Ecological and evolutionary implications of immunological priming in invertebrates

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Invertebrates have an immune response that differs considerably from the acquired immune response found in vertebrates. However, new studies indicate that past experience with a pathogen can provide individual invertebrates, or their descendants, with enhanced immunity. This prophylactic effect, termed immunological priming, is functionally similar to the acquired immune response in vertebrates. This new-found complexity of invertebrate immunity begs investigation into the conditions under which immunological priming should evolve, and its consequences for population dynamics.

Invertebrates have primitive immune systems, worthy of only passing mention in the major immunology textbooks, and long assumed to lack the acquired or memory form of defense that is seen in vertebrates. Although there have been some indications in the older literature that insects can be immunized [1,2], increasing recent evidence suggests that inveretebrate immunity is much more complex than was generally believed. The latest example of an 'acquired' response in an invertebrate comes from a new paper by Moret and Siva-Jothy [3]. They showed that, when an insect (the beetle *Tenebrio molitor*) encounters a pathogen, its past experience with pathogens might increase its chance of survival. Basically, it matters if the host is naïve to pathogens (or parasites). This 'acquired' phenomenon is remarkably similar to what occurs during encounters of vertebrates with pathogens.

The acquired facet of vertebrate immune systems has long been assumed to not apply to invertebrates because they lack immunoglobulins. This dogma might now fall, not because homologous genes have been revealed, but because studies such as those by Moret and Siva-Jothy show that at least some invertebrates have functional equivalents to the acquired response of vertebrates. To remind us that different mechanisms underlie acquired immunity in vertebrate taxa, we use the general term 'immunological priming' when referring to invertebrates.

To study immunological priming, Moret and Siva-Jothy injected individual beetles with lipopolysaccharide (LPS), a molecular signature of bacteria that is highly immunogenic. When these same hosts encountered a fungal pathogen, past exposure to LPS endowed them with greater resistance than was found in control hosts that had not been injected with LPS. Moret and Siva-Jothy hypothesized that LPS acted as a prophylactic that stimulated an immune response, thereby mimicking a primary encounter with a pathogen. When the fungal pathogen was later encountered, components of the immune system were still upregulated because of previous exposure to LPS, and thus the host was better primed to deal with the pathogen.

LPS is a signature of gram-negative bacteria, but it is not found in fungi. Thus, that a previous application of LPS had an effect on fungal infections indicates that LPS upregulates a very general response in hosts. But a general response is not the only feat that invertebrates are capable of. Studies of other taxa have shown that specific responses in individual hosts also occur, that is, responses might be finely tuned to particular pathogen types or strains [4–6], and these specific responses might also be prophylactic [7]. Both specific and general immunity can also be transmitted across generations [8–10], endowing the offspring of pathogen-exposed parents with improved immunity. The use of the word 'specific' here refers only to a scenario of specialized host and parasite genotypes, and does not necessarily imply acquired immunity, as it does in vertebrate immunology [11]. Clarification of the variant use of these words, as well as examples of experimental designs to elucidate specificity and immunological priming are given in Box 1.

Thus, the recent outbreak of studies of invertebrate–pathogen (or parasite) interactions has revealed several nuances of immunological priming that protect against pathogens. How does it work? Although the job of identifying the cells and proteins involved will fall to molecular immunologists, immunological priming in invertebrates will have substantial implications for both evolutionary ecologists and epidemiologists. In particular, it will be important to consider (i) the conditions under which immunological priming is expected to evolve; and (ii) the consequences of immunological priming for the dynamics of populations and gene frequencies.

Under what circumstances should we expect to find immunological priming in an invertebrate? The life-history characteristics of an organism could provide a first clue. One argument as to why invertebrates should lack acquired immunity is their short life span; most invertebrates will have died before a secondary exposure occurs. Therefore, long-lived invertebrates, such as members of the genus *Nautilus* or the horseshoe crab, seem

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Box 1. Generality, specificity and priming of immune responses

Definitions from vertebrate immunology

In vertebrate immunology, the specific response is synonymous with the acquired response and is mediated by immunoglobulins, which invertebrates lack. Acquired (or 'adaptive') responses are based on the proliferation of specialized cells following the capture of a foreign antigen, whereas general responses utilize the innate immune system.

