Immune Defense and Host Life History

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ABSTRACT: Recent interest has focused on immune response in an evolutionary context, with particular attention to disease resistance as a life-history trait, subject to trade-offs against other traits such as reproductive effort. Immune defense has several characteristics that complicate this approach, however; for example, because of the risk of autoimmunity, optimal immune defense is not necessarily maximum immune defense. Two important types of cost associated with immunity in the context of life history are resource costs, those related to the allocation of essential but limited resources, such as energy or nutrients, and option costs, those paid not in the currency of resources but in functional or structural components of the organism. Resource and option costs are likely to apply to different aspects of resistance. Recent investigations into possible trade-offs between reproductive effort, particularly sexual displays, and immunity have suggested interesting functional links between the two. Although all organisms balance the costs of immune defense against the requirements of reproduction, this balance works out differently for males than it does for females, creating sex differences in immune response that in turn are related to ecological factors such as the mating system. We conclude that immune response is indeed costly and that future work would do well to include invertebrates, which have sometimes been neglected in studies of the ecology of immune defense.

Keywords: immunocompetence, life history, parasite resistance.

Until the 1970s, parasites and pathogens were of interest mainly to parasitologists, who focused on descriptions of life cycles, pathology, and control of pests and disease-causing agents. Then, over a span of perhaps 10 years, parasites became interesting to ecologists and evolutionary biologists, many of whom realized that they had neglected an important force in the life history of the more visible host organisms (Price 1980; Grenfell and Dobson 1995; Clayton and Moore 1997). Current topics in evolutionary biology involving parasites include the evolution of virulence, the endosymbiotic relationships discussed by others in this issue, and investigation of the role of parasites in sexual reproduction.

Other research has begun to focus not on the interactions between parasites and their hosts per se but on how host response to infection has shaped the host's life history. This shift has turned the efforts of many researchers to the immune system, the major physiological means by which animals resist disease. If selective pressure from parasites is important, one would expect that the ability to resist disease is a critical component of life history. As a result, a number of studies in the last decade have examined how resistance to disease is associated with fitness, and in turn how investment in disease resistance affects other traits such as survival or reproduction.

For example, Moret and Schmid-Hempel (2000) examined immune defense in bumblebees. If the bees are fed ad lib., they do not show any change in survival when they are mounting an immune response, but when they are starved, two methods of immune system challenge result in sharply reduced survival compared with starved controls. Using a different, comparative approach, Møller et al. (2001) examined the degree of investment in immune parameters such as T and B cell response to challenge in species of swallows varying in sociality; more social species appeared to invest more heavily but also had longer developmental time for nestlings. Such use of immunity as a life-history trait has proved quite productive, and recent interest has therefore focused on immune response in an evolutionary context (Sheldon and Verhulst 1996; Coustau et al. 2000; Norris and Evans 2000).

The basic idea is that fending off disease is advantageous, and therefore we ought to see selection for it. Despite the obvious advantage of being resistant to disease, however, susceptibility is of course rampant. As with many life-history traits, it has seemed logical to conclude that resistance is traded off against the need for investment in other important characters, such as competitive ability or development time (Roff 1992). Researchers have assumed that animals still are vulnerable to disease because being invulnerable, being resistant, is costly and that immune defense can be placed alongside other life-history traits.

It is important to distinguish between a response to being infected and the actual resistance to a pathogen or

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parasite. Upon infection, animals may exhibit many changes in physiology and behavior; they may lose weight, become anemic, develop enlarged or atrophied organs, and so forth. These signs or symptoms of disease may be caused by the action of the disease agent or by the host's effort to defeat the infection. For example, fever, a rise in body temperature following infection, is suggested to be a host response that creates a hostile environment for many pathogenic bacteria and viruses, whereas the diarrhea that accompanies diseases such as cholera may be an adaptation to increase the transmission of the infective agent at the expense of the host (Nesse and Williams 1995).

The distinction is important because if we are to evaluate resistance as a life-history trait, we need to be sure that we are focusing on host immune response. In addition, studies of the ecological and evolutionary significance of resistance usually strive for generality so that they are not limited to a single disease or parasite. As a result, most researchers attempt to isolate the effects of mounting an immune response from the effects of the disease process itself, usually by administering a foreign substance to the animal that will trigger the immune system without causing disease symptoms. Common agents for such tests include injected sheep red blood cells, the plant protein phytohemagglutinin, and bovine serum albumin. Some aspect of immune response is frequently measured after challenge with one of these antigens. On the positive side, the novelty of the substance assures that the experimental subject is responding to it for the first time and that the test is of the immune system itself. On the negative side, however, it is possible that a strong response to a given antigen does not indicate an overall ability to resist actual disease agents.

Our goal here is to examine how resisting disease fits into life history. We will look at the broader context of host defense by looking at resistance to disease as a life-history characteristic, with the usual issues of costs and trade-offs as well as some unique problems. Our intent is not to set up a straw man and attempt to show that immune defense is necessarily unlike traits such as clutch size or age at maturity but to see the ways in which resisting infection poses unique problems for a host. These problems in turn influence how we can study immunity in an ecological or evolutionary context. In addition, the literature on immune defense in life history has been relatively segregated into those studying invertebrates, mainly insects, and those studying vertebrates, mainly birds, with little interaction or consideration of common problems. By using examples from both groups, we hope to encourage a less taxonomically narrow perspective. We begin by briefly reviewing the operation of the immune system.

