

## Refining approaches and diversifying directions in ecoimmunology

Lynn B. Martin II,<sup>1,\*</sup> Zachary M. Weil,<sup>\*,†,‡</sup> and Randy J. Nelson<sup>\*,†,‡</sup>

<sup>\*</sup>Department of Psychology, <sup>†</sup>Department of Neuroscience, and <sup>‡</sup>Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43210, USA

**Synopsis** Ecoimmunologists have made many important discoveries about the immune systems of wild animals including (1) immune activity is usually costly, (2) counter-intuitive decrements in immune activity are often due to trade-offs with other physiological activities or behaviors, and (3) immune activity is a currency by which sexually selected traits are indices of individual quality. The use of single assays to characterize “immunocompetence,” however, as was, and is, the common practice in ecoimmunology, ignores the inherent complexity of the immune system and may have led to inappropriate conclusions or even positive publication bias. Recently, some have suggested that ecoimmunologists measure disease resistance or the fitness consequences of immunological insults instead of the immune system itself. We propose that researchers continue to use the techniques that have already been fruitful in ecoimmunology, but better incorporate the underlying immunophysiology of such techniques into their study designs and interpretation. We review the benefits and some recent successes of such an approach. Then, we discuss several under-explored but potentially rewarding areas of ecoimmunology, including development of the immune system, immunosenescence, and immunoredistribution. All three areas are well studied in biomedicine and are likely to be relevant in ecological contexts. For instance, because of the inherent costliness of immune defense and reproduction, variation in rates of development and senescence of the immune system likely impacts the ways in which individuals of different species mature and/or breed. Likewise, differential capacity to redistribute immune resources in response to changes within the endocrine system may explain some of the inconsistencies regarding the immunocompetence handicap hypothesis of sexual selection.

Since the inception of the field, ecoimmunologists have relied predominantly on one or two measures of immune activity to characterize “immunocompetence.” Studies using single indices, such as phytohemagglutinin (PHA) induced wing-web swelling (Lochmiller and others 1993), agglutination of heterologous red blood cells (Deerenberg and others 1997), or even simple quantification of circulating leukocytes (Saino and others 1995), have been informative, but their value has recently been challenged. Some have called for the abandonment of conventional immune measures in favor of “fitness consequences” of infection (Viney and others 2005). As currently available immune assays only allow for coarse qualification of weak versus strong responses, the potential auto-immunological damage that may be caused by misdirected or overly strong immune responses may be missed. In other words, optimal immunocompetence may be most favorable in terms of fitness in many cases, but this possibility is rarely considered. Another

perspective proposes that resistance to disease should be measured in conjunction with immune parameters (Adamo 2004). Although such information would likely be valuable, disease resistance as with immune activity is not monolithic. Indeed, resistance of some pathogens may have no bearing on resistance of others (Kaufmann and others 2002). Undoubtedly, however, simultaneous consideration of disease resistance and the immunological mediators of these outcomes are needed.

These two new propositions for conducting ecoimmunology highlight an important and recurrent shortcoming of the discipline. Indeed, how does one adequately measure the immune defenses of wild animals given the technical constraints of doing so? Several reviews have already proposed solutions to this problem (Norris and Evans 2000; Grasman 2002) and many have proposed means for doing so (Salvante 2006). We propose that in conjunction with increased efforts to study disease resistance and incorporate

From the symposium “Ecological Immunology: Recent Advances and Applications for Conservation and Public Health” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2006, at Orlando, Florida.

<sup>1</sup> E-mail: lmartin@mail.psy.ohio-state.edu

*Integrative and Comparative Biology*, volume 46, number 6, pp. 1030–1039

doi:10.1093/icb/icl039

Advance Access publication September 16, 2006

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an optimality perspective for the immune system, the most productive direction in the near term will involve heightened appreciation of the inherent complexity and redundancy of the immune system itself when designing experiments and interpreting results (Schmid-Hempel and Ebert 2003; Matson and others 2006a). Any single measure of immune activity no more embodies immunocompetence than does gonad size embody reproductive condition. The two may be related, but in different directions, or sometimes not at all, depending on the context (Matson and others 2006a).

This directive is, of course, not new. For more than 10 years, the importance of understanding the immunology underlying chosen techniques has been emphasized (Sheldon and Verhulst 1996). Generally, though, the extent of incorporating this perspective has consisted of categorization of immune assays as cell-mediated, humoral or innate and little else. Recently, a conceptual model was proposed to promote appreciation of the intricacies of immune defense. This immune-defense-component model (IDCM) emphasizes that immune defenses of all species can be broken down into four basic quadrants (Schmid-Hempel and Ebert 2003). In essence, immune responses can be directed towards particular components of pathogens (specific) or be broadly effective against many targets (nonspecific), and they can either be expressed at all times (constitutive) or appear only in response to immunological insult (inducible). Organisms are predicted to favor certain types depending on both the costs each variant imparts and the benefits each provides.

