Invertebrate Ecological Immunology

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Ecological immunology is a rapidly expanding field that examines the causes and consequences of variation in immune function in the context of evolution and of ecology. Millions of invertebrate species rely solely on innate immunity, compared with only 45,000 vertebrate species that rely additionally on an acquired immune system. Despite this difference in diversity, most studies of ecological immunology focus on vertebrates. Here we review recent progress derived largely from the mechanistic analysis of invertebrate innate immunity. Using this empirical base, we pose general questions in areas that are of central importance for the development of ecological immunology.

The field of immunology traditionally examines the physiological and molecular mechanisms underpinning host defense against pathogens and is usually conducted on laboratory models under ideal conditions (1), often in the absence of pathogens. By contrast, ecologists are becoming interested in the role immune effector systems play in determining host fitness in the wild (i.e., under the constraints imposed by ecology and life history). This emerging field is concerned with understanding the microevolutionary processes that create and maintain variation in immune effector systems and the coordinated host response to pathogens (2). Most studies have focused on vertebrates and have mainly tested predictions arising from Hamilton and Zuk’s (3, 4) and Fostad and Karter’s (5) ideas about honest signals of parasite resistance. These theories propose that secondary sexual traits indicate the bearer’s genetic ability to resist the negative effects of pathogens (3) and that these signals are honest because their expression is mediated by physiologically active compound(s) that simultaneously modulate immunity (5). The hypothesis of parasite-mediated sexual selection (3) offers an explanation for the maintenance of secondary sexual traits in vertebrates and invertebrates. However, the mechanistic refinement of the theory, the immunocompetence handicap (5, 6), is implicitly vertebrate-based and so focuses on acquired immunity. Despite the pre-

(i) the costs associated with evolving immunity [e.g., antagonistic pleiotropy (10)] and (ii) the physiological costs of maintaining and utilizing immune effector systems.

Perhaps the best evidence for evolutionary costs comes from work on Drosophila melanogaster, in which selection for resistance against parasitoids produced the correlated response of reduced larval ability to acquire food when in competition with conspecifics (11). Resistance is all-or-nothing in this system: If the host fails to encapsulate the parasitoid [by smothering it with a layer of hemocytes (insect immune cells), which die and become melanized], the host will die. Kraaijeveld et al. (12) have since shown that resistant hosts have twice as

Fig. 1. A study on leafcutter ants (15) assayed the metabolic costs of immune function as respiration rate over a 2-hour period. In some colonies, the glands producing antimicrobial peptides were experimentally blocked (black bars) and so were prevented from producing the immune effector peptides. The controls were allowed to produce antimicrobial peptides (open bars), and have a much higher respiration rate [photo courtesy of D. Nash; graph reprinted with permission from (15)].

A central tenet of ecological immunology is that immune responses are costly for the host. There are, broadly, two types of cost: occupation with sexual selection and vertebrates, there has been a recent shift toward investigating ecological immunity in a wider evolutionary context that has focused on invertebrates (7, 8). Invertebrates are attractive subjects for study because of the simple mechanisms underpinning their innate immune systems [a system that is being studied with renewed interest in vertebrates (9)] and their amenability to physiological manipulations in the context of life history and ecology.

Costs

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response reduces defense capacity through hemocyte activity (19).

Another potential cost of using immune effector systems is autoimmunity. The insect phenoloxidase (PO) cascade is switched on inside the open hemocoel of insects in response to nonself. The cascade produces a number of cytotoxic by-products (e.g., phenols) that attack the pathogen and, because of the open hemocoel, are believed to attack “self” as well (20). Because these rapid-acting, constitutive cytotoxic defenses might bear high costs, they usually coexist with low-cost, inducible, but relatively specific defenses like antibacterial and antifungal peptides. Theory (21) predicts when a host should switch between constitutive and inducible defenses or when they should combine those defenses (21). Constitutive defenses should be prevalent when the challenge is large and the parasite growth rate is high. Having permanent defenses in place under these conditions has obvious benefits over the need to produce time-delayed induced effector responses (22). Other recent theory predicts when and how hosts should combine specific and/or general responses (23) and when and how trade-offs between different pathways within an immune system should operate (24). Studies have demonstrated that different immune effector systems are independent, so trade-offs between them can only be expected if the different systems share resources. For example, melanism and immunity share the melamin-producing pathway, so we might expect a trade-off. Despite this physiological link, melanic morphs of the moth Spodoptera exempta are more resistant to fungal infections and have a stronger encapsulation response to parasitoid eggs compared to pale morphs (25, 26).

