Linking immune defenses and life history at the levels of the individual and the species

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Synopsis Immune defenses have been suggested to play an important role in mediating life history trade-offs. Detecting and understanding such trade-offs, however, is complicated by the complexity of the immune system. The measurement of multiple immune indices in studies of "eco-immunology" has only recently become more common, but has great potential for furthering an understanding of the ecological and evolutionary forces driving immunological variation. Building on previous proposals, I create a framework integrating immunological and life history axes that can be used to formulate predictions and interpret variation in multiple types of immune defense at both the individual and species levels in vertebrates. In particular, this framework predicts that "fast-living" species (those with high reproductive and low survival rates) should rely more heavily on nonspecific and inflammatory immune defenses, while "slow-living" species should exhibit stronger specific and especially antibody-mediated immunity. At the level of individuals within species, nonspecific and inflammatory responses should be downregulated, and specific defenses upregulated (1) in individuals experiencing the greatest demands on their resources (for example, undertaking large reproductive efforts); (2) in the sex investing more in a particular activity (for example, females during reproduction); and (3) during the most demanding periods of the year (for example, the breeding season). A review of the literature reveals that incorporating multiple facets of the immune system into a model of the relationship between immune defense and life histories brings disparate questions and systems into a common context, and helps explain empirical results that are sometimes counterintuitive.

Introduction

The place of immunology in ecological research has become well established over the past decade, and many new insights have emerged from "eco-immunology" (Sheldon and Verhulst 1996; Norris and Evans 2000; Zuk and Stoehr 2002). There are still important gaps, however, in our understanding of immunological variation and how it relates to life history. Part of the difficulty of integrating immunology and life history into a single cohesive theory could be due to the simplified view of "immuno-competence" with which the field began (Norris and Evans 2000; Zuk and Stoehr 2002), as well as the (until recently) very limited techniques available for measuring immunological parameters in nonmodel species. Here, I suggest a comprehensive framework integrating vertebrate immunology and life history, building on ideas proposed by Klasing and Leshchinsky (1999). Within this context I explicitly consider how patterns in ecological, individual-level variation in immunity might differ from evolutionary, species-level patterns. I then summarize empirical studies describing relationships between different components of the immune system and life history traits at different scales. Overall, the goals of this paper are to provide a model for understanding variation in immune defenses at multiple levels, assess the state of our understanding of such relationships and highlight the importance of considering multiple aspects of immunity in ecological studies.

The idea of trade-offs in ecological immunology

The central assumption underlying almost all ecological immunology is that trade-offs exist between immune defenses and other functions or activities that share common resources and contribute to an animal's fitness (Sheldon and Verhulst 1996; Zuk and Stoehr 2002). Among these other functions are prominently reproduction, growth and development. Negative correlations between the magnitude of a single immune response and one of these life history components have been reported by several authors (e.g., Deerenberg and others 1997; Ardia 2005; Mauck and others 2005). There have also been studies, however, that report no correlation, or even a positive correlation between what were expected to be competing functions (Birkhead

and others 1998; Apanius and Nisbet 2006). How can these discordant results be explained?

There are many potential causes of negative or positive correlations, or lack of correlation, between an immune parameter and a life history trait. If 2 processes do not share important resources, or if resources are not limiting, trade-offs might not be found (Moret and Schmid-Hempel 2000; Sandland and Minchella 2003). Alternatively, a trade-off might exist but does not involve the immune parameter being measured. Because the immune system is made up of a large number of interrelated components, unexpected negative or positive correlations might arise when a single immune measure provides only one piece of a larger picture (Norris and Evans 2000; Zuk and Stoehr 2002). Taking into account relationships or trade-offs between potentially competing or cross-regulated defense mechanisms, each with different inherent costs, could uncover adaptive shifts of emphasis or resource use within the immune system (Braude and others 1999; Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003; Martin and others 2006a), and perhaps help explain seemingly contradictory results. Such an approach increases the probability of detecting a trade-off if one exists, and does not require observing wholesale immunosuppression in the face of extreme conditions to draw conclusions about relationships between fitness-related traits.

The measurement of multiple immunological parameters has recently begun to be more prevalent in vertebrate ecological immunology (Martin, Hasselquist and others 2006; Matson and others 2006; Mendes and others 2006), but it has a long history in studies of domestic and laboratory animals. Therefore, although I include much of the available data from recent ecologically oriented studies in this review, I also draw from the veterinary and biomedical literature.

Components of the immune system: A brief overview of costs and benefits

Understanding how different types of immune defense might vary with life history requires knowledge of the costs and benefits of defense components. Below is a brief general summary of these costs and benefits in general terms that are relevant to an immunology-life history framework (also see Table 1).

The innate immune system
Constitutive innate immune defenses
Constitutive components of the vertebrate nonspecific, or innate, immune system include macrophages, granulocytes, natural killer (NK) cells, and complement, lysozyme, defensins and other antimicrobial proteins. These cells and proteins are constitutively present at low levels in the blood and provide rapid first-line defenses (Janeway and others 1999). Macrophages and granulocytes ingest pathogens, produce reactive oxygen species and produce cytokines that recruit additional white blood cells and help organize induced immune responses. NK cells recognize and destroy infected or abnormal host cells. Complement proteins form complexes that lyse pathogens, or tag them for recognition by antibodies and phagocytic cells (Janeway and others 1999). The costs of constitutive innate immunity have not been definitively measured, but are thought to be comparatively low, because of the lack of a diversification process such as that required for lymphocyte development, low rates of cell turnover when an immune response is not being mounted, and the small tissue mass accounted for by constitutive innate cells and proteins (Klasing and Leshchinsky 1999).

