating the diversion of energetic resources from somatic maintenance to reproductive output (**Figure 1**).

The balance between JH and 20E dictates the progression of oogenesis, with JH promoting egg production and provisioning (reviewed in 57, 133). JH levels are responsive to diet and to mating in female insects. Increased JH levels promote the expression of vitellogenin or yolk protein genes

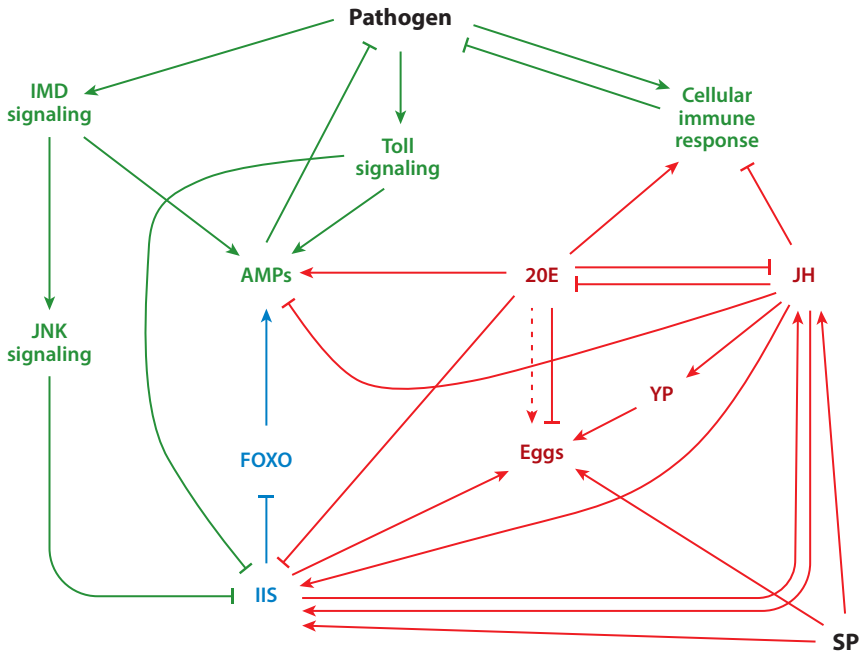

Figure 1

A generalized mechanism for the interactions between immunity and reproduction. Reproductive pathways are red, metabolic signaling pathways are blue, and immunity pathways are green. A female insect host responds to a pathogen via IMD and Toll pathways and cellular immune activation. Activation of the IMD pathway activates JNK signaling, which inhibits IIS signaling. The end result is the production of AMPs. The receipt of SP during mating alters the typical response to a pathogen. SP stimulates JH synthesis, which negatively regulates the cellular immune response and the production of AMPs. Abbreviations: 20E, 20-hydroxyecdysone; AMPs, antimicrobial peptides; FOXO, forkhead box, subgroup O; IIS, insulin/insulin-like growth factor-like signaling; IMD, immune deficiency; JH, juvenile hormone; JNK, c-Jun N-terminal kinase; SP, Sex Peptide; YP, yolk protein.

in the fat body of female insects such as red flour beetles (*Tribolium castaneum*) (118), cockroaches (*Leucophaea maderae*, *Blattella germanica*) (21, 130), and Oriental fruit flies (*Bactrocera dorsalis*) (28). JH also promotes the uptake of vitellogenin or yolk protein into oocytes by creating intercellular spaces between the follicle cells (2, 52) and aids in the progression of developing follicles (124). In contrast, high 20E titers typically result in the resorption of immature vitellogenic eggs (124). However, not all organisms may conform to these patterns of JH/20E signaling. For example, although both JH and 20E are essential for oogenesis in mosquitoes, 20E is more important for regulating vitellogenesis (reviewed by 60). Thus, reproductive aspects such as the requirement for blood meals may influence the generality of molecular mechanisms.

JH and 20E also have opposite effects on immunity in most insects (**Figure 1**). Ectopic application of methoprene, a synthetic JH analog, reduces the activation of antimicrobial peptide genes in response to infection in *D. melanogaster* (49). JH reduces phenoloxidase activity in *T. molitor* (112) and inhibits the spreading behavior of hemocytes in *Tribolium castaneum* and *Spodoptera exigua* (62, 74). In contrast, 20E potentiates the expression of antimicrobial peptide genes in *D. melanogaster* (36, 49, 96, 146) and signals through the ecdysone receptor (EcR) to drive expression of the peptidoglycan-recognition protein LC (PGRP-LC), a primary activator of the IMD humoral

immune signaling pathway (114). 20E also regulates embryonic immunity in *D. melanogaster* (131) and ecdysone signaling is required for cellular immunity in *D. melanogaster* (107, 125).