Basics of Immunity

Although both invertebrates and vertebrates can recognize and attack foreign particles that invade the body, the mechanisms by which they do so differ. Immunity is considered to be either innate (i.e., present regardless of the diseases in the environment of an individual) or acquired (i.e., activated only in response to challenge). Perhaps confusingly for evolutionary biologists, immunologists sometimes refer to acquired immunity as "adaptive immunity," meaning not that it is the result of selection but that it is a facultative response. In vertebrates, the acquired immune system is further divided into two arms, cell mediated and humoral. Invertebrates also have cell-mediated and humoral (sometimes called "inducible") immune responses, but authors differ on whether they possess true acquired immunity. Cell-mediated immunity involves generalized responses to foreign substances and to wounding and includes inflammation, phagocytosis, and the graft versus host response important in transplants (Roitt et al. 1998). In vertebrates, cell-mediated immunity is associated with a type of white blood cell called a T cell or T lymphocyte. Invertebrates do not have the same type of blood cells (hemocytes) as vertebrates and lack true T cells; their cell-mediated immune response consists mainly of the ability to encapsulate and melanize foreign substances such as parasitoid eggs (Gupta 1991).

The humoral immune response is more specific than the cell-mediated response and relies on molecules produced by the host when foreign material is detected in the body. In the case of vertebrates, humoral immunity is the means by which antibodies specific to disease entities are manufactured. After the first time that antibodies are produced, an immunological memory retains the ability to replicate them again if the same antigen is introduced; this is why vaccination works—a weakened version of the pathogen is used to stimulate the primary response by antibodies, and then if the "real" disease is contracted, having the memory makes the response so quick that the host never actually gets sick, at least in principle (Roitt et al. 1998).

Invertebrates by and large lack this immunological memory, although they can produce substances that attack invading bacteria or other particles and therefore possess humoral immunity as well (Gupta 1991; Pathak 1993). They do not generally retain an ability to fight a pathogen to which they have previously been exposed, as do the vertebrates. Nevertheless, the insect humoral immune system is proving to be more complex than previously thought, with more types of antibacterial compounds being discovered each year (Vass and Nappi 2001).

Note that the lack of specific antibodies and the subsequent memory reduces the risk of confounding exposure

with resistance. In other words, if an individual vertebrate is not sick and shows no signs of infection, it is difficult if not impossible to distinguish between it being resistant to the pathogen of concern or simply never having been exposed to it. This distinction is worth bearing in mind for tests of field-derived organisms.

Trade-offs and Costs in Immunity

The study of life histories, and adaptation in general, is motivated by the simple observation that organisms do not do all things perfectly. Given the scrutinizing eye of natural selection, we might expect to see organisms with unlimited fecundity, but of course we do not. This leads to the general hypothesis that adaptation is not free—adaptive changes come with costs. The basis for life-history theory is the idea that this cost arises from the "competitive allocation of resources to growth, maintenance and reproduction" (Reznick 1992, p. 42). These costs are the basis for what are commonly referred to as "trade-offs" in life-history theory. Thus, it is a major goal of life-history studies to understand the nature of these trade-offs because they are key to explaining the basic observation described above (Roff 1992; Stearns 1992).

As important as an understanding of trade-offs and the costs responsible for them is, answers do not come easily. This is because the limited resources responsible for these costs are many and varied and not always easy to identify or measure. Furthermore, although in theory it is clear that the currency of ultimate importance is fitness, fitness is difficult to measure, and so we are forced to make do by measuring life-history costs in other currencies, such as metabolic rate, number of offspring fledged, or age at maturity, in the hope that they are reasonable estimates of true fitness costs. Our studies proceed based on our hypotheses about what resources are limited in a given biological system and how the allocation of these resources will affect the various components of fitness.

Costs (or constraints) in life-history theory are usually divided into two basic categories, internal costs and external costs. Internal costs include genetic and physiological trade-offs as well as mechanical and phylogenetic constraints (Roff 1992). Currently, focus on the life-history costs of immune defense concentrates on the genetic and physiological costs. Most often, we think of physiological costs as arising from limited energy, protein, nutrients, and so forth. External or ecological costs, in contrast, arise from things such as increased risk of predation due to conspicuous reproductive behaviors or increased risk of disease due to mating (Roff 1992; Stearns 1992). Although categorizing costs as either physiological or ecological may be useful, in some ways it is a false dichotomy. Increased disease susceptibility, for example, may be one of the costs of reproduction because reproduction affects both the likelihood of exposure to disease (e.g., increased risk of contracting a sexually transmitted disease—an ecological cost) and the ability to fight disease (e.g., by reducing resistance—a physiological cost).

Our interest in immunity and its relationship to the evolution of life histories stems from another basic observation mentioned above: in spite of what is almost certainly a strong and constant selection pressure—damage and mortality from parasites and pathogens—no organism is entirely resistant to all of the parasites and pathogens that may infect it. Why, after such long periods of strong selection, have host immune systems not eliminated them once and for all? Rapid and cyclical host-parasite coevolutionary arms races have been offered as one possible explanation for this basic observation (Thompson 1994). An additional, though not necessarily mutually exclusive, explanation is that resistance to disease comes at a cost, a cost that arises from the same things responsible for the costs and trade-offs addressed by life-history theory (Sheldon and Verhulst 1996). This hypothesis suggests that an understanding of the evolution of disease resistance might come from an understanding of basic life-history trade-offs because they will share many features. These "features" will be partly theoretical; that is, many of the ideas from life-history theory should apply to the evolution of disease resistance. In addition, however, we expect the evolution of life histories and of disease resistance to be linked biologically. Fighting disease requires resources, and these resources may be the same ones required by other components of fitness such as growth and reproduction.

The hypothesis of "costs of immunity" suggests that optimal immune defense is not necessarily maximum immune defense (Sheldon and Verhulst 1996; Råberg et al. 1998; Westneat and Birkhead 1998). If we assume that disease is not beneficial to the host, why would maximum defense not be optimal? This is the key to understanding the costs of immunity. That is, what are the negative effects, on fitness, of a maximal (or substantial) immune defense? The literature suggests that costs of immunity generally fall under one or more of three nonmutually exclusive categories. One type of cost of resistance is the risk of immunopathology that might result from maximal immune response. Examples of possible immunopathological effects of maximal immune response include hypersensitivity reactions and autoimmunity in vertebrates (Roitt et al. 1998), as well as possible damage to sperm cells (Hillgarth et al. 1997) and developing embryos (Wegmann et al. 1993). In insects, the encapsulation of parasites results in the production of potentially toxic compounds such as quinones (Nappi and Vass 1993). Råberg et al. (1998) suggest that the risk of immunopathology may be particularly high during times of stress. In fact, the reduced

immunocompetence often associated with reproduction (or other forms of stress) might be a result not of the allocation of limited resources away from defense but, rather, an adaptive response to an increased risk of immunopathology (Råberg et al. 1998).