Induced innate defenses
Constitutive components of the innate immune system can induce local inflammation via the production of inflammatory cytokines, and, if the challenge is sufficiently strong, the highly costly systemic inflammatory response. The systemic inflammatory response is characterized by increased production of acute phase proteins by the liver, changes in energy and nutrient metabolism, decreased locomotor and social activities, anorexia and fever (Hart 1988; Klasing and Leshchinsky 1999). These large-scale behavioral and physiological changes can speed recovery by enhancing some immune defenses and restricting access of pathogens to limiting nutrients (Hart 1988), but they are also highly costly to the host in terms of energy and nutrients and potential autoimmune damage (Klasing and Leshchinsky 1999).

The adaptive immune system
Induced adaptive immune defenses
The vertebrate adaptive immune system is generally divided into cell-mediated and humoral components. The major effectors of cell-mediated immunity, class one T-helper cells (Th1) and cytotoxic T-lymphocytes (CTL), can recognize and destroy infected host cells, and so cell-mediated immunity primarily defends against intracellular pathogens such as viruses (Janeway and others 1999). A subset of T-cells can also retain memory of past pathogen encounters, which allows more rapid responses to subsequent exposures (Janeway and others 1999). However, similar to induced innate immunity, cell-mediated responses are

Table 1 Immune defense costs and benefits, and common assays

	Functions	Relative costs	Assays	Citations
Innate defenses				
Constitutive: Phagocytic cells, granulocytes, NK cells, complement proteins, antimicrobial proteins	First line of defense: ingest and destroy pathogens; destroy infected host cells (NK cells); opsonize pathogens	Developmental, maintenance and use costs thought to be low	Differential cell counts; phagocytosis; complement activity; NK cell activity; whole blood bacterial killing	Sanderson (1981); Sandgren and others (1991); Luster and others (1992); Matson and others (2005); Tieleman and others (2005); Millet and others (2006)
Induced: local and systemic inflammatory responses	Increase rates of many immunological processes, sequester nutrients from pathogens	Developmental cost: low Use cost: very high	Following challenge (for example, LPS), measurement of APP, fever, sickness behavior, cytokines	Adler and others (2001); Bonneaud and others (2003); Leshchinsky and Klasing (2001a); Corris and Kirby (2005)
Adaptive defenses				
Constitutive: Natural antibodies	First line of defense: opsonize or neutralize pathogens	Developmental cost: thought to be low Maintenance and use costs: low	Hemagglutination assay	Matson and others (2005)
Induced: Cell-mediated responses (Th1)	Kill infected host cells; memory of intracellular pathogens	Developmental cost: high Use cost: medium to high	*In vivo*: PHA-induced swelling*; DTH (T-cell memory) *In vitro*: mitogenic proliferation (PHA, PWM†, ConA)	McCorkle and others (1980); Martin and others (2006d); Leshchinsky and Klasing (2001b); Fairbrother and others (2004)
Induced: B-cell, Th2 and antibody responses	Immunological memory of pathogens; neutralize or opsonize pathogens	Developmental cost: high Use cost: low	*In vivo*: IgM and IgG titers measured after primary and secondary exposure *In vitro*: mitogenic proliferation (LPS, PWM†)	Grasman (2002); Leshchinsky and Klasing (2001b); Fairbrother and others (2004)

NK cells, natural killer cells; Th1, type 1 T helper cells; Th2, type 2 T helper cells; LPS, lipopolysaccharide; APP, acute phase proteins; PHA, phytohemagglutinin; DTH, delayed-type hypersensitivity; PWM, pokeweed mitogen; ConA, concanavalin A; Ig, immunoglobulin.
*The in vivo response to PHA involves T-cells, granulocytes and inflammation, and so it is a combination of cell-mediated and innate immune responses.
†PWM is both a T-cell and B-cell mitogen.

accompanied by the secretion of proinflammatory cytokines, and are sometimes associated with the energetically and nutritionally expensive systemic inflammatory response (Halloran and others 1992; Janeway and others 1999). Additionally the rapid expansion of T-cells during development and later diversification likely require substantial time and nutrients (George and Ritter 1996; Butler and others 2006).

The effectors of the humoral component of the adaptive immune system are B-cells and type 2 T-helper cells (Th2 cells). Pathogens are recognized by B-cells, and Th2-cells stimulate B-cells to differentiate and produce antibodies [immunoglobulins (Ig)], which then bind and neutralize pathogens or mark them for phagocytosis (Janeway and others 1999). A major benefit of humoral immunity is the ability to recognize a wide variety of extracellular parasites and pathogens and to store this recognition in the form of immunological memory. The costs of using humoral responses are thought to be small compared with those of innate and cell-mediated defences, because humoral immunity is associated with the production of anti-inflammatory (Th2-type) cytokines (Janeway

and others 1999). In many vertebrates, however, the burst of lymphocyte proliferation and diversification is mostly restricted to the developmental period (McCormack and others 1991; Jasper and others 2003; Butler and others 2006) and requires substantial energy and nutrients during this critical time (Klasing and Leshchinsky 1999).

Constitutive adaptive immunity

Humoral defenses have a constitutive component, circulating nonspecific antibodies (IgM) (Ochsenbein and others 1999; Ochsenbein and Zinkernagel 2000). These constitutive antibodies can provide some immediate protection against a pathogenic attack (Ochsenbein and Zinkernagel 2000), while the induced antibody response requires several days to reach effective levels but is highly specific for a given pathogen (Janeway and others 1999).

Levels of circulating lymphocytes (B-cells and T-cells) in a healthy animal are also in some sense constitutive adaptive immunity, and higher concentrations might increase the rate of pathogen detection by the adaptive immune system. Considering lymphocytes as part of constitutive immune defense is problematic, however, because unlike cells of the innate system that can destroy pathogens, the defensive role of individual B-cells and T-cells relies upon recognition of a pathogen and subsequent stimulation of an induced adaptive immune response. Therefore, circulating lymphocyte concentration might be better considered another measure of investment in induced adaptive responses.