The opposite effects of JH and 20E on reproduction and immunity raise the possibility that JH and 20E levels may mediate the physiological trade-off between the two processes. In light of this, it is intriguing that both JH and 20E can be transferred to female mosquitoes in the male seminal fluid (30, 54). Most insects activate synthesis of JH in the corpora allata after mating. Transplantation of corpora allata from mated *T. molitor* females into virgins resulted in lower levels of phenoloxidase activity, similar to the mating-induced suppression of phenoloxidase observed in mated *T. molitor* females (112). In *D. melanogaster*, JH synthesis is activated when the protein Sex Peptide (Acp75) is transferred to the female in the male's seminal fluid (42). Short et al. (121) demonstrated that females mated to males lacking Sex Peptide were as resistant as virgin females to a bacterial infection, whereas females mated to wild-type males showed depressed immunity.

FOXO: transcription factor that is active under low IIS activity

In summary, strong evidence across a breadth of insects exists to support a model in which mating increases JH titers and suppresses 20E, promoting egg development and inhibiting immune capability. This provides perhaps the clearest example to date of widespread endocrinological regulation of a life-history trade-off through a pleiotropic signal (**Figure 1**). Although a general model is presented here, future comparisons among insect taxa are encouraged to test the universality of this mechanism.

Insulin/Insulin-Like Growth Factor-Like Signaling

As described above, both egg provisioning and immune response are sensitive to the insect's nutritional status and metabolism. As in vertebrates, IIS is a major regulator of metabolic status in response to dietary nutrition (reviewed in 141), and both reproduction and immunity are responsive to IIS. Elevated IIS promotes oogenesis and inhibits immune responses. Thus, IIS serves as a potential control switch for regulating the physiological trade-off between reproduction and immunity.

Insects use flux through the insulin signaling pathway to indicate whether the female has sufficient nutrient stores to provision eggs (13). High IIS activity promotes oogenesis, and under reduced IIS activity (indicative of nutritional deprivation) egg production is reduced or halted (40, 69, 80, 102, 109). In contrast, IIS and immune signaling are reciprocally antagonistic. In *D. melanogaster*, genetic activation of the Toll immune response pathway results in reduced IIS, even in the absence of infection (35). IIS is inhibited also by the immune-responsive JNK pathway (137). Low IIS activity may enhance immunity through increased nuclear localization of the transcription factor FOXO, which can bind to the promoters of antimicrobial peptide genes and positively regulate their expression (19). Moreover, IIS pathway mutants survive bacterial infection better than wild-type flies do (87), possibly through rescue from energetic loss associated with fighting infection (37). Infection of skimmer dragonflies (*Libellula pulchella*) by protozoan parasites results in metabolic disruption and pathology, which can be reversed by blocking IIS (116).

The effect of IIS signaling on reproduction and immunity may not be independent of JH and 20E; it may in fact partially act through JH and 20E. *D. melanogaster* insulin receptor (*dInR*) mutants exhibit low JH titers (135), indicating that IIS promotes JH synthesis. Nuclear FOXO—an indication of low IIS activity—also increases JH titers and vitellogenin production in the German cockroach (*B. germanica*) (1, 130). Moreover, JH and 20E can regulate IIS activity. 20E promotes FOXO activity in *Bombyx mori* (67), and reduction of JH titers via ablation of the corpus allatum in *D. melanogaster* reduces IIS signaling and increases 20E levels (98). Thus, JH and IIS may form a positive feedback loop that promotes oogenesis and inhibits immunity in response to the nutritional environment and reproductive cues (**Figure 1**).

CONCLUDING REMARKS

Immune defense and reproduction are each central to organismal fitness, yet they trade off at both the physiological and evolutionary levels. Here we propose a model (**Figure 1**) whereby endocrine and metabolic signaling may cooperatively mediate a physiological trade-off between reproduction and immunity via JH, 20E, and IIS. More research is needed to fully test this model and to determine its mechanistic detail. For example, although ample evidence exists that JH inhibits immune system activity in multiple insects, it is unknown whether this is a direct or indirect effect.

Most of this review has focused on how a trade-off can be generated by competition between two physiological processes for a limiting resource, with some thought to the mechanics of how allocation of that resource might be managed. We emphasize, though, that both reproduction and immune performance are critical to evolutionary fitness, and the trade-off between them is therefore likely subject to strong natural selection. Environmental fluctuations in resource availability or pathogen pressure could shift the selective pressure between reproduction and immunity, and a few examples exist in which genetically distinct individuals in a population vary in their hardwired bias, favoring one process or the other. However, at this time, the research community has virtually no understanding of the mechanistic basis for evolutionary trade-offs and we emphasize that they may or may not be the same as those underlying physiological trade-offs. More research is needed in this vein to understand how evolution shapes investment in and trade-offs between reproduction and immunity.