Definitions from evolutionary ecology

For evolutionary ecologists, general resistance refers to the observation that host genotypes differ in their capacity to defend against parasites or pathogens, regardless of parasite or pathogen genotype. Specificity in resistance implies that host genotypes differ, but that this is dependent on which parasite or pathogen genotype is encountered. In a system with two host genotypes (H1, H2) and two parasite or pathogen genotypes (P1, P2), specificity is present when H1 is susceptible to P1 but is resistant to P2, whereas H2 is susceptible to P2 but resistant to P1.

Tests for immunological priming

Tests for immunological priming have so far used a few designs, which are detailed in Figure I. Experiment (a) tests single individuals with a prechallenge (e.g. lipopolysaccharide, a pathogen mimic) and a secondary challenge of a pathogen [3]. Experiment (b) is similar to (a), but tests the offspring of individuals subjected to the prechallenge, and thus tests for transgenerational effects [9,10]. Experiments (c) and (d) test for strain specific effects on individuals (c) [7] or as a transgenerational effect (d) [8]. The final row indicates the predicted outcome of the second challenge if priming effects are present: '+' indicates a relatively severe infection; '−' indicates a relatively minor infection.

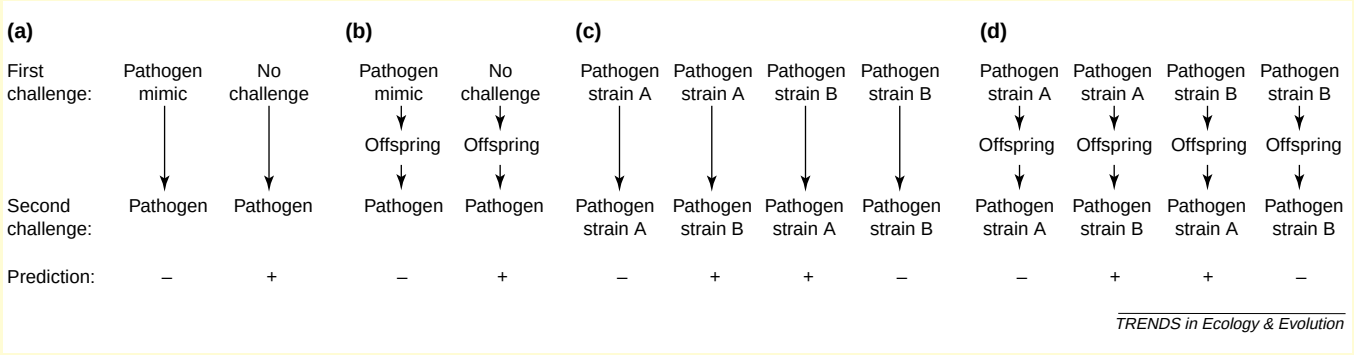

Figure I.

more likely candidates for immunological priming than does a rotifer, especially if we are looking for priming responses within individuals (as opposed to across generations). What is probably crucial, however, is not absolute life span, but life span relative to time between exposures. In addition, although individuals perish, each has an interest in the success of their offspring, and so transgenerational-priming effects might be expected in most invertebrates. This might be particularly relevant for clonal or selfing organisms, which cannot produce genetically diverse offspring that have novel abilities to resist pathogens that have adapted to exploit the parental generation.

All of these conditions will be subsumed by ecological considerations. For organisms that occupy a time and place where secondary encounters are likely to both occur and have an impact on fitness, there should be selection for mechanisms that reduce the impact of secondary exposure. Thus, taxa that colonize new, biologically impoverished habitats might not be ideal candidates for immunological priming. For example, pioneer species of copepod invading recently deglaciated and parasite-free arctic habitats [12] are unlikely candidates for selection on immunological priming, whereas the relatively long-lived octopus living on an established coral reef is a prime candidate.