Table 1 summarizes the costs and benefits of different types of immune defense, and lists some of the assays most commonly used to measure them. For a more complete summary of immunological assays that can be used in ecological studies, see Salvante (2006).

Multiple measures of immunity in relation to life history: A theoretical framework

In light of the varying costs of different immune defenses, and the organism's need to protect itself against disease-causing agents, it might be expected that in some situations some types of defenses are favored in general over others, depending on life history traits and life stage (Norris and Evans 2000; Sandland and Minchella 2003). It is unlikely that a small number of predicted patterns will hold in all cases, and differences in pathogen exposure, body size and phylogeny (discussed in more detail below) are all likely to be involved in shaping a species' immune

defenses. However, despite the number of variables that could affect the immune system, there is enough generality in the costs and benefits of different types of immune defenses across taxa for a common heuristic model to be useful as a starting point for understanding broad patterns. The following predictions begin with the simplifying assumptions that all species face similar classes of pathogens (for example, viruses, bacteria and macroparasites) and a similar frequency of challenge; these are followed by a discussion of the consequences of relaxing these assumptions.

1. Immunological variation at the population or species scale

A hypothetical model relating life history traits to immune defense profile, based in part on that of Klasing and Leshchinksky (1999), is outlined in Figure 1. Life history variation is reduced to a single axis describing the "speed" of life (Promislow and Harvey 1990; Ricklefs and Wikelski 2002). Relatively speaking, "fast-living" species have high reproductive rates, low investment per offspring, short developmental times and short adult life spans, while "slow-living" species invest more per offspring, have slower reproductive rates, and higher adult survival. Examples of the extreme ends of this continuum are very large, slowly reproducing species such as African elephants (*Loxodonta Africana*), which produce one offspring every 2–5 years (Moss 2001) ("slow-living"), and species with high reproductive rates, short life spans and typically small body sizes such as the house mouse (*Mus musculus*), which have litters of 6 (on average) several times per year (Laurie 1946) ("fast-living"). Fast-living species can be said to favor current reproduction more heavily than survival and future reproduction, while slow-living species should favor survival and maintenance of immune defenses over current reproduction when a trade-off is required (Promislow and Harvey 1990).

Induced immune defenses

Trade-offs between reproduction and "immunocompetence" have been the focus of many ecological immunology studies, and there are exciting results suggesting that such trade-offs do exist (e.g., Hasselquist and others 2001; Ardia 2005). Different components of immune defense, however, are likely to vary in different ways along the life history axis. Induced defenses that require substantial time and resources during development of the young animal and are mostly beneficial against repeated infections, such as responses mediated by B- and T-cells, are more likely to be favored in longer-lived species that invest highly in each offspring they produce than they are in
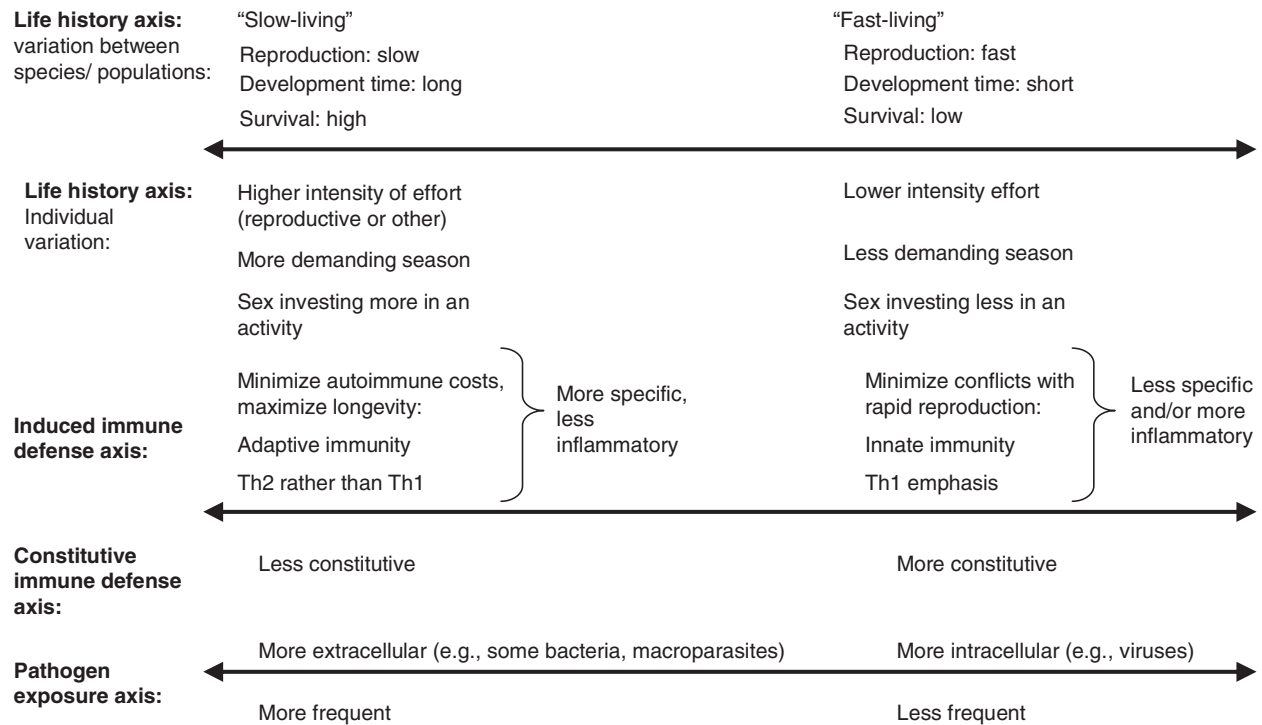
Life history axis: variation between species/populations:	"Slow-living" Reproduction: slow Development time: long Survival: high	"Fast-living" Reproduction: fast Development time: short Survival: low
Life history axis: Individual variation:	Higher intensity of effort (reproductive or other) More demanding season Sex investing more in an activity	Lower intensity effort Less demanding season Sex investing less in an activity
Induced immune defense axis:	Minimize autoimmune costs, maximize longevity: Adaptive immunity Th2 rather than Th1 } More specific, less inflammatory	Minimize conflicts with rapid reproduction: Innate immunity Th1 emphasis } Less specific and/or more inflammatory
Constitutive immune defense axis:	Less constitutive	More constitutive
Pathogen exposure axis:	More extracellular (e.g., some bacteria, macroparasites) More frequent	More intracellular (e.g., viruses) Less frequent