SUMMARY POINTS

1. The trade-off between female reproduction and immunity has been detected in a diverse range of insect species.
2. Physiological costs of immunity include reduced egg production and viability. Physiological costs of reproduction often include reduction in both basal and induced levels of immunity.
3. The energetic requirement of reproduction and immunity suggests that the reallocation of a common resource may be the basis for the trade-off between the traits.
4. Hormonal signaling, including JH and 20E, are critical for modulating egg production and immunity. Such signals may hold a central position in the allocation of resources required for both reproduction and immunity.

FUTURE ISSUES

1. How does reproduction suppress immunity? Are all components of immunity suppressed by reproduction? How does immunity suppress reproduction?
2. Are other postmating changes (e.g., reduced siesta sleep or increased feeding) important for the trade-off? Is egg production the only point of conflict?
3. How do nutrition and metabolic state affect the physiological trade-off between reproduction and immunity? What limiting resources are shared between the two processes?
4. What is the mechanistic basis of evolutionary trade-offs between reproduction and immunity? Do physiological and evolutionary trade-offs have the same mechanistic basis? Are these mechanisms shared across insect taxa?

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LITERATURE CITED

1. Abrisqueta M, Süren-Castillo S, Maestro JL. 2014. Insulin receptor-mediated nutritional signalling regulates juvenile hormone biosynthesis and vitellogenin production in the German cockroach. *Insect Biochem. Mol. Biol.* 49:14–23
2. Abu-Hakima R, Davey KG. 1977. Two actions of juvenile hormone on the follicle cells of *Rhodnius prolixus*: the importance of volume changes. *J. Exp. Biol.* 69:33–44
3. Adamo SA, Fidler TL, Forestell CA. 2007. Illness-induced anorexia and its possible function in the caterpillar, *Manduca sexta*. *Brain Behav. Immun.* 21:292–300
4. Ahmed AM, Baggott SL, Maingon R, Hurd H. 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos* 97:371–77
5. Ahmed AM, Hurd H. 2006. Immune stimulation and malaria infection impose reproductive costs in *Anopheles gambiae* via follicular apoptosis. *Microbes Infect.* 8:308–15
6. Armitage SAO, Siva-Jothy MT. 2005. Immune function responds to selection for cuticular colour in *Tenebrio molitor*. *Heredity* 94:660–66
7. Armitage SAO, Thompson JJW, Rolff J, Siva-Jothy MT. 2003. Examining costs of induced and constitutive immune investment in *Tenebrio molitor*. *J. Evol. Biol.* 16:1038–44
8. Arrese EL, Soulages JL. 2010. Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* 55:207–25
9. Attardo GM, Hansen IA, Raikhel AS. 2005. Nutritional regulation of vitellogenesis in mosquitoes: implications for anautogeny. *Insect Biochem. Mol. Bio.* 35:661–75
10. Avila FW, Sirot LK, LaFlamme BA, Rubinstein CD, Wolfner MF. 2011. Insect seminal fluid proteins: identification and function. *Annu. Rev. Entomol.* 56:21–40
11. Awmack CS, Leather SL. 2002. Host plant quality and fecundity in herbivorous insects. *Annu. Rev. Entomol.* 47:817–44
12. Ayres JS, Schneider DS. 2009. The role of anorexia in resistance and tolerance to infections in *Drosophila*. *PLOS Biol.* 7:e1000150
13. Badisco L, Van Wielendaele P, Vanden Broeck J. 2013. Eat to reproduce: a key role for the insulin signaling pathway in adult insects. *Front. Physiol.* 4:1–16
14. Baer B, Armitage SAO, Boomsma JJ. 2006. Sperm storage induces an immunity cost in ants. *Nature* 441:872–75
15. Bajgar A, Kucerova K, Jonatova L, Tomcala A, Schneedorferova I, et al. 2015. Extracellular adenosine mediates a systemic metabolic switch during immune response. *PLOS Biol.* doi: 10.1371/journal.pbio.1002135
16. Barnes AI, Wigby S, Boone JM, Partridge L, Chapman T. 2008. Feeding, fecundity and lifespan in female *Drosophila melanogaster*. *Proc. Biol. Sci.* 275:1675–83
17. Bascuñán-García AP, Lara C, Córdoba-Aguilar A. 2010. Immune investment impairs growth, female reproduction and survival in the house cricket, *Acheta domesticus*. *J. Insect Physiol.* 56:204–11
18. Bashir-Tanoli S, Tinsley MC. 2014. Immune response costs are associated with changes in resource acquisition and not resource reallocation. *Funct. Ecol.* 28:1011–19