Another important consideration for determining the conditions under which immunological priming should evolve is that of costs. For example, there is likely to be an energy-related cost of an upregulated immune system. Studies have shown a fitness cost for both having a potentially effective immune system [13], and for its initial upregulation [14]. For studying costs of immunological priming, it will be necessary to determine both the cost of initial upregulation and the additional cost of keeping it upregulated for an extended period of time. This second cost is the crucial one and should be compared with the cost of upregulating repeatedly, from the ground up, with each new challenge. The speed of reaction is also an issue; even if it is costly to maintain an upregulated immune system, this might pay for itself if it is crucial that a harmful pathogen be eliminated as quickly as possible. It could be, however, that there is little cost associated with keeping immune systems upregulated, if immune proteins, such as antimicrobial peptides, are relatively stable. Similarly, immunological priming could also be attributable to the cellular immune system and, following the rapid proliferation of haemocytes that many taxa show after injury or parasitic attack, it could be relatively inexpensive to keep haemocyte populations high, given that they have already been boosted. Again, this depends on the shelf life of haemocytes.

Once in place, how will priming effects influence the population dynamics of invertebrates? This will need to be modeled, but we predict that priming effects will dampen the amplitude of cyclical dynamics. If a host population is, through experience, becoming increasingly resistant to pathogens, this should lessen pathogen- or parasite-mediated effects on population sizes. This might become important for predictions regarding the severity and

persistence of epidemics. The most interesting implications for host–parasite (or pathogen) dynamics could occur in systems that incorporate genetic specificity, and where past challenges will lead hosts to be increasingly resistant to dominant pathogen strains. For example, the Red Queen Hypothesis proposes that parasitic interactions select for recombination, and rests on the notion that the most successful parasite strains will be those that can infect common host genotypes. The resulting proliferation of parasites that can infect common host genotypes should result in the demise of those hosts, but strain-specific immunological priming could put a brake on this if experience endows common host genotypes and their offspring with better, specific immune responses. It might be less likely that parasites can select for recombination under these conditions, because frequency-dependent selection will be weaker, but, to gain clarity on this, it will also be necessary to model feedback into parasite genetic dynamics [15].

The functional similarity between vertebrate and invertebrate immune responses might even provide greater generality to experimental studies of model invertebrates. Laboratory microcosms involving even the smallest vertebrate are cumbersome, but many invertebrates lend themselves well to experimental evolution and epidemiology [16]. Thus, this new understanding of invertebrate immunology might open a new and exciting chapter in our ability to estimate the important parameters influencing the dynamics arising from antagonistic interactions.

Until both the mechanisms and consequences of immunological priming in invertebrates have been more deeply explored, it is worth bearing in mind that these phenomena might not be the direct result of natural selection on the immune system. These 'adaptive' responses might simply be caused by stable immune system molecules that linger in the haemolymph following primary immune system stimulation. This is particularly true for studies of single individuals. Immunological priming might just be immunological loitering. Persistent immune reactions might seem adaptive when studied in the laboratory, but it is conceivable that this persistence was not under natural selection, and that, in the wild, secondary encounters with pathogens are inconsequential. However, given that some systems not only show priming, but have also linked general or specific immune responses to a system of epigenetic inheritance [8–10], it becomes somewhat difficult to accept that these phenomena are a coincidental by-product of an innate immune system with no adaptation for secondary encounters.

Conclusion

Both vertebrates and invertebrates exhibit immunological priming. The highly sophisticated priming system of vertebrates (i.e. acquired immunity) is well understood both functionally and mechanistically. So far, invertebrate immunological priming is known only from phenomenological studies of whole organisms, and its mechanistic basis is not known. The genes and enzyme cascades of the invertebrate immune system are rapidly being elucidated [17–20], just not yet for priming effects. Linking functional to phenomenological studies will be exciting indeed, and should foster a new era in the evolutionary ecology and epidemiology of immunity and disease.

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