Fig. 1 Proposed relationships between life history and immune defense axes. "Fast-living" species with high reproductive rates and short development times are expected to have less well-developed adaptive immune system than "slow-living" species, and to rely more heavily on nonspecific defenses. Constitutive defenses are predicted to be greater when adaptive defenses are less well-developed, in order to decrease the induction of costly inflammatory responses. Amongst individuals within species and across seasons, a shift toward the specific/less inflammatory side of the axis is expected to accompany increased intensity of reproductive or other effort, to minimize the competition for resources between, for example, reproduction and inflammatory responses.

"fast-living" animals with low per-offspring investment, short developmental times and shorter life spans (Klasing and Leshchinsky 1999). If adaptive immune defenses are decreased in faster-reproducing species, they might instead rely more heavily on induced innate defenses, including inflammatory responses, despite their high costs (Klasing and Leshchinsky 1999). This could result in a somewhat counterintuitive pattern, in which induced immune responses in species or populations that produce the most offspring are more likely to be high-cost, nonspecific defenses rather than less expensive, induced specific defenses (Figure 1). Though such a strategy might appear maladaptive, it could be an essential component of life histories characterized by rapid development and early reproductive maturity, and therefore favored in species with high mortality rates and short adult life spans.

There could also be trade-offs within the adaptive immune system (Graham 2001; Graham 2002). Antibody-mediated defenses are carried out by B-cells and Th2-cells, and are accompanied by production of noninflammatory cytokines (Mosmann and Fowell 2002). In contrast, cell-mediated defenses are orchestrated by Th1-cells, which are associated with the production of inflammatory cytokines (Mosmann and Fowell 2002). Th1-cells and Th2-cells are reciprocally downregulated (Mosmann and Moore 1991), and there is evidence that organisms can favor one over the other (Charles and others 1999). The autoimmune costs of inflammatory responses are potentially high, and to avoid the accumulation of such damage, longer-lived species might exhibit a tendency to mount Th2-type responses more often than Th1. Conversely, the presence of inflammatory cytokines favors the development of Th1-type responses and suppresses the development of Th2-cells (Glimcher and Murphy 2000). If fast-living species more often mount inflammatory responses they might also have a greater Th1 bias in their adaptive immune systems compared with slow-living species. While there are few explicit data on Th1 versus Th2 biases in wild animals, there are many studies that have found negative relationships between antibody-mediated immunity (Th2) and measures of cell-mediated immunity (Th1) (Klein and others 1997; Gonzalez and others 1999; Johnsen and Zuk 1999; Klein and Nelson 1999; Buchanan and others 2003; Faivre and others 2003; Martin, Hasselquist and others 2006), suggesting that

such biases might exist. If this is the case, the life history axis could be mirrored by a specific-nonspecific and less inflammatory-more inflammatory induced immune defense continuum (Figure 1).

Constitutive defenses

A second immunological axis describing the degree of reliance on constitutive immune defenses might also exist, but how it should be oriented toward life history is less clear. The way different constitutive immune defenses relate to life history will likely depend on the cost of developing and maintaining a given defense, and how frequently an animal is challenged by infectious agents (over evolutionary time) (Shudo and Iwasa 2001). In Figure 1 I have hypothesized that on average, strong constitutive immune defense should be favored in fast-living species because this might minimize the use of costly induced inflammatory responses. Possible exceptions are constitutive defenses that are expensive to maintain; there should be selection for these as the frequency of challenge increases (Shudo and Iwasa 2001; Poitrineau and others 2004). The costs of most constitutive defenses are unknown, however, making predictions about particular components highly speculative. Recent studies suggest that relationships between components of constitutive immunity might be lacking or highly complex (Matson and others 2006; Mendes and others 2006).

2. Seasonal and individual-level immunological variation within species or populations

The fast-living versus slow-living dichotomy more commonly occurs at the scale of evolutionarily distinct units, for example, between isolated populations of the same species, or at the species level or above. The great majority of eco-immunological studies, however, are concerned with variation that occurs within a single population of a single species. The framework in Figure 1 can be used to understand individual variation within populations, but the predictions are ecological rather than evolutionary in nature, and this results in very different expectations. Below I discuss how immune defenses might vary within and between individuals as a function of season and condition. Martin and colleagues (2006a) discuss an additional aspect of immunological variation, that occurring as individual age (immunosenescence).

Induced defenses

As described above, *species* with high reproductive rates are predicted to rely more heavily on induced innate immune responses. Within species (where developmental timing should be less variable), however, the expected pattern is reversed: among *individuals* a negative relationship between the intensity of "effort" (reproductive or otherwise) and nonspecific/inflammatory immune defenses is predicted. This is because such a strategy could relieve some of the demands on an individual's resources during stressful times. Such reasoning can be applied to the interpretation of intraspecific immunological variation among seasons, among individuals within a season and between sexes.