19. Becker T, Loch G, Beyer M, Zinke I, Aschenbrenner AC, et al. 2010. FOXO-dependent regulation of innate immune homeostasis. *Nature* 463:369–73
20. Boots M, Begon M. 1993. Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct. Ecol.* 7:528–34
21. Brookes VJ. 1969. The induction of yolk protein synthesis in the fat body of an insect, *Leucophaea maderae*, by an analog of the juvenile hormone. *Dev. Biol.* 20:459–71
22. Büning J. 1994. *The Insect Ovary: Ultrastructure, Previtellogenic Growth, and Evolution*. London: Chapman and Hall
23. Carvalho GB, Kapahi P, Anderson DJ, Benzer S. 2006. Allocrine modulation of feeding behavior by the sex peptide of *Drosophila*. *Curr. Biol.* 16:692–96
24. Castella G, Christe P, Chapuisat M. 2009. Mating triggers dynamic immune regulations in wood ant queens. *J. Evol. Biol.* 22:564–70
25. Castillo JC, Reynolds SE, Eleftherianos I. 2011. Insect immune responses to nematode parasites. *Trends Parasitol.* 27:537–47
26. Catalán TP, Barceló M, Niemeyer HM, Kalergis AM, Bozinovic F. 2011. Pathogen- and diet-dependent foraging, nutritional and immune ecology in mealworms. *Evol. Ecol. Res.* 13:711–23
27. Cavaliere V, Bernardi F, Romani P, Duchi S, Gargiulo G. 2008. Building up the *Drosophila* eggshell: First of all the eggshell genes must be transcribed. *Dev. Dyn.* 237:2061–72
28. Chen S-L, Lin C-P, Lu K-H. 2012. cDNA isolation, expression, and hormonal regulation of yolk protein genes in the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *J. Insect Physiol.* 58:763–70
29. Chinzei Y, Wyatt GR. 1985. Vitellogenin titre in haemolymph of *Locusta migratoria* in normal adults, after ovariectomy, and in response to methoprene. *J. Insect Physiol.* 31:441–45
30. Clifton M, Correa S. 2014. Male *Aedes aegypti* mosquitoes use JHIII transferred during copulation to influence previtellogenic ovary physiology and affect the reproductive output of female mosquitoes. *J. Insect Physiol.* 64:40–47
31. Clifton ME, Noriega FG. 2011. Nutrient limitation results in juvenile hormone-mediated resorption of previtellogenic ovarian follicles in mosquitoes. *J. Insect Physiol.* 57:1274–81
32. Cotter SC, Kruuk LEB, Wilson K. 2004. Costs of resistance: genetic correlations and potential trade-offs in an insect immune system. *J. Evol. Biol.* 17:421–29
33. Cotter SC, Simpson SJ, Raubenheimer D, Wilson K. 2011. Macronutrient balance mediates trade-offs between immune function and life history traits. *Funct. Ecol.* 25:186–98
34. Cressler CE, Nelson WA, Day T, McCauley E. 2013. Disentangling the interaction among host resources, the immune system and pathogens. *Ecol. Lett.* 17:284–93
35. DiAngelo JR, Bland ML, Bambina S, Cherry S, Birnbaum MJ. 2009. The immune response attenuates growth and nutrient storage in *Drosophila* by reducing insulin signaling. *PNAS* 106:20853–58
36. Dimarcq JL, Imler JL, Lanot R, Ezekowitz RAB, Hoffmann JA, et al. 1997. Treatment of l(2)mbn *Drosophila* tumorous blood cells with the steroid hormone ecdysone amplifies the inducibility of antimicrobial peptide gene expression. *Insect Biochem. Mol. Biol.* 27:877–86
37. Dionne MS, Pham LN, Shirasu-Hiza M, Schneider DS. 2006. *Akt* and *foxo* dysregulation contribute to infection-induced wasting in *Drosophila*. *Curr. Biol.* 16:1977–85
38. Dixon A. 1963. Reproductive activity of the sycamore aphid, *Drepanosiphum platanoides* (Schr.) (Hemiptera, Aphididae). *J. Anim. Ecol.* 32:33–48
39. Domanitskaya EV, Liu H, Chen S, Kubli E. 2007. The hydroxyproline motif of male sex peptide elicits the innate immune response in *Drosophila* females. *FEBS J.* 274:5659–68
40. Drummond-Barbosa D, Spradling AC. 2001. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* 231:265–78
41. Du Plessis H, Byrne MJ, Van den Berg J. 2012. The effect of different host plants on the reproduction and longevity of *Nysius natalensis*. *Entomol. Exp. Appl.* 145:209–14
42. Fan Y, Rafaeli A, Moshitzky P, Kubli E, Choffat Y, Applebaum SW. 2000. Common functional elements of *Drosophila melanogaster* seminal peptides involved in reproduction of *Drosophila melanogaster* and *Helicoverpa armigera* females. *Insect Biochem. Mol. Biol.* 30:805–12