The second type of cost associated with immunity in the context of life history is also the one that has received the most attention and is related to the allocation of essential but limited resources, such as energy, protein, or nutrients. We refer to such costs, that is, those costs paid in the currency of resources, as "resource costs." Studies examining changes in metabolic rate after immune system challenge are examples of those measuring resource costs. A third type of cost is that which is paid not in the currency of resources but in options, that is, an "option cost" (not to be confused with a similar use of the term in economic theory). An option cost would arise, for example, if newly evolved resistance to a particular pathogen was the result of the alteration of a receptor that allowed the recognition of the pathogen as nonself but altered the host's ability to recognize another pathogen as nonself. A functional change in a protein that allowed its use in immune defense but prevented its use in other aspects of the host's biology would also be an option cost. Again, it is important to recognize that resource and option costs are not two entirely separate costs. For example, given unlimited resources, we might expect an organism to have all the functional protein types (or receptor types) needed to perform its biological duties. Recognition that resource and option costs are not identical, however, is important because these cost types will lead to different predictions about the evolution of disease resistance and life histories (Janzen 1981). Coustau et al. (2000) suggest that if resistance (in their example, to xenobiotics such as pesticides) is the result of a single major mutation, it should be associated with a physiological cost and subject to counterselection in the absence of the xenobiotic; eventually, however, the cost should be reduced or eliminated with the accumulation of modifier mutations. This eventual elimination of a cost of resistance seems less likely if the cost is based on the adaptive allocation of resources.

Resource costs and option costs are likely to apply to different aspects of resistance. Fully effective resistance to a parasite or pathogen requires not only the successful recognition of the invader as foreign but also the successful elimination (or disablement) of the invader if the host is to reduce or eliminate the detrimental effects of infection. Recognition costs are likely to be primarily option costs, whereas the active process of parasite elimination may incur resource costs.

The two costs should interact to influence the evolution of life histories. Consider the relationship between *Drosophila melanogaster* and two of its hymenopteran parasitoids. The ability to encapsulate the eggs of both *Leptopilina boulardi* and *Asobara tabida* varies widely across the geographical distribution of *Drosophila* (Kraaijeveld and van Alphen 1995). Although the basic process of encapsulation is, presumably, very similar regardless of the parasitoid species, there is no correlation across populations between the ability to encapsulate one species of parasitoid and the other (Kraaijeveld and van Alphen 1995). Furthermore, *Drosophila* selected for increased encapsulation of *A. tabida* are not better at encapsulating *L. boulardi* (Fellowes et al. 1999). However, the converse is not true: lines selected for increased resistance to *L. boulardi* do encapsulate *A. tabida* better than less resistant lines (Fellowes et al. 1999). Fellowes et al. (1999) suggest that these results are due to an increase in general encapsulation ability combined with a specific response to some aspects of *Leptopilina*'s ability to resist encapsulation. Thus, ecological conditions that determine parasitoid diversity and prevalence will interact with the conditions affecting larval competitive environment to shape *Drosophila* immunity.

These two aspects of costs (diversity of parasites being most related to option costs, elimination of parasites being resource costly) are addressed in a model by Jokela et al. (2000). They argue that as the diversity of attack increases, investment in defense becomes less effective—given certain trade-offs between immune function and other important tasks—and therefore investment in defense decreases (fig. 1). According to this notion, when effectiveness (α) is low, the optimal strategy is to tolerate damage. As effectiveness increases, the optimal allocation flips rapidly from no defense (tolerance) to high allocation to defense and then decreases at a decelerating pace (Jokela et al. 2000). Full understanding of the relationship between resistance, costs, and life histories depends critically on knowledge of the mechanisms, from the molecular to the physiological, responsible for resistance and its potential costs (Coustau et al. 2000).

Note that the "cost of immunity," in its broadest sense, encompasses many things. There may be the costs of having an immune system at all, or of having specific kinds of resistance (i.e., innate or acquired). Furthermore, the cost of having the potential to mount a successful immune response (of being "potentially resistant") to specific parasites or pathogens and the cost of actually mounting an immune response in the face of antigenic challenge may not be identical. These different aspects of immune costs may be related, but because they are not identical they must be addressed in different ways. Costs of actually mounting an immune response are, obviously, absent if the relevant parasites are absent and will be paid only as often as the parasites are encountered. Such inducible defenses are expected to evolve whenever there are significant

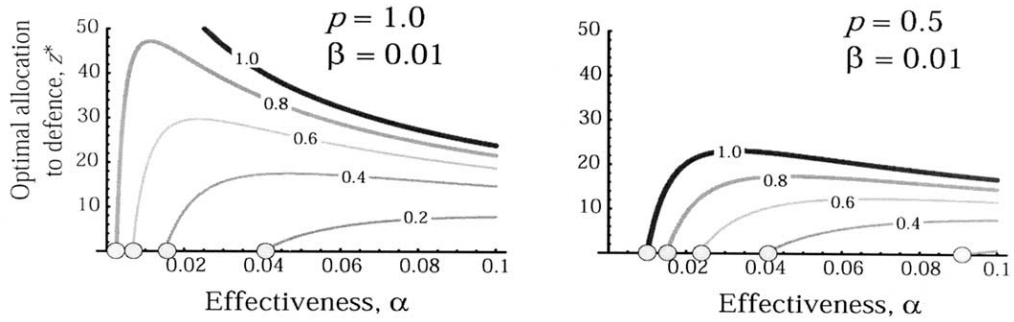

Figure 1: Relationship between the diversity of attack by pathogens and investment in defense by the host. As effectiveness (α) increases, the optimal allocation (shown on the *Y*-axis) flips rapidly from no defense (tolerance) to high allocation to defense and then decreases at a decelerating pace as effectiveness increases. β is the cost of defense, and p is the probability of attack, which is different in the two graphs. Each line represents a response given a different cost of defense, shown by the number on the line. Adapted from Jokela et al. 2000.