In seasonally breeding animals, changes in resource demand, and availability over the year likely influence immune responses (Martin and others 2006b). Following Figure 1, individuals would be expected to show weaker nonspecific and/or Th1 responses associated with inflammation, and a greater reliance on specific/Th2 immune defenses, during the reproductive season. This is based on the assumption that the breeding season is the most demanding time of the year for many species; in cases where this is not true (for example, in areas with very severe winters), the opposite pattern might hold. Within a single reproductive season, individuals undertaking a large reproductive effort might first show suppression in costly innate responses involving inflammation. Specific defenses might not become compromised until very high levels of reproductive effort and stress are reached, and mild increases in effort might even result in increased specific defenses as the animal shifts from the inflammatory to the less inflammatory side of the immune defense continuum.

A similar mechanism could explain sex-specific immune defense strategies (Rolff 2002). The costs of many activities are unevenly divided between the sexes: physiological costs of producing young usually fall more heavily on females, while costs of sexually selected characters such as large body size, ornaments or territory maintenance are usually greater for males. These sex-specific costs might underlie gender differences in immune system function at different life history stages; the sex engaged in more costly activities should show a greater shift toward specific, noninflammatory defenses. Therefore, in many species females should have decreased inflammatory responses and increased antibody responses relative to males, at least during reproduction. Such sex-specific trade-offs need not be restricted to breeding activities, however, and they might differ during the developmental period compared with adulthood, and depend on factors such as mating system.

Constitutive immunity

As at the species level, patterns in constitutive defenses among individuals will depend on the cost of the defense. Overall, the predicted relationships between

induced and constitutive immunity are the same as those between species: individual animals at the nonspecific/inflammatory end of the induced defense spectrum should maintain higher levels of constitutive protection in order to minimize the use of costly inflammatory responses (Figure 1).

The shape of the life history–immune defense relationship

It is important to note that we do not yet know the shape a relationship between "speed of living" and the innate–adaptive balance of the immune system should take. The shape of any such function is important because it could affect how study species are chosen and results interpreted. The relationship between life history and investment in adaptive versus innate immunity could be linear; alternatively, there might be upper and lower thresholds at points along the life history continuum beyond which all species invest either fully or minimally in adaptive immunity. Under either scenario, differences in innate and adaptive immune responses should be most pronounced when comparing extreme ends of the life history spectrum (for example, elephant versus mouse). Finer scale differences in life histories such as those between sister species or even populations of the same species should always be accompanied by immunological differences if the adaptive immunity–life history relationship is linear; if instead there are upper and lower thresholds of investment in adaptive immunity, such correlations could still be found, but only if one or more populations or species falls above or below a threshold. An analogous argument can be made for the shape of the relationship between stress or effort and immune responses at the level of an individual; extreme differences in effort between individuals should yield the greatest differences in immune responses and more subtle differences may or may not be detected, depending on the shape of the relationship.

Empirical studies: 1. Relationships between life history and immune defenses at the population/species level

Induced immune defenses

The ideas outlined above and in Figure 1 are relatively new and untested. There are only a few studies that have explicitly examined the relationship between life history characteristics and multiple components of immune defense among different species or populations, but for the most part they seem to support the existence of the relationships described in Figure 1. For example, "fast-living" temperate populations of house sparrows (*Passer domesticus*), which lay on average 2 clutches of 4–5 eggs during a shorter breeding season, had slower specific antibody responses (Th2) but stronger antigen-specific T-cell memory and stronger T-cell-mediated local inflammation to phytohemagglutinin (PHA) (Th1) than did "slow-living" tropical populations, which lay 4 clutches of 2–3 eggs throughout the year (Martin and others 2004; Martin, Han and others 2006), in agreement with Figure 1. We found that tree sparrows (*Passer montanus*), which appear to have higher reproductive rates and lower survival rates than do house sparrows (Anderson 1978; Summers-Smith 1988; Lowther and Cink 1992), have weaker antibody responses and specific T-cell memory, but stronger nonspecific T-cell-mediated inflammation (Lee and others 2006). Additionally, tree sparrows but not house sparrows decreased reproductive output when challenged with an inflammatory stimulus, indicating a more vigorous inflammatory response (Lee and others 2005). Ash-throated flycatcher (*Myiarchus cinerascens*) nestlings, which have a shorter incubation period and higher field metabolic rates ("fast-living" traits) (Mock and others 1991) show stronger nonspecific T-cell-mediated inflammation than do western bluebirds (*Sialia mexicana*) of the same age, but similar antibody responses (Fair and others 2003). Positive associations between nonspecific T-cell-mediated inflammation and fecundity are also found across a larger number of species spanning a greater range of life history values: the swelling response to PHA is greater in species that lay larger clutches (Martin and others 2001) and have shorter incubation periods (Palacios and Martin 2006). Comparable data on antibody responses or T-cell memory, however, are not available.

It should be emphasized that despite the general agreement of the above studies with the orientation of life history and immune axes in Figure 1, some studies have found conflicting patterns. Across 6 species of *Peromyscus* mice, there was no relationship between life history characteristics and antibody responses or specific T-cell memory (LB Martin, ZM Weil, and RJ Nelson unpublished data). Both *in vivo* PHA (Th1 plus nonspecific inflammation) and antibody responses (Th2) were higher in hirundinid species with longer developmental periods (Moller and others 2001); this is in line with the predicted relationship between life history and inducible immune defense, but not with predictions regarding inflammatory defenses. Additionally, although we found longer-lived house sparrows to have stronger antibody responses and weaker nonspecific T-cell-mediated inflammation than did tree sparrows, antigen-specific T-cell memory

was also higher in house sparrows (Lee and others 2006). Perhaps the relationship between life history traits and adaptive versus innate immune defenses is more robust than that between life history and the Th1–Th2 dichotomy, at least between closely related species.