43. Fedorka KM, Linder JE, Winterhalter W, Promislow D. 2007. Post-mating disparity between potential and realized immune response in *Drosophila melanogaster*. *Proc. Biol. Sci.* 274:1211–17
44. Fedorka KM, Zuk M, Mousseau T. 2004. Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*. *Evolution* 58:2478–85
45. Fellowes MDE, Kraaijeveld AR, Godfray HCJ. 1999. The relative fitness of *Drosophila melanogaster* (Diptera, Drosophilidae) that have successfully defended themselves against the parasitoid *Asobara tabida* (Hymenoptera, Braconidae). *J. Evol. Biol.* 12:123–28
46. Ferdig MT, Beerntsen BT, Spray FJ, Li J, Christensen BM. 1993. Reproductive costs associated with resistance in a mosquito-filarial worm system. *Am. J. Trop. Med. Hyg.* 49:756–62
47. Finch CE, Rose MR. 1995. Hormones and the physiological architecture of life history evolution. *Q. Rev. Biol.* 70:1–52
48. Fisher RA. 1930. *The Genetical Theory of Natural Selection*. Oxford, UK: Oxford Univ. Press
49. Flatt T, Heyland A, Rus F, Porpiglia E, Sherlock C, et al. 2008. Hormonal regulation of the humoral innate immune response in *Drosophila melanogaster*. *J. Exp. Biol.* 211:2712–24
50. Flatt T, Kawecki T. 2007. Juvenile hormone as a regulator of the trade-off between reproduction and life span in *Drosophila melanogaster*. *Evolution* 61:1980–91
51. Flatt T, Tu M-P, Tatar M. 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. *BioEssays* 27:999–1010
52. Fleig R. 1995. Role of the follicle cells for yolk uptake in ovarian follicles of the honey bee *Apis mellifera* (Hymenoptera: Apidae). *Int. J. Insect Morphol. Embryol.* 24:427–33
53. Foley K, Fazio G, Jensen AB, Hughes WOH. 2012. Nutritional limitation and resistance to opportunistic *Aspergillus* parasites in honey bee larvae. *J. Invertebr. Pathol.* 111:68–73
54. Gabrieli P, Kakani EG, Mitchell SN, Mameli E, Want EJ, et al. 2014. Sexual transfer of the steroid hormone 20E induces the postmating switch in *Anopheles gambiae*. *PNAS* 111:16353–58
55. Gadgil M, Bossert WH. 1970. Life historical consequences of natural selection. *Am. Nat.* 104:1–24
56. González-Santoyo I, Córdoba-Aguilar A. 2012. Phenoloxidase: a key component of the insect immune system. *Entomol. Exp. Appl.* 142:1–16
57. Gruntenko NE, Rauschenbach IY. 2008. Interplay of JH, 20E and biogenic amines under normal and stress conditions and its effect on reproduction. *J. Insect Physiol.* 54:902–8
58. Gupta V, Ali ZS, Prasad NG. 2013. Sexual activity increases resistance against *Pseudomonas entomophila* in male *Drosophila melanogaster*. *BMC Evol. Biol.* 13:185
59. Gwynn DM, Callaghan A, Gorham J, Walters KFA, Fellowes MDE. 2005. Resistance is costly: trade-offs between immunity, fecundity, and survival in the pea aphid. *Proc. R. Soc. B* 272:1803–8
60. Hansen IA, Attardo GM, Rodriguez SD, Drake LL. 2014. Four-way regulation of mosquito yolk protein precursor genes by juvenile hormone-, ecdysone-, nutrient-, and insulin-like peptide signaling pathways. *Front. Physiol.* 5:1–8
61. Harshman LG, Zera AJ. 2007. The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* 22:80–86
62. Hepat R, Kim Y. 2014. JH modulates a cellular immunity of *Tribolium castaneum* in a Met-independent manner. *J. Insect Physiol.* 63:40–47
63. Hoffmann JA. 2003. The immune response of *Drosophila*. *Nature* 426:33–38
64. Hogg JC, Hurd H. 1995. *Plasmodium yoelii nigeriensis*: the effect of high and low intensity of infection upon the egg production and bloodmeal size of *Anopheles stephensi* during three gonotrophic cycles. *Parasitology* 111:555–62
65. Hosken DJ. 2001. Sex and death: microevolutionary trade-offs between reproductive and immune investment in dung flies. *Curr. Biol.* 11:R379
66. Hosken DJ, Garner TWJ, Ward PI. 2001. Sexual conflict selects for male and female reproductive characters. *Curr. Biol.* 11:489–93
67. Hossain MS, Liu Y, Zhou S, Li K, Tian L, Li S. 2013. 20-hydroxyecdysone-induced transcriptional activity of *FoxO* upregulates *brummer* and *acid lipase-1* and promotes lipolysis in *Bombyx* fat body. *Insect Biochem. Mol. Biol.* 43:829–38
68. Howick VM, Lazzaro BP. 2014. Genotype and diet shape resistance and tolerance across distinct phases of bacterial infection. *BMC Evol. Biol.* 14:56