costs to the defense but the threat is variable and unpredictable (Tollrian and Harvell 1999). However, being potentially resistant may exact costs even in the absence of the parasites. If resistance depends, at least in part, on possessing the machinery necessary to mount a defense should infection occur, then counterselection in the absence of the parasite is likely. This is again well illustrated by the mechanisms of resistance to *A. tabida* in *Drosophila*. Recent work has shown that flies resistant to the parasitoid have more circulating hemocytes (Kraaijeveld et al. 2001). These blood cells are believed to arise from the same precursors responsible for the muscles that make up the chewing apparatus of the larvae (Tepass et al. 1994). If this hypothesis is correct, it could explain why resistant larvae are poor competitors: resources allocated to building hemocytes are made unavailable for building chewing muscles (Kraaijeveld et al. 2001). The point here is that individuals with the potential to successfully encapsulate parasitoids (i.e., those that possess more circulating hemocytes and are thus potentially resistant) pay the price of this superior immune defense even when they are not directly using it. However, if, for example, resistance is conferred through molecular changes that prevent molecular mimicry by parasites (i.e., parasites mimic the host molecules that determine "self"), counterselection is likely to occur only if the change increases susceptibility to another parasite; if this is not the case, or if other parasites are not present, counterselection may not occur (Coustau et al. 2000). Thus, the concept of "costs of resistance" is, like immunocompetence, one that is of limited value unless defined precisely in each case.

These different types of costs not only have important implications for life histories, they must be measured in different ways. First, researchers have taken advantage of mutant lines of organisms with disabled immune systems, including so-called knockout organisms that lack genes for producing particular immune compounds and "nude" mice that lack the lymphocyte-producing thymus gland. Genetic knockouts may be particularly valuable for understanding the mechanisms of resistance. However, as a means for studying life-history costs that arise because of correlations between traits, such knockouts may be less useful because eliminating one component of a complex pathway does not eliminate the entire correlation. Caution must also be exercised if knockout organisms are to be used as proxies for the ancestral state of a complex immune system. Second, one can compare species and/or populations that differ in the types and/or magnitudes of immune defense (Kraaijeveld and Godfray 1999; Fellowes and Godfray 2000; Klein 2000). These studies carry the same difficulties as any comparative tests, but they are promising given the current sophistication of analysis of large groups of taxa, particularly because they also add to the understanding of the diversity of immune defenses.

Most experimental approaches to studying the costs of immune defense fall under one or more of four types. Selected examples of these four approaches are outlined in table 1. A few studies have created or taken advantage of selected lines with differing degrees of investment in immune response (Boots and Begon 1993; Kraaijeveld and Godfray 1997; Verhulst et al. 1999). Less common are selection experiments that measure the correlated response of immunity to selection on other life-history traits; one good example of such a study is that of Hosken (2001). Both of these types of selection experiments are useful because they allow us to address the costs of immunity in the absence of immune challenge.

The costs of mounting an immune response are more amenable to experimentation, and for this reason, much of the recent focus on the costs of immunity has been on

Table 1: Selected examples of the four primary experimental approaches to studying costs of immunocompetence (IC)

Experimental approach and examples	Cost found?[a]	Reference
1. Manipulate some aspect of host biology, measure corresponding changes in IC:		
Zebra finches (*Taeniopygia guttata*) rearing larger broods have reduced IC	Yes	Deerenberg et al. 1997
Collared flycatchers (*Ficedula albicollis*) rearing larger broods have reduced IC	Yes	Nordling et al. 1998
Male barn swallows (*Hirundo rustica*) with elongated tail feathers have reduced IC	Yes	Saino et al. 1997
Relationship between IC and ornamentation (comb size) in male red jungle fowl (*Gallus gallus*) changes with change in social environment, and direction depends on dominance status	Conditional	Zuk and Johnsen 2000
Low-protein diet reduces cell-mediated but not humoral IC in northern bobwhite (*Colinus virginianus*) chicks	Conditional	Lochmiller et al. 1993
Blue tits (*Parus caeruleus*) subjected to cold stress show reduced IC	Yes	Svensson et al. 1998
Female tree swallows (*Tachycineta bicolor*) with clipped flight feathers have reduced IC	Yes	Hasselquist et al. 2001
Predator avoidance behavior (blood expulsion) reduces IC in pond snails (*Lymnaea stagnalis*)	Yes	Rigby and Jokela 2000
Increased sexual activity reduces IC in male fruit flies (*Drosophila melanogaster*)	Yes	McKean and Nunney 2001
Reproductive activity reduces IC in damselflies (*Matrona basilaris japonica*)	Yes	Siva-Jothy et al. 1998
Bumblebees (*Bombus terrestris*) that forage have lower IC than those that do not forage	Yes	König and Schmid-Hempel 1995
Bumblebees (*B. terrestris*) in variable and poor environments have IC similar to that of controls	No	Schmid-Hempel and Schmid-Hempel 1998
2. Challenge host immunologically, measure corresponding changes in other traits (including IC) compared with controls:		
Challenged female blue tits (*P. caeruleus*) feed offspring less often	Yes	Råberg et al. 2000
Challenged female pied flycatchers (*Ficedula hypoleuca*) show reduced offspring feeding rates and fewer and lower-quality offspring	Yes	Ilmonen et al. 2000
Challenged great tits (*Parus major*) have higher metabolic rates and greater weight loss	Yes	Ots et al. 2001
Challenged blue tits (*P. caeruleus*) do not have higher metabolic rates	No	Svensson et al. 1998
Challenge does not affect egg mass, clutch size, or laying interval in European starlings (*Sturnus vulgaris*)	No	Williams et al. 1999
Experimental immune challenge (but not infection by a parasite) reduces IC in bumblebees (*B. terrestris*)	Conditional	Allander and Schmid-Hempel 2000
Challenge alters relationship between parasite levels and IC in gut but not hemocytes in damselflies (*Mnais costalis*)	Conditional	Siva-Jothy et al. 2001
Challenged bumblebees (*B. terrestris*) suffer higher mortality—but only if starved	Conditional	Moret and Schmid-Hempel 2000
Challenged pond snails (*L. stagnalis*) do not show reduced survival but do reduce probability of breeding, number of eggs, and fat reserves; however, response depends upon magnitude of predator avoidance behavior	Conditional	Rigby and Jokela 2000
3. Select for increased IC, measure corresponding changes in other traits:		
More resistant fruit fly (*D. melanogaster*) larvae less competitive under crowding	Yes	Kraaijeveld and Godfray 1997
More resistant Indian meal moths (*Plodia interpunctella*) have lower egg viability, longer development	Yes	Boots and Begon 1993
More resistant male chickens (*Gallus domesticus*) have smaller combs and lower testosterone levels	Yes	Verhulst et al. 1999
4. Select for change in host trait(s), measure corresponding change in IC:		
Yellow dung flies (*Scathopaga stercoraria*) selected under polyandry have larger reproductive organs but lower IC than lines selected under monogamy	Yes	Hosken 2001