Perhaps the fields that have best grasped the nettle of costs associated with resistance are those of the evolution of pesticide and antibiotic resistance. Selection against resistance in the absence of pesticide in the sheep blowfly (27) reveals directly the selective cost of generating resistance. This example reveals the evolutionary cost of generating resistance. However, these fields have also demonstrated that compensating mechanisms evolve in response to the costs. The two most important routes to cost-free pesticide resistance are the expression of modifier genes and allelic substitutions (28); see also (17). Surprisingly, the existence of such counter mechanisms has hardly been studied in relation to parasite resistance. If such mechanisms exist, which seems likely they would alter the way we think about host-parasite coevolution by shifting attention away from costs onto how those costs, or their absence, affect the host’s response to pathogens.

**Host Life History and Investment in Immunity**

Because the likelihood of encountering parasites and pathogens increases with increasing population density (29, 30), selection will favor hosts that invest in prophylactic immune function under these conditions. Moths, beetles, and locusts reared under crowded (but parasite-free) conditions invest in immune defense (usually manifested as darker, melanized, exocuticle) that affords them greater protection than individuals reared at low densities (29).

In addition to this plastic response, it is likely that intraspecific variation in immune function will be driven by longevity through its correlation with infection risk. Because females gain fitness largely through longevity (whereas males gain through mating success), there should be major gender differences in lifetime investment strategies in immunity (8, 31). At present, few studies have examined the relation between investment in life-history traits such as longevity, and immunity (32). An important generic question arising from this aspect of ecological immunity is, how is investment in immunity likely to be shaped by differences in life history between species, morphs, and/or sexes?

**Hormones and Immune Suppression**

Recent studies have shown that processes analogous to those envisaged by the immunocompetence handicap hypothesis (5, 6) can underpin immune outcomes in insects. For example, Juvenile Hormone (JH) is a key insect hormone that has several different dose-dependent functions in insect ontogeny. Recent work has revealed that it down-regulates PO activity during development (33), as well as after mating (34). It has also been implicated in reducing the encapsulation response toward parasitoid eggs (35). In contrast, ecdysone, the main antagonist of JH during insect ontogeny, affects immune function by promoting the proliferation of hemocytes (36). Moreover, neurotransmitters have recently been shown to regulate immune function (37). Given that hormones affect immune function through largely unidentified pathways, we need to examine the chain of hormonal events linking an individual’s perception of environmental factors with the immunological outcomes of those perceptions.

**Correlation Between Immunity and Other Traits**

When empiricists examine the microevolution of immune systems, they usually focus on the effects of parasites. However, the physiological systems involved in resistance are likely to be involved in other functions as well (38). For example, selection on PO activity in insects may arise from its immunological effects, as well as the need to heal wounds and to melanize exocuticle after eclosion (38). It has only recently become apparent that there are immune consequences to variation in cuticular melanism, a discovery that may lead to a reinterpretation of the function of melanism in some systems. For example, swallowtail butterflies have darker larvae in the hibernating generation (39). The classic interpretation is that the darker larvae achieve higher solar gain during the colder autumn (39). However, because overwintering larvae cannot avoid micropathogens through behavior and so are exposed to them for longer, they also need a more robust immune defense. Clearly, the important goal here is to determine the degree to which selection acts directly on immune traits or is a correlated response to selection on nonimmunological function.

**Genetics**

A genomewide analysis of immune responses in Drosophila using microarrays revealed that a single species of bacterial or fungal infection caused the up-regulation of up to 350 genes (40). Many of these genes had no known immunological function. Given that genomics and ecological immunology are in their infancy, it is not surprising that we know relatively little about the coordinated molecular genetic response to pathogens. Combining these two fields will, we anticipate, provide insight into the microevolution of immune systems.

Our understanding of the population genetics of host resistance in insects is also relatively poor. Insights into the genetic response of populations of host organisms to selection for resistance have, again, been provided by studies of pesticide use (27). When selection is very strong (e.g., high pesticide concentrations), the target population develops resistance based on monogenic mechanisms. When selection is weaker, the target population usually responds by polygenic change. Response by hosts to pathogens is more likely to reflect the latter case (41).

We are not aware of any analysis of genetic variation in immunity in wild invertebrates [compare vertebrates (42)]. However, studies of aphids and their natural enemies demonstrated genetic differences in resistance to parasitoids between populations from different host plants (43). It is known that genetic variation for resistance is high (44), and there are two possible explanations for the patterns we see. First, negative frequency–dependent selection may be operating; as pathogen genotypes evolve that target predominant host genotypes, rare host genotypes will be favored (44). Alternatively, antagonistic pleiotropy (10) might maintain additive genetic variation in these traits in wild populations; genes advantageous in resistance
against one parasite may have detrimental effects in the absence of parasites.