Constitutive immunity

Few eco-immunological studies have measured constitutive immune defenses other than circulating white blood cells and heterophil:lymphocyte ratios across multiple species (Matson and others 2006; Mendes and others 2006), and such measurements are difficult to interpret because they can be strongly affected by current health status. One exception is the study of *Peromyscus* mice by Martin and colleagues (unpublished data) discussed above, in which fast-living species had higher constitutive whole-blood bactericidal capacity than did slow-living species. This pattern parallels that predicted in Figure 1. A second study, however, found the reverse pattern; constitutive bactericidal capacity of whole blood was negatively correlated with mass-corrected basal metabolic rate across 12 tropical bird species, and the authors suggested that high constitutive bactericidal activity is a characteristic of slow-living species (Tieleman and others 2005). This discrepancy could reflect a difference in frequency or diversity of pathogen challenge in the tropics compared with the temperate zone, or a difference between mammals and birds. In their study of tropical and temperate house sparrows, however, Martin, Hasselquist and colleagues (2006) found no differences in natural antibody titers and complement activity between populations, despite populational differences in antibody and cell-mediated responses. More studies measuring additional types of constitutive immunity in a life history context are clearly needed before general conclusions can be drawn.

Empirical studies: 2. Relationships between life history and immune defenses at the level of the individual

Seasonal variation in immune defense and life history within populations

The above examples suggest that there might be a tendency for species with greater reproductive rates and shorter lifetimes to use nonspecific and inflammatory immune defenses more often than do longer-lived, less fecund species. Within species, the opposite pattern is expected, with animals shifting more toward the adaptive, noninflammatory end of the axis as they move into the breeding season or other especially demanding periods.

Induced defenses

There are data on seasonal variation in immune function in a diverse group of organisms. For example, mallards (*Anas platyrhynchos*) produce 2 types of IgY during antibody responses, a full-length form and a truncated form. The truncated form minimizes the extent of inflammation that accompanies recognition of bacteria (Humphrey and others 2004). This truncated form is produced in greater amounts during the breeding season than during the nonbreeding season, indicating a possible downregulation of inflammatory immune responses during breeding (K.C. Klasing, unpublished data). Rhesus monkeys (*Macaca mulatta*), which breed in the fall and winter, decrease Th1-type cytokine production in the winter, but increase lymphocyte proliferation (Mann and others 2000). Although not seasonal breeders, human females exhibit a shift toward Th2-dominated (noninflammatory) immunity during pregnancy (Lin and others 1993; Wegmann and others 1993; Shurin and others 1999). Also as expected, in temperate (fast-living) but not tropical (slow-living) house sparrows, nonspecific T-cell-mediated inflammation is suppressed in the early breeding season (Martin and others 2004). In general, a number of animals, including some mammals, reptiles and birds, have more robust antibody responses during the reproductive season (Stone 1956; Sidky and others 1972; Hussein and others 1979; Elridi and others 1981; Peters 2000; Hadley and others 2002), while cell-mediated responses are elevated in the nonbreeding season (Shifrine and others 1980; Zapata and others 1992; Sinclair and Lochmiller 2000).

Seasonal patterns in induced immune function might differ in species that are not strongly seasonal breeders, or in species for which the nonbreeding season is more stressful. During the winter some small rodent species favor adaptive immunity and down-regulate induced nonspecific defenses, including fever (Nelson 2004). This could be an adaptation for a hard winter; many of the species that show this pattern can breed at any time during the year, and Nelson (2004) suggested that winter might be their most demanding season.

Constitutive defenses

According to Figure 1, constitutive immune defenses should be lowest at more demanding times of the year when lower inflammatory responses might render them less beneficial. There is limited evidence that constitutive immunity varies seasonally, in tandem with Th1–Th2 shifts in adaptive immune defenses. Rhesus monkeys (mentioned above) that decreased Th1-type cytokine production during the breeding

season also decrease NK cell activity (Mann and others 2000). Complement activity shows no clear temporal pattern in prairie voles (*Microtus ochrogaster*), but females in reproductive state have significantly lower complement activity than nonreproductive females (Sinclair and Lochmiller 2000). In a cyprinid fish (*Rutilus rutilus*), measurements of constitutive innate immune function (granulocyte chemotaxis and phagocytosis) were highest during the nonspawning season (Kortet and others 2003).

As was the case with induced defenses, some small rodent species held under laboratory conditions exhibit patterns that are opposite of those predicted. In male Siberian hamsters (*Phodopus sungorus)*, exposure to short days (winter photoperiod) resulted in increased NK cell activity, as expected if summer (peak breeding) is the more stressful period (Yellon and others 1999; Bilbo and others 2002); however, phagocytosis and oxidative bursts of granulocytes were also suppressed (Yellon and others 1999). As previously mentioned, such patterns could be related to the relative demands placed on these species during the winter compared with the breeding season. For a more extensive review of seasonal changes in immune defenses, see Martin and colleagues (2006b).

Immune defenses and effort within season

Induced immune defenses

If the orientation of life history and immune-defense axes in Figure 1 is correct, reproductive output or other types of effort should be positively correlated with antibody-mediated defenses, but negatively correlated with nonspecific and/or inflammatory immune responses. In common terns (*Sterna hirundo*), parents of faster-growing chicks had higher circulating "baseline" specific antibody (IgY) levels, and chicks from broods of 2 were lighter and had higher IgY titers than did lone chicks (Apanius and Nisbet 2006). These results suggest a positive relationship between antibody titers and reproductive effort in parents, and a negative relationship between antibodies and condition in chicks. There is evidence from multiple studies that nonspecific T-cell-mediated inflammation (for example, the *in vivo* PHA response) is more sensitive to changes in host condition and/or workload than are antibody responses. For example, in the pied flycatcher (*Ficedula hypoleuca*), experimentally increasing clutch size decreased chicks' nonspecific swelling response to PHA, but did not affect antibody responses of the parents (Moreno and others 1999; Ilmonen and others 2002). House sparrows on a poor-quality diet mounted increased antibody responses (Buchanan and others 2003), while sparrows on a protein-rich diet decreased antibody responses but increased the swelling response to PHA (Gonzalez and others 1999). In humans, there is a shift from a Th1 immune profile to a Th2 bias following excessive exercise (Smith 2003) and in general T-cell-mediated immunity is very sensitive to nutritional deficiencies, while antibody responses are relatively insensitive (McDade 2005). Other studies have found similar patterns in male peacocks (*Pavo cristatus*) (Moller and Petrie 2002) and jungle fowl (*Gallus gallus*) (Johnsen and Zuk 1999).