Note: This table is not meant to be exhaustive but rather is intended to provide selected examples of the four primary experimental approaches to studying the costs of immune defense. For this reason as well, details of the various measures of immune response used in the different studies are not given; instead, the general term "immunocompetence" (abbreviated IC) is used. Readers are referred to the original references for details. Note also that studies using one immune challenge and then measuring a corresponding change in another measure of immunocompetence are categorized as experimental approach type 2 experiments although they are, by our definitions, a combination of both types 1 and 2.

[a] "Conditional" means that whether costs of immune defense were found depended on the different experimental conditions reported in the study.

this aspect rather than on the cost of maintaining the immune system itself. The experimental approach to addressing the costs of immune response usually takes one of two forms. In some cases, the immune system is challenged with a substance that will elicit a response but is not pathogenic, and the subsequent effects on the traits of interest are measured (growth, clutch size, etc.). The other approach is to manipulate some other aspect of host biology (clutch size, nutritional state, etc.) and measure the resulting effects on immune function. If mounting an immune response is costly, one would predict that in the former approach, an immune challenge will result in negative effects on the traits of interest. The latter approach predicts that those individuals forced to perform costly activities (or deprived of resources) will exhibit reduced immune responses compared with controls.

Evidence of costly immune responses has been found using both approaches in a variety of invertebrate and vertebrate species. Female pied flycatchers (*Ficedula hypoleuca*; Ilmonen et al. 2000) and female blue tits (Råberg et al. 2000) reduce nestling feeding rates if they are challenged immunologically. Male great tits show increased metabolic rates when challenged immunologically (Ots et al. 2001). In the snail *Lymnaea stagnalis*, investment in immune defense reduces reproductive success by reducing the number of eggs laid and the probability of reproduction (Rigby and Jokela 2000). Bumblebees (*Bombus terrestris*) mounting an immune defense show reduced survival compared with controls, provided that they are not allowed access to food (Moret and Schmid-Hempel 2000). Reduced immunity following diet manipulation has been demonstrated in northern bobwhite quail (*Colinus virginianus*), in which a low-protein diet reduces cell-mediated immunity (Lochmiller et al. 1993), and in barn swallows (*Hirundo rustica*), in which a high-protein diet increases cell-mediated immunity (Saino et al. 1997). Experimental increases in brood size have been shown to reduce immunocompetence in adults in zebra finches (*Taeniopygia guttata*; Deerenberg et al. 1997) and in both collared (Nordling et al. 1998) and pied (Moreno et al. 1999) flycatchers. Foraging activity reduces immunocompetence in bumblebees (König and Schmid-Hempel 1995), and sexual activity reduces humoral immune function in male *Drosophila melanogaster* (McKean and Nunney 2001).

While both of these approaches are, in principle, straightforward, they are in practice fraught with their own problems. First, these types of experimental manipulations are rarely able to address the genetic basis of the possible life-history costs of immunity, which is essential to a complete understanding of life-history trade-offs (Reznick 1985). Furthermore, one can rarely measure all fitness-related traits, so any hypothesis of costs must be specific to both the traits of interest and the conditions of the experiment. It might be reasonable to conclude that mounting an immune response does not negatively affect a specific life-history trait, but no single study can address whether an immune response is, in general, costly. The same problem applies to measuring the immune response. Because immunity is a complex trait, we do not necessarily expect all aspects of immunity to respond (i.e., trade off) in identical ways. Various aspects of immune defense may compete among themselves for limited resources.

Unfortunately, measuring all the components of the immune system is difficult to do and thus rarely done. Therefore, overall immunocompetence is rarely, if ever, measured. These problems may explain the failure of several studies to find costs of immunity. For example, in contrast to the studies of birds outlined above, Williams et al. (1999) found no evidence for costs of immune defense when they immunologically challenged female starlings and looked for subsequent effects on clutch size, egg mass, and the interval between egg laying. Likewise, Svensson et al. (1998) did not detect statistically significant increases in metabolic rate in challenged blue tits (*Parus caeruleus*). The effects of immune challenge on survival in bumblebees described above were apparent only under conditions of starvation (Moret and Schmid-Hempel 2000), and diet manipulation did not reduce encapsulation ability in another study of bumblebees (Schmid-Hempel and Schmid-Hempel 1998). The effects of immune challenge in the snails studied by Rigby and Jokela (2000) depended in part on whether the snails had previously performed a costly "predator-avoidance behavior" (expulsion of blood), and even in these cases the heightened immune response did not result in reduced survival.