69. Hsu H-J, Drummond-Barbosa D. 2009. Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*. *PNAS* 106:1117–21
70. Hurd H. 2001. Host fecundity reduction: a strategy for damage limitation? *Trends Parasitol.* 17:363–68
71. Innocenti P, Morrow EH. 2009. Immunogenic males: a genome-wide analysis of reproduction and the cost of mating in *Drosophila melanogaster* females. *J. Evol. Biol.* 22:964–73
72. Isaac PG, Bownes M. 1982. Ovarian and fat-body vitellogenin synthesis in *Drosophila melanogaster*. *Eur. J. Biochem.* 123:527–34
73. Kelly CD. 2011. Reproductive and physiological costs of repeated immune challenges in female Wellington tree weta (Orthoptera: Anostostomatidae). *Biol. J. Linn. Soc.* 104:38–46
74. Kim Y, Jung S, Madanagopal N. 2008. Antagonistic effect of juvenile hormone on hemocyte-spreading behavior of *Spodoptera exigua* in response to an insect cytokine and its putative membrane action. *J. Insect Physiol.* 54:909–15
75. Klemola N, Klemola T, Rantala MJ, Ruuhola T. 2007. Natural host-plant quality affects immune defence of an insect herbivore. *Entomol. Exp. Appl.* 23:167–76
76. Knell RJ, Webberley KM. 2004. Sexually transmitted diseases of insects: distribution, evolution, ecology and host behaviour. *Biol. Rev. Camb. Philos. Soc.* 79:557–81
77. Kokoza VA, Martin D, Mienaltowski MJ, Ahmed A, Morton CM, Raikhel AS. 2001. Transcriptional regulation of the mosquito vitellogenin gene via a blood meal-triggered cascade. *Gene* 274:47–65
78. Kraaijeveld AR, Elrayes NP, Schuppe H, Newland PL. 2011. L-arginine enhances immunity to parasitoids in *Drosophila melanogaster* and increases NO production in lamellocytes. *Dev. Comp. Immunol.* 35:857–64
79. Kraaijeveld AR, Ferrari J, Godfray HCJ. 2002. Costs of resistance in insect-parasite and insect-parasitoid interactions. *Parasitology* 125:S71–82
80. LaFever L, Drummond-Barbosa D. 2005. Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science* 309:1071–73
81. Law R. 1979. Optimal life histories under age-specific predation. *Am. Nat.* 114:399–417
82. Lawniczak MKN, Barnes AI, Linklater JR, Boone JM, Wigby S, Chapman T. 2007. Mating and immunity in invertebrates. *Trends Ecol. Evol.* 22:48–55
83. Lawniczak MKN, Begun DJ. 2004. A genome-wide analysis of courting and mating responses in *Drosophila melanogaster* females. *Genome* 47:900–10
84. Lazzaro BP, Flores HA, Lorigan JG, Yourth CP. 2008. Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster*. *PLOS Pathog.* 4:e100025
85. Lazzaro BP, Little TJ. 2009. Immunity in a variable world. *Philos. Trans. R. Soc. B.* 364:15–26
86. Lee KP, Simpson SJ, Wilson K. 2008. Dietary protein-quality influences melanization and immune function in an insect. *Funct. Ecol.* 22:1052–61
87. Libert S, Chao Y, Zwiener J, Pletcher SD. 2008. Realized immune response is enhanced in long-lived *puc* and *chico* mutants but is unaffected by dietary restriction. *Mol. Immunol.* 45:810–17
88. Luong LT, Polak M. 2007. Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. *Evolution* 61:1391–402
89. Mack PD, Kapelnikov A, Heifetz Y, Bender M. 2006. Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. *PNAS* 103:10358–63
90. McGraw LA, Gibson G, Clark A, Wolfner MF. 2004. Genes regulated by mating, sperm, or seminal proteins in mated female *Drosophila melanogaster*. *Curr. Biol.* 14:1509–14
91. McKean KA, Lazzaro BP. 2010. The costs of immunity and the evolution of immunological defense mechanisms. In *Mechanisms of Life History Evolution*, ed. T Flatt, A Heyland, pp. 299–310. Oxford, UK: Oxford Univ. Press
92. McKean KA, Nunney L. 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. *PNAS* 98:7904–9
93. McKean KA, Nunney L. 2005. Bateman's principle and immunity: Phenotypically plastic reproductive strategies predict changes in immunological sex differences. *Evolution* 59:1510–17
94. McKean KA, Yourth CP, Lazzaro BP, Clark AG. 2008. The evolutionary costs of immunological maintenance and deployment. *BMC Evol. Biol.* 8:76–95