Contrary to the above results, tree swallows (*Tachycineta bicolor*) from a population characterized by low survival decreased both the T-cell-mediated inflammatory response to PHA and antibody responses when broods were experimentally enlarged (Ardia 2005). Additionally, barn swallow (*Hirundo rustica*) chicks from second broods had slower mass gain but higher PHA responses than did chicks from first broods (Merino and others 2000) and circulating IgY levels and the nonspecific T-cell-mediated inflammation response to PHA were positively correlated in female pied flycatchers in the late breeding season (Morales and others 2004). Clearly, although less sensitive, antibody responses are not immune to the effects of increased reproductive effort. Increasing brood size or work load decreased antibody responses in a number of studies (Deerenberg and others 1997; Nordling and others 1998; Svensson and others 1998; Hasselquist and others 2001). These studies, however, did not assay inflammatory or cell-mediated immune responses.

Constitutive immune defenses

If a greater reliance on induced inflammatory relative to adaptive responses is accompanied by higher constitutive defenses, constitutive defenses should be higher in animals undertaking relatively smaller reproductive or other efforts, and/or in animals in better condition. In agreement with this, turkeys selected for high egg production had lower complement activity than did their parent line (Bayyari and others 1997); as mentioned above, female prairie voles in reproductive condition had lower complement activity compared with nonreproductive females regardless of time of year (Sinclair and Lochmiller 2000); and in general low body weight and nutritional stress can decrease complement activity (Pomeroy and others 1997). Heterophil:lymphocyte ratio was also positively correlated with body condition in peacocks (Moller and Petrie 2002). In zebra finches (*Taeniopygia guttata*), however, granulocyte:lymphocyte ratios were higher in birds subjected to increased workloads (Birkhead and others 1998); and body

condition and natural antibody titers (IgM) were negatively correlated in storm-petrel (*Oceanodroma leucorhoa*) chicks (Mauck and others 2005). It will not be possible to rigorously assess the relationship between "effort" and constitutive immunity until more data become available.

Immunological variation between sexes

Induced immune defenses

In many species, females invest more of their resources in reproduction than do males, and if the scheme in Figure 1 is valid females should fall more often toward the specific/less inflammatory end of the induced immune-defense spectrum, at least during reproduction. A number of studies show Th2 biases in females, and greater Th1 or inflammatory responses in males. For example, female rats mount weaker fevers than do male rats (Murakami and Ono 1987), and female Siberian hamsters mount stronger antibody responses than do males, and their T-cell proliferative response is less affected by a prior inflammatory challenge (LPS) than are males' responses (Bilbo and Nelson 2001). In general, female mammals are often reported to have stronger antibody responses and weaker cell-mediated immunity than do male mammals (Schuurs and Verheul 1990; Klein 2000a). A few studies, however, have found higher indices of some types of nonspecific immune defense in females. For example, female rats under stressful conditions mounted a stronger inflammatory response than did male rats (Hermes and others 2006).

There also appear to be interactions between sex, breeding system and immune defenses (Zuk and Stoehr 2002). As predicted in Figure 1, during long days (summer photoperiod), monogamous prairie vole females have higher antibody responses and lower T-cell mitogenic responses than do conspecific males. In polygynous meadow voles (*Microtus pennsylvanicus*), however, this pattern is reversed; males have higher antibody responses and lower T-cell proliferation (Klein and others 1997; Klein and Nelson 1998). Perhaps in polygynous species males, not females, are under greater stress during the time of peak breeding.

The data on sex differences in immune function of adult birds are much scarcer. Male white-crowned sparrows (*Zonotrichia leucophrys*) showed a greater decrease in locomotor activity than did females following an inflammatory challenge (lipopolysaccharide) during long days (breeding season), although other indices of the inflammatory responses did not differ between the sexes (Owen-Ashley and others 2006). Male Magellanic penguins (*Spheniscus magellanicus*) had lower PHA responses than did females early in the breeding season (Moreno and others 2001); the authors suggested that the early arrival on the breeding grounds and territory establishment makes the early breeding season more stressful for males than for females. Contrary to expectations, we found that during long days, male house sparrows tended to have stronger primary antibody responses and stronger swelling responses to PHA than did females (Lee and others 2006). However, in our experiments birds were held in long-term captivity with *ad libitum* food, and were not experiencing the demands of breeding. Martin and colleagues (2004) also found no differences in the nonspecific swelling response to PHA in wild or captive male and female house sparrows in any season.

At different life stages, sex differences in immune strategies might flip-flop. It has been suggested that because size and condition are often very important for determining mating success in males, and because that success can be quite variable, it is more important for young males to invest in fast growth and development than it is for females (Trivers and Willard 1973). Therefore, in contrast to the situation in reproductive adults, during growth and development males' resources might be stretched more thinly than those of females; thus, during this life stage males should decrease reliance on inflammatory defenses. Empirical data support such a distinction; male great tit (*Parus major)* nestlings have weaker nonspecific T-cell-mediated inflammatory responses than females (Tschirren and others 2003), and in both European starlings (*Sturnus vulgaris*) and Eurasian kestrels (*Falco tinnunculus*), male but not female nestlings exhibited decreased PHA responses under suboptimal conditions (Fargallo and others 2002; Chin and others 2005).