This is not to say that the search for costs is hopeless, or that studies failing to find them are flawed. Costs may be difficult to detect for a number of reasons; for example, they may be expressed in different currencies in different systems, or they may simply be small and only detectable as they accumulate over a lifetime. We do suggest, however, that given the complex nature of immune defense the term "immunocompetence" is virtually meaningless unless very narrowly and explicitly defined in every case. Individuals are not "highly immunocompetent" in the same sense that they are "very large." We argue for a more limited and, we hope, more meaningful definition of immune response under a given set of circumstances and suggest that the word "immunocompetence" be abandoned except when discussing resistance in a broad hypothetical context. In specific instances, referring to "immune defense against pathogen type *X*" may make more sense than a blanket assumption of strong immune responses under a variety of circumstances.

Even in those cases where we expect life-history costs to resistance and/or immune response because of resource

allocation trade-offs, can we predict which life-history traits are likely to be most affected by the costs of immunity? Because it is difficult to measure all of the possibly important components of fitness in any one study, some understanding of where we might expect costs will certainly help guide our investigations. This sometimes requires knowledge of the physiological mechanisms responsible for both life-history traits and resistance. For example, several studies have demonstrated that immune challenge reduces parental effort in birds. Presumably, both parental effort and immune response require energy in the form of calories, and thus the mechanistic basis of this trade-off may be the allocation of limited energy. This hypothesis predicts elevated basal metabolic rates during immune challenge. This prediction has been tested, but the results are not always consistent. Ots et al. (2001) found that immune-challenged great tits (*Parus major*) had basal metabolic rates about 9% higher than controls, but in blue tits, metabolic rates were not significantly different between immune-challenged and control birds (Svensson et al. 1998), leading the latter authors to conclude that limited energy is not the basis of some immune-defense/life-history trade-offs.

Energy, however, is but one of several possibly limited resources. Proteins and other specific nutrients may also form the basis for such trade-offs. Recent investigations into possible trade-offs between reproductive effort, particularly sexual displays, and immunity have suggested interesting functional links between the two. In insects, the primary means of eliminating macroparasites involves encapsulation via the phenoloxidase cascade and subsequent melanization of the encapsulated parasite (Pathak 1993). The same biochemical precursors involved in this defense mechanism are responsible for the dark, melanin-based markings of some insects. Thus, it is possible that simultaneous investment in immune defense and sexual display requires allocation trade-offs. Evidence for this hypothesis comes from several independent studies of damselflies. Male *Calopteryx splendens* possess dark melanin-based wing spots that may function in female choice (Siva-Jothy 1999) and, in a related species (*Hetaerina americana*), function in male-male competition (Grether 1996). In southern France, male *C. splendens* with lighter, less homogenous wing pigmentation were less resistant to eugregarine parasites and showed a greater increase in prophenoloxidase activity in response to immune challenge with nylon implants but no difference in the encapsulation of the implant (Siva-Jothy 2000). However, in a Finnish population of *C. splendens*, males with larger and more symmetrical wing spots were better able to encapsulate nylon filaments (Rantala et al. 2000). The reasons for the differences between these studies are not clear, but both studies suggest a possible and intriguing mechanistic link between sexual display and immune function in these insects.

Similar ideas might also apply to the carotenoid-based sexual displays of many vertebrates, particularly birds and fish (Olson and Owens 1998). Carotenoids cannot be synthesized by these organisms and must be ingested in the diet. Some evidence also suggests that carotenoids may function to enhance immune defense, leading to the hypothesis that carotenoid-based sexual displays may signal immunocompetence (Lozano 1994), much like the melanin-based displays of insects. Both of these hypotheses require detailed knowledge of the physiology of both display and immune response, and until such knowledge is complete, the assumptions and predictions of these hypotheses will remain controversial (Olson and Owens 1998; Hill 1999).

Intergenerational life-history trade-offs, which have received less attention than they deserve (Stearns 1989), may also result from balancing the costs and benefits of immunocompetence. For example, the compounds responsible for immune defense and sexual displays in insects described above are also responsible for the tanning of the egg chorion in some insect species (Li and Christensen 1993). Assuming that this affects the strength and/or disease resistance of these eggs, we might expect to find relationships between adult immunocompetence, or even sexual display, and egg viability. Indeed, Boots and Begon (1993, 1995) found significant correlations between resistance to a granulosis virus and egg viability in Indian meal moths. The correlation was negative in an experiment in which moths were selected for resistance (Boots and Begon 1993) but positive in a study involving different strains that varied in resistance but had not been specifically selected for resistance (Boots and Begon 1995). The inconsistency of these results and the lack of a clear mechanism for the relationship between resistance and egg viability, however, make firm conclusions premature.

Like melanins, carotenoids may link resistance to intergenerational trade-offs. Carotenoids function as important antioxidants in bird eggs and are important for nestling health (Blount et al. 2000). Similar functions in insects might explain why, for example, the spermatophores of male bushcrickets contain carotenoids (Heller et al. 2000). The precise roles of adult-derived resources such as melanins and carotenoids in the potential trade-off between adult and offspring health and survival are unknown, but the research to date suggests that simply counting offspring numbers when examining such trade-offs is likely to miss part of the picture.