95. McNamara KB, Van Lieshout E, Simmons LW. 2013. Females suffer a reduction in the viability of stored sperm following an immune challenge. *J. Evol. Biol.* 27:133–40
96. Meister M, Lagueux M. 2003. *Drosophila* blood cells. *Cell. Microbiol.* 5(9):573–80
97. Miest TS, Bloch-Qazi MC. 2008. Sick of mating: sexual transfer of a pathological bacterium in *Drosophila melanogaster*. *Fly* 2:215–19
98. Mirth CK, Tang HY, Makohon-Moore SC, Salhadar S, Gokhale RH, et al. 2014. Juvenile hormone regulates body size and perturbs insulin signaling in *Drosophila*. *PNAS* 111:7018–23
99. Moret Y, Schmid-Hempel P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* 290:1166–68
100. Nijhout HF. 1994. *Insect Hormones*. Princeton, NJ: Princeton Univ. Press
101. Nystrand M, Dowling DK. 2014. Dose-dependent effects of an immune challenge at both ultimate and proximate levels in *Drosophila melanogaster*. *J. Evol. Biol.* 27:876–88
102. Parthasarathy R, Palli SR. 2011. Molecular analysis of nutritional and hormonal regulation of female reproduction in the red flour beetle, *Tribolium castaneum*. *Insect Biochem. Mol. Biol.* 41:294–305
103. Peng J, Zipperlen P, Kubli E. 2005. *Drosophila* Sex-peptide stimulates female innate immune system after mating via the Toll and Imd pathways. *Curr. Biol.* 15:1690–94
104. Polak M. 1996. Ectoparasite effects on host survival and reproduction: the *Drosophila-Macrocheles* association. *Ecology* 77:1379–89
105. Povey S, Cotter SC, Simpson SJ, Wilson K. 2013. Dynamics of macronutrient self-medication and illness-induced anorexia in virally infected insects. *J. Anim. Ecol.* 83:245–55
106. Reaney LT, Knell RJ. 2010. Immune activation but not male quality affects female current reproductive investment in a dung beetle. *Behav. Ecol.* 21:1367–72
107. Regan JC, Brandão AS, Leitão AB, Mantas Dias AR, Sucena E, et al. 2013. Steroid hormone signaling is essential to regulate innate immune cells and fight bacterial infection in *Drosophila*. *PLOS Pathog.* 9:e1003720
108. Ribeiro C, Dickson BJ. 2010. Sex Peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in *Drosophila*. *Curr. Biol.* 20:1000–5
109. Richard DS, Rybczynski R, Wilson TG, Wang Y, Wayne ML, et al. 2005. Insulin signaling is necessary for vitellogenesis in *Drosophila melanogaster* independent of the roles of juvenile hormone and ecdysteroids: Female sterility of the *chico1* insulin signaling mutation is autonomous to the ovary. *J. Insect Physiol.* 51:455–64
110. Roff DA. 1992. *Evolution of Life Histories: Theory and Analysis*. London: Chapman and Hall
111. Rolff J. 2002. Bateman's principle and immunity. *Proc. R. Soc. B* 269:867–72
112. Rolff J, Siva-Jothy MT. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. *PNAS* 99:9916–18
113. Rose MR, Bradley TJ. 1998. Evolutionary physiology of the cost of reproduction. *Oikos* 83:443–51
114. Rus F, Flatt T, Tong M, Aggarwal K, Okuda K, et al. 2013. Ecdysone triggered PGRP-LC expression controls *Drosophila* innate immunity. *EMBO J.* 32:1626–38
115. Sander JD, Joung JK. 2014. CRISPR-Cas systems for editing, regulating, and targeting genomes. *Nat. Biotechnol.* 32:347–55
116. Schilder RJ, Marden JH. 2006. Metabolic syndrome and obesity in an insect. *PNAS* 103:18805–9
117. Schmid-Hempel P. 2005. Evolutionary ecology of insect immune defenses. *Annu. Rev. Entomol.* 50:529–51
118. Sheng Z, Xu J, Bai H, Zhu F, Palli SR. 2011. Juvenile hormone regulates vitellogenin gene expression through insulin-like peptide signaling pathway in the red flour beetle, *Tribolium castaneum*. *J. Biol. Chem.* 286:41924–36
119. Shoemaker KL, Parsons NM, Adamo SA. 2006. Mating enhances parasite resistance in the cricket *Gryllus texensis*. *Anim. Behav.* 71:371–80
120. Short SM, Lazzaro BP. 2010. Female and male genetic contributions to post-mating immune defence in female *Drosophila melanogaster*. *Proc. Biol. Sci.* 277:3649–57
121. Short SM, Wolfner MF, Lazzaro BP. 2012. Female *Drosophila melanogaster* suffer reduced defense against infection due to seminal fluid components. *J. Insect Physiol.* 58:1192–201