Constitutive immune defenses

Constitutive immunity is often found to be stronger in males than in females, as expected if females bear a greater reproductive burden and fall on the specific/less inflammatory end of the immune defense spectrum. For example, Bouman and others (2004) found that human males had more circulating monocytes but fewer circulating T-cells than did women, and monocytes from males produced more inflammatory cytokines than those from females when stimulated *in vitro*. Similarly, Moxley and others (2002) found that in healthy human females, induced production of tumor necrosis factor (TNF), an inflammatory cytokine, in whole blood, was lower than in males. Female mammals maintain higher levels of natural antibodies than do males, however, Schuurs and Verheul (1990) and we found no sex differences in natural antibody titers in either house sparrows or tree sparrows (Lee and others 2006).

Additional factors influencing the immune system

Though they have not been the focus of this review, factors other than life history traits undoubtedly shape immune defenses. Three important factors, pathogen exposure, body size and phylogeny, are likely to influence immune defense strategies, and taken together with life history data should provide a more detailed understanding of immune system variation than life history traits alone. Phylogeny is often treated by ecologists as a variable to be controlled for rather than an influence that is interesting to explore in its own right, and to my knowledge there are no data on the relationship between phylogeny and vertebrate immune defense strategies. The following brief overview emphasizes the possible influences and available data on how the remaining 2 factors, pathogen exposure and body size, relate to vertebrate immune responses.

Pathogen exposure

If a certain pathogen or class of pathogens is especially important for a given host species or population, that selective pressure might take precedence over those imposed by the more generic costs and benefits of immune defenses. For example, T-cell-mediated defenses are essential for fighting viral infections, while antibody-mediated defenses are often needed to clear extracellular pathogens (Janeway and others 1999), and if one or another type of pathogen dominates, interesting deviations from the predicted patterns might be observed. Graham (2002) discusses how the Th1–Th2 balance is struck within an individual in the presence of multiple infectious agents; such a process at the level of a population or species could create Th1–Th2 profiles that would not be predicted based on life history information alone.

As mentioned earlier, frequency of challenge should also affect the selective advantage of a particular defense strategy, with a high frequency of attacks favoring greater adaptive and constitutive immunity and decreased reliance on costly induced innate responses (Shudo and Iwasa 2001; Poitrineau and others 2004). Differential exposure is often hard to predict or quantify, but sometimes ecological differences between species provide clues. For example, differences in reproductive strategies, social organization and diet and feeding behavior are all likely to affect hosts' exposure to pathogens (Altizer and others 2003; Ezenwa 2004) and have all been found to correlate with some measures of immune system function (Klein 2000b; Nunn and others 2000; Blount and others 2003). Examples of pathogen-related population-level differences in immune defenses can be found in the biomedical literature; for example, human populations in which tuberculosis is endemic differ in their immune responses from populations that have not been exposed to the disease (Sousa and others 1997). Populations of small ground finches (*Geospiza fuliginosa*) in the Galapagos archipelago experiencing higher parasite prevalence and intensity had stronger antibody responses but weaker T-cell-mediated inflammatory responses than did populations with lower parasite loads (Lindstrom and others 2004). The "pathogen axis" in Figure 1 illustrates how large species or population differences in pathogen exposure might influence the evolution of immune defense strategies.

Body size

Life history traits correlate with body size (Blueweiss and others 1978), but imperfectly, and so including body size in models of immune defense variation could in some cases improve predictive power. Generally, body mass decreases and mass-specific metabolic rate increases from the "slow-living" to the "fast-living" ends of the life history continuum (Blueweiss and others 1978; Hennemann 1983). The development, maintenance, and use of the immune system are metabolic processes and should also scale with body size (Wiegel and Perelson 2004). In particular, larger-bodied animals with lower mass-specific metabolic rates should be more able to "afford" costly induced immune responses than small-bodied animals, regardless of their life histories. Small-bodied animals might rely more on constitutive defenses than induced defenses in order to minimize their proportionally larger costs. The data available thus far suggest there might be an effect of body size on immune responses that is independent of other life history traits; Tella and colleagues (2002) and Palacios and Martin (2006) found positive relationships between body mass and the PHA response at the species level, the relationship expected if larger animals invest more in induced immune defenses. In contrast, Tieleman and colleagues (2005) found no relationship between a measure of constitutive immunity and body mass at the level of species or individual. Additional work is needed to tease apart the effects of body size and life history traits on immune defenses.

Conclusions

The goal of eco-immunology is to explain the ecological and evolutionary causes and consequences of variation in immune defense strategies within and among species and higher taxa. To achieve this goal, we need to (1) more thoroughly describe the degree of

variation in the many different functions of the immune system, and where that variation occurs (that is, within or between species, genera or higher taxa); (2) identify potential ultimate causes of this variation (important correlates of the variation); and (3) once relationships have been found, attempt to determine the proximate mechanisms driving these differences to allow more rigorous evaluation of hypothesized ultimate driving factors. Of these 3, much work has focused on (2), but there is a great need for more descriptive work (Matson and others 2006), and investigations into the mechanistic bases of trade-offs.

The immune system consists of a number of interrelated functions, each with its own costs and benefits. The most beneficial combination of these components should depend on the life history of the organism. Here, I have proposed a comprehensive framework for formulating hypotheses and predictions about how immune defenses should vary with life history across scales. Although this framework is more detailed than earlier ones proposing trade-offs between "immunocompetence" and life history traits, it is undoubtedly still an oversimplification, and will continue to be refined as more data become available. The aim of this paper was to highlight the importance of considering multiple immune defense types and relationships among them in ecological studies, and to point out the potential benefit of such an approach for developing a more complete theoretical underpinning for the field. The studies summarized here suggest that such a framework will be useful for interpreting diverse and, at times, seemingly contradictory empirical results, and for shaping the questions addressed by future research.

Acknowledgments

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