Where our knowledge of the physiological mechanisms that might result in trade-offs is lacking, perhaps a fishing trip is in order. The study of life histories has produced, especially in *Drosophila*, a multitude of lines selected for

virtually every conceivable life-history variant. Techniques are available that allow the measurement of both humoral and cell-mediated immunocompetence in insects, and lines lacking components of the immune system are available. It would be interesting to examine lines of *Drosophila* for genetic correlations between aspects of their life histories and their immune defenses. The resulting discoveries might inspire the search for the mechanistic bases for the trade-offs (see, e.g., Kraaijeveld et al. 2001) as well as the search for similar trade-offs in other organisms.

Finally, even when costs are demonstrated, it may be difficult to see where selection is acting. In a study of the effects of mounting an antibody response, Demas et al. (1997) found that laboratory mice had significantly higher metabolic rates when they were immunized with keyhole limpet antigen. The difference was not simply due to a rise in body temperature and appeared to reflect the actual cost of producing the antibodies. Nelson et al. (1998) suggest that selection should help animals compensate for times of upcoming stress by increasing immune response just before those times—"winter's coming, better up-regulate the immune system." Indeed, immune function seems to be enhanced before winter comes or when days grow shorter, at least in the mammals from temperate regions that have been tested (Nelson et al. 1998). This interpretation, however, is only one possibility (fig. 2). What if the immune system is at baseline not during reproduction but during nonreproductive periods, and is reduced during breeding because of the cost of immunity? Alternatively, immune response could be enhanced to counteract the stressful effects of winter, the nonreproductive period, with a baseline during reproduction. Without understanding which of these models is correct, it is difficult to see how selection has influenced immune response in the context of other demands of life history.

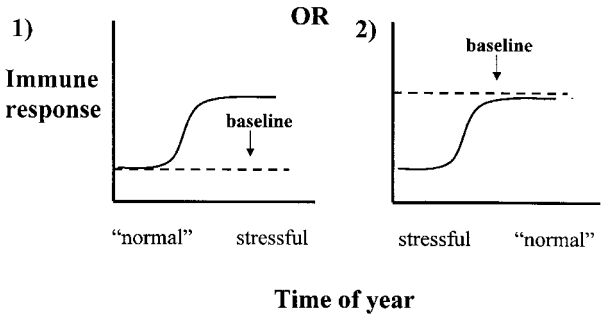

Figure 2: Two possible scenarios for the regulation of immune defense during the course of a breeding cycle. *1*, Immune defense is up-regulated during a time of stress, such as winter for temperate climate organisms. *2*, Reproduction is considered to be the stressful period, and immune defense is down-regulated because of competing needs, such as sexual competition. Both patterns appear the same, but selection acts in different ways in each.

Sex Differences, Life History, and Immunity

Some life-history components, such as clutch size, are obviously sex limited, while others, such as body size at maturity, have clearly different consequences for members of either sex. Immune defense may seem at the outset to be an exception, since both males and females are expected to benefit from resisting disease. However, although all organisms balance the costs of immune defense against the requirements of reproduction, this balance works out differently for males than it does for females. Although obvious exceptions occur, male fitness is limited by the number of mates fertilized, while female fitness is limited by the number of offspring produced and (where relevant) reared (Trivers 1972). Males are thus generally expected to have a "live hard, die young" strategy, at least where polygyny is common and where males that take large risks may also accrue large gains. Selection for investment in reproduction at the expense of disease resistance may therefore be more pronounced in males than females, particularly so in species where male reproductive competition is extreme and males invest relatively little in offspring. Where male and female investment is relatively equal, the difference between the sexes in immune response/disease susceptibility should be slight (Zuk 1990; Zuk and McKean 1996). We can think of this as a continuum, so that the mating system, or intensity of sexual selection, is expected to reflect the ability of the sexes to defend themselves against pathogens.

Among many vertebrates, males tend to suffer more from parasitic infections and to have reduced immune responses compared with females (Poulin 1996; Zuk and McKean 1996). On a proximate level, these differences have been attributed either to ecological differences between the sexes (males and females are exposed to different pathogens because of what they do or where they go) or to the generally immunosuppressive effects of testosterone, the major male sex hormone (Alexander and Stimson 1988; Schuurs and Verheul 1990; Zuk 1990). For example, females of a variety of mammal species display higher immunoglobulin concentrations than males and can also respond more vigorously to antigenic challenge (Schuurs and Verheul 1990; Klein 2000). On an ultimate level, however, and especially from a life-history perspective, has differential selection on the sexes favored different investment levels in disease resistance? Do species differing in mating system also differ in how the sexes resist infection?

Studies of these questions in vertebrates have focused on the endocrine mediation of the interaction between

reproduction and immunity. They have also rarely compared male and female immune responses either within or among species. One noteworthy exception is the research of Klein and colleagues (Klein et al. 1997; Klein and Nelson 1998, 1999*a*, 1999*b*; Klein 2000) examining sex differences in immune function of voles in the genus *Microtus*. These rodents are useful for addressing questions about the relationship between mating system and immunity because the species differ in mating system but remain similar in most other aspects of life history. Individually housed voles show no consistent patterns of sex difference in immunity, but when housed with conspecifics, polygynous voles show a more pronounced sex difference in both cell-mediated and humoral immune responses than do monogamous species (Klein 2000). In other rodents, polygynous males appear to be more susceptible to infection than conspecific females (Klein 2000). Nevertheless, the relationship among hormone levels, time of year, and social environment is not straightforward, and it has been difficult to generate predictions involving all of these variables and their effect on disease resistance.

The situation with invertebrates has been somewhat more illuminating. Although a study of four species of damselfly revealed no sex differences in immune response (Yourth et al. 2001), two species of crickets showed the expected sexual dimorphism, with males being more susceptible than females to infection with the bacteria *Serratia liquefaciens* (Gray 1998; Adamo et al. 2001). Similarly, females of the scorpion fly *Panorpa vulgaris* were better able to phagocytose injected particles and had higher levels of antibacterial compounds than males (Kurtz et al. 2000).