Two different theoretical approaches (Fig. 2) try to capture coevolution between hosts and parasites. Gene-for-gene models [e.g., (45)] assume that a single parasite genotype can infect all host genotypes and that resistance is costly to maintain. By contrast, matching-alleles models assume that successful infection only occurs if the parasite and host genotype match; consequently, polymorphism in immune function is maintained by negative frequency--dependent selection. Indeed, these two types of model are opposites in a continuum of possible interactions (45). Resistance only goes to fixation in the strict gene-for-gene case. In natural conditions, we might not expect such traits to go to fixation. A generally held view is that essential genes (46) evolve slowly, because they are part of a balanced genome. Surprisingly, immune genes appear to evolve relatively rapidly (46).

Future studies in ecological immunology should provide the links between the laboratory-based identification of immune genes with the natural forces driving the evolution of those genes in natural populations. We know that invertebrate hosts are able to resolve (and resist) specific pathogen genotypes (44) despite their simple innate immunity. By contrast, the resolution at the molecular level is fairly coarse; i.e., receptors can only distinguish Gram-positive from Gram-negative bacteria (47). Bridging this disparity has potential far-reaching applications, such as the medical use of antimicrobial peptides synthesized by animals and plants. Even though these peptides are highly conserved, they are incredibly successful in dealing with a huge range of bacteria (48).

Cross-Resistance, Multiple Infection

Many studies of ecological immunology [but see (49)] examine the consequences of an isolated immune insult provided by a single parasite. However, hosts in the wild are likely to face multiple, overlapping insults. For example, parasitoids use different methods to circumvent the host’s immune defenses. They try to hide in host tissues or even attack the host’s immune defense directly, using venom or virus-like particles (VLPs) (49). Hosts can become resistant against each of these types of attack. However, in a study of D. melanogaster and three of its parasitoids, cross-resistance did not occur in all host-parasitoid combinations (49). Whereas host lines selected for resistance against Leptopilina boulardi, a species that uses VLPs, were resistant against Asobara tabida, which does not use VLPs, lines selected against A. tabida were not resistant against L. boulardi. It appears that being resistant against a sophisticated attacker may automatically provide resistance against the less-developed offense.

Multiple infections are usually assumed to be cumulatively bad for the host, but this may not always be the case. A recent study (50) shows that a single infection of a bacterium managed to stay undetected by the immune system of Drosophila. But when antibiotic peptides were produced in response to another pathogen, the previously undetected bacteria were killed.

As well as dealing with concomitant infections, organisms in the wild have to cope with additional ecological stressors. Studies that have looked at how immune function is modulated in the field and/or under stressful conditions suggest that the action of such stressors has important consequences for immune function (49, 51, 52). Ecological factors such as diet (51), temperature (49), and habitat (53) are known to affect immune function.

In the wild, where hosts are under continual risk of predation, as well as infection, minor fluctuations in immune function may have important fitness consequences. Ecologists have successfully incorporated multilayered interactions, and their consequences for life-history traits (e.g., development time, age, and size) are naturally recognized by the experimental designs. Questions about how predators, and/or other sources of environmental stress, disrupt investment and maintenance of immune function need to be addressed.

Prospects

Ecological immunology is generating some remarkable biological insights [e.g., collective medication in ants (54)]. Until recently, it has been driven by informal models (2, 5) and has produced an expanding base of relatively diffuse empirical work. Studying invertebrates has furthered our understanding of the mechanisms underpinning ecological immunology. The field is ultimately seeking to address the question “Why is there variation in immune function?” and it seeks the answer at levels of biological organization from the cell to the phylum. To be successful, it needs to link its key subdisciplines with (i) a formalized theoretical framework and (ii) a focused empirical approach directed at the open questions posed herein and elsewhere (55). With respect to the first need, life-history theory provides a well-defined template that lacks physiological dimensions (56); synergism between the empty areas of overlap will benefit both fields.

References and Notes
4. The hypothesis of parasite-mediated sexual selection (3) predicts that within a species, females should prefer to mate with “bright” males in order to gain indirect genetic benefits—the assumption being that “brightness” is correlated with parasite resistance. This prediction should also hold among species, such that species with higher average parasite burdens should have more elaborate ornamentation in males.
6. The immunocompetence handicap proposes that males honestly signal their parasite resistance. This is due to the assumed, but highly debated, dual function of testosterone: It mediates the expression of secondary sex traits but compromises the immune system. Only the “best” males are able to express attractive traits and to withstand parasitic infections.
10. Pleiotropy occurs when one allele has effects on two or more traits. Antagonistic pleiotropy occurs when the effects increase fitness through one trait and decrease it through another trait (57).
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32. Most long-lived animals (i.e., vertebrates) rely on innate and acquired immunity. The reason may be that the metabolic costs of maintaining an innate immune system are disproportionately higher in larger animals because basal metabolic rate scales exponentially with size (59).


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