The utility of the IDCM is reflected in recent studies on house sparrows (*Passer domesticus*). Comparison of indices of immune defense from different quadrants of the IDCM between two populations of house sparrows suggested that organisms favor defenses that they can afford in light of the other physiological costs to which they are subject (Martin, Hasselquist, and Wikelski 2006). In other words, one population of sparrows, which lays small clutches over a long breeding season (that is, a slow pace of life), invested more in immune defense, in terms of elevation in energy expenditure in response to an inflammatory challenge and rate of antibody production to a novel antigen (see below), than another population that lays larger clutches over a shorter breeding season (that is, a fast pace of life). Similar discoveries in domesticated fowl led to the speculation that the costs of different types of immune defense shape the immunological portfolio of organisms in a life history context (Leshchinsky and Klasing 2001). Lines of chickens selected for abundant egg production favored

inducible, nonspecific immune defenses associated with the innate arm of the immune system. Broiler lines, however, selected for size and meat production, avoided these defenses, presumably because of better-developed adaptive defenses. More recently, similar organization was discovered in small mammals. Species of deer mice (genus *Peromyscus*) that live slow-paced reproductive lives exhibited stronger antibody responses (inducible, specific) but weaker nonspecific, constitutive defenses than reproductively prolific species (L. B. Martin, Z. M. Weil and R. J. Nelson, unpublished data). Based on these studies, the general pattern seems to be that developmentally expensive defenses (inducible-specific) are robust in species living slow-paced lives thereby improving the chances of survival to subsequent breeding events via increased immune investments. Conversely, developmentally inexpensive, but operationally costly immune defenses (inducible, nonspecific) are probably more robust in species living fast-paced reproductive lives.

From the perspective of the practicing ecoimmunologist, these studies reveal that the immune system is comprehensible in spite of its complexity. For this reason, we support the continued use of currently favored immune techniques while using the IDCM to guide selection and interpretation of assays. In the remainder of this paper, we review areas ripe for study in ecoimmunology in light of well-known phenomena from the biomedical and veterinary literature. Although most of these ideas have been raised before, and some have even obtained empirical support, all three are understudied but likely to yield exciting insight.

## Development of the immune system

One of the first phenomena described regarding development of the immune system in wild animals involved a positive correlation detected between prevalence of malaria and the duration of incubation among avian species (Ricklefs 1992). The author proposed that the coupling of lower prevalence of infection and longer developmental periods might represent greater investment in immune defense in some species. Although this pattern was later replicated (Tella and others 1999), only a few studies have provided immunological support for it, and this support is only indirect. In one, slow-lived house sparrows produced primary antibodies more rapidly to an antigenic challenge than did fast-lived house sparrows (Martin, Hasselquist, and Wikelski 2006). In another, slow-lived species of *Peromyscus* generated more antibodies faster (during both primary and secondary responses) than did related fast-living species (L. B. Martin, Z. M. Weil, and R. J. Nelson, unpublished data).

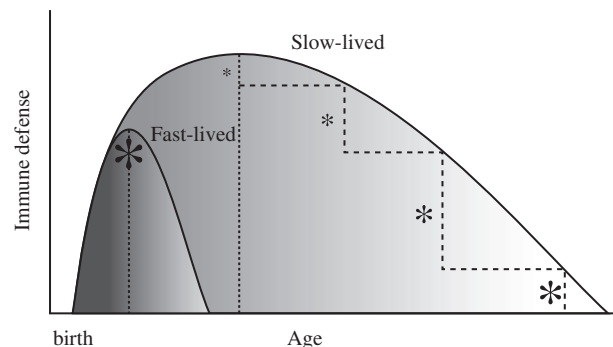
A third study detected the opposite pattern: passerines with longer incubation periods had weaker swelling responses to the mitogen, phytohemagglutinin (PHA) (Palacios and Martin 2006) than did more rapidly developing species.

On the one hand, these results may represent a valid contradiction of the original hypothesis. On the other, they may represent an excellent example of the utility of the IDCM. T-cell-mediated skin swelling in response to PHA does not measure the same type of immune activity as was measured in the studies on house sparrows and deer mice (Martin, Han, and others 2006). PHA swelling is more an inducible, nonspecific response; indeed, it is an index of basophil-mediated inflammation. Inflammatory responses are likely to be robust in rapidly developing species (Leshchinsky and Klasing 2001), as Palacios and Martin found. A follow-up study correlating a humoral immune response (inducible, specific) and incubation period would therefore be informative here.

The above studies represent most of the work conducted on immunological development in free-living vertebrates to date, and these offer only indirect evidence that the immune system develops differently among species. A few studies have shown that activation of the immune system during development retards reproductive and somatic development (Fair and Myers 2002; Prendergast and others 2004). Further, appreciable physiological costs of immune activity during development have been discovered in domesticated fowl (Henken and Brandsma 1982; Siegel and others 1982). To date however, few studies regarding development of the immune system in wild animals have been conducted. Work in biomedicine and veterinary science indicates that development of immune defense is probably very important in evolutionary and ecological contexts. Domesticated species with short gestation periods (for example, mice, rats, rabbits, and hamsters) have relatively immature immune systems at birth compared to dogs, primates, and humans (Felsburg 2002; Holsapple and others 2003). Further, fetal thymocytes from mice do not respond to mitogen stimulation until 85% of gestation is complete. In humans, however, responsiveness occurs at 30% of gestation (Holsapple and others 2003). In birds, similar patterns have been found (Haussmann and others 2005). Immature turkeys (*Meleagris gallopavo*), for example, exhibit weaker humoral and cell-mediated responses than do adults (Moore and Siopes 2002).

Such broad-scale patterns suggest that the rate of immunological development is effectively fixed across species, although more studies are undoubtedly

necessary. More rapid (reproductive) maturation may infringe on maximization of immunological potential, but to begin to address this possibility, ecoimmunologists would do well to characterize variability within and among species in the duration of time the immune system requires to become competent. Technological limitations currently make these studies difficult, particularly measurement of the rate of generation of the B-cell and T-cell repertoires. Use of common immune assays, however, could be informative in the meantime. For example, challenges with commonly used antigens in ecoimmunology [keyhole limpet hemocyanin (KLH), diphtheria-tetanus toxoid (DPT), or sheep red blood cells (SRBC)] and subsequent measurement of antibody production as animals mature could indicate whether development of