To examine the effect of mating effort on immune response, McKean and Nunney (2001) did not use different species varying in mating system but instead subjected male *Drosophila melanogaster* to different social conditions. Virgin males were housed either alone or with one or four virgin females. The idea was to see whether the flies invested less in immune response when they were performing the demanding tasks of courtship and mating. To measure humoral immunity, the flies were injected with a strain of *Escherichia coli*, which carries chromosomal resistance genes to both ampicillin and streptomycin. The rate of clearance of the bacteria from the *Drosophila* was then quantified by homogenizing each individual 4 d after injection and then plating the solution on agar containing streptomycin. The plates were then scored for the number of *E. coli* colonies; if the flies mount a vigorous immune response, they produce antibacterial compounds that make the *E. coli* unable to multiply, and hence flies with better immunity produce fewer colonies. As predicted, males housed with no females were able to clear substantially more bacteria than those housed with one female, which in turn had more robust immune responses than those kept with four females.

But what if it is just the presence of other flies, rather than sexual activity per se, that caused this result? If mere social proximity results in reduced immune function, then males kept with females should have rates of clearance similar to males housed with other males. Contrary to this hypothesis, males with females exhibited reduced immune function compared with males housed with other males (McKean and Nunney 2001). Although males housed with nonvirgin females mated far less often than those kept with virgins, there was no evidence that food availability (at least across the nonstarvation levels studied), female mating status (virgin or nonvirgin), or their interaction had any significant effect on male clearance rates. Immune suppression probably results either from increased courtship activity or (perhaps more likely) as a consequence of increases in both mating and courtship. Such a direct phenotypic trade-off between male sexual activity and the humoral immune response supports the view that immune system function and levels of disease susceptibility are traits shaped by trade-offs with other costly fitness components.

Conclusions and Future Directions

Two recent reviews concerning the costs of immunity to vertebrates in general (Lochmiller and Deerenberg 2000) and specifically in birds (Norris and Evans 2000) both concluded that evidence supports the notion that immunity is costly. Lochmiller and Deerenberg (2000) go so far as to argue that the interaction between host environment (nutritional and pathogenic) and immunity may be the most important factor shaping host life history. Norris and Evans (2000) are more conservative in their conclusions and suggest that more research is needed before the broad generality of the "costly immunity" hypothesis can be determined. Norris and Evans (2000) point out, in particular, the lack of studies addressing the relationship between immunocompetence and fitness in wild populations.

Our own view is more similar to that of Lochmiller and Deerenberg (2000); the evidence to date suggests, to us, that the hypothesis that immune defense is costly and is therefore likely to shape life histories has broad generality. Significant and substantial costs of immunity have been demonstrated in a variety of organisms, both vertebrates and invertebrates. Furthermore, the literature suggests that costs are found more often than they are not. However, it is also apparent that the problem is complex. The nature of immunity–life-history trade-offs appears to depend on a wide variety of factors including, but probably not limited to, the host species and/or population involved, the

type of immune challenge, the component of immunocompetence measured, the life-history trait of interest, the nutritional condition of the subjects, and whether the study was conducted on wild or captive animals (which is likely to influence all the previous factors). Thus, we agree with Norris and Evans (2000) that many gaps remain.

Filling these gaps will require a multipronged approach. We encourage evolutionary ecologists interested in these questions to examine as many life-history traits as possible and under a variety of conditions—it is not entirely clear which traits we expect to compete most directly with immune defense, and under what conditions. Furthermore, it is essential that as many components of the immune system are examined as is possible, for not only may these trade off with other traits in different ways, they are likely to be related in complex and unpredictable ways. In particular, selection experiments can provide a greater understanding of the genetic constraints underlying immunity–life-history trade-offs. Because such experiments are costly and time consuming, we encourage researchers to take advantage of selection lines already in existence (see, e.g., Verhulst et al. 1999). Given the generally simpler immune systems of insects and the relative ease with which they can be experimentally manipulated and monitored over multiple generations, we think the most promising research will use invertebrates as subjects.

How important is it to understand the details of the physiological mechanisms involved in immune system function and in the trade-offs between immune defense and other traits? Interest in proximate causation has ebbed and flowed in evolutionary ecology; at the moment enthusiasm seems to be relatively high (Drickamer 1998; Drickamer and Gillie 1998). We suggest that any rush for evolutionary biologists to delve into the details of immunology be tempered by a consideration of why knowledge of the mechanisms might be important. Sometimes understanding the mechanism leads to insights otherwise unobtainable. For example, the underlying mechanism linking melanin production to both immune function and wing spot formation in insects provides a hitherto unrecognized link between sexual selection and disease resistance (Siva-Jothy 2000). However, in trying to understand whether selection is up- or down-regulating immune defense as described in figure 2, knowledge of which classes of cells are increasing and which are decreasing is not necessarily very helpful.

Finally, it is essential that we examine the costs of immunity in the ecological contexts in which they are most important. This means that we must consider not only the diversity and prevalence of parasites encountered by the hosts but also the other ecological factors that will influence how the costs and benefits of immune defense are balanced. Host density, for example, may influence investment in immune defense not only because it may increase the probability of infection (Reeson et al. 1998; Barnes and Siva-Jothy 2000) but also because it may increase intraspecific competition (Kraaijeveld and Godfray 1999; Svensson et al. 2001). Furthermore, the vast majority of organisms are at the same time both prey and host to their respective predators and parasites. Thus, they must balance the "life-dinner" asymmetry (Dawkins and Krebs 1979) in both directions simultaneously—if one fails to successfully evade a predator, the result is fatal, whereas the result of failing to successfully defeat a parasite may not be. But of course, from the perspective of most parasites, failure to establish in a host is invariably fatal. Most organisms, then, are imposing strong selection on their own parasites from below and, at the same time, responding to selection pressures from their predators from above. When the adaptations that result from such interspecific dynamics are costly, balancing the costs and benefits of immune defense with other defenses becomes particularly interesting (Rigby and Jokela 2000).

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