122. Simmons LW. 2011. Resource allocation trade-off between sperm quality and immunity in the field cricket, *Teleogryllus oceanicus*. *Behav. Ecol.* 23:168–73
123. Siva-Jothy MT, Yoshitaka T, Hooper R. 1998. Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. *Physiol. Entomol.* 23:274–77
124. Soller M, Bownes M, Kubli E. 1999. Control of oocyte maturation in sexually mature *Drosophila* females. *Dev. Biol.* 208:337–51
125. Sorrentino RP, Carton Y, Govind S. 2002. Cellular immune response to parasite infection in the *Drosophila* lymph gland is developmentally regulated. *Dev. Biol.* 243:65–80
126. Stahlschmidt ZR, Rollinson N, Acker M, Adamo SA. 2013. Are all eggs created equal? Food availability and the fitness trade-off between reproduction and immunity. *Funct. Ecol.* 27:800–6
127. Stearns SC. 1992. *Evolution of Life Histories*. Oxford, UK: Oxford Univ. Press
128. Stearns SC. 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* 87:476–86
129. Strand M. 2008. Insect hemocytes and their role in immunity. In *Insect Immunology*, ed. NE Beckage, pp. 25–47. San Diego, CA: Elsevier
130. Süren-Castillo S, Abrisqueta M, Maestro JL. 2012. FoxO inhibits juvenile hormone biosynthesis and vitellogenin production in the German cockroach. *Insect Biochem. Mol. Biol.* 42:491–98
131. Tan KL, Vlisidou I, Wood W. 2014. Ecdysone mediates the development of immunity in the *Drosophila* embryo. *Curr. Biol.* 24:1–8
132. Terashima J, Bownes M. 2004. Translating available food into the number of eggs laid by *Drosophila melanogaster*. *Genetics* 167:1711–19
133. Toivonen JM, Partridge L. 2009. Endocrine regulation of aging and reproduction in *Drosophila*. *Mol. Cell. Endocrinol.* 299:39–50
134. Tsukamoto Y, Kataoka H, Nagasawa H, Nagata S. 2014. Mating changes the female dietary preference in the two-spotted cricket, *Gryllus bimaculatus*. *Front. Physiol.* 5:95
135. Tu M-P, Yin C-M, Tatar M. 2005. Mutations in insulin signaling pathway alter juvenile hormone synthesis in *Drosophila melanogaster*. *Gen. Comp. Endocrinol.* 142:347–56
136. Valtonen TM, Viitaniemi H, Rantala MJ. 2010. Copulation enhances resistance against an entomopathogenic fungus in the mealworm beetle *Tenebrio molitor*. *Parasitology* 137:985–89
137. Wang MC, Bohmann D, Jasper H. 2005. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121:115–25
138. Wheeler D. 1996. The role of nourishment in oogenesis. *Annu. Rev. Entomol.* 41:407–31
139. Wigglesworth VB. 1960. Nutrition and reproduction in insects. *Proc. Nutr. Soc.* 19:18–23
140. Williams G. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am. Nat.* 100:687–90
141. Wu Q, Brown MR. 2006. Signaling and function of insulin-like peptides in insects. *Annu. Rev. Entomol.* 51:1–24
142. Yan G, Severson DW, Christensen BM. 1997. Costs and benefits of mosquito refractoriness to malaria parasites: implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution* 51:441–50
143. Ye YH, Chenoweth SF, McGraw EA. 2009. Effective but costly, evolved mechanisms of defense against a virulent opportunistic pathogen in *Drosophila melanogaster*. *PLOS Pathog.* 5:e1000385
144. Zera AJ, Harshman LG, Williams TD. 2007. Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. *Annu. Rev. Ecol. Evol. Syst.* 38:793–817
145. Zerofsky M, Harel E, Silverman N, Tatar M. 2005. Aging of the innate immune response in *Drosophila melanogaster*. *Aging Cell* 4:103–8
146. Zhang Z, Palli SR. 2009. Identification of a *cis*-regulatory element required for 20-hydroxyecdysone enhancement of antimicrobial peptide gene expression in *Drosophila melanogaster*. *Insect Mol. Biol.* 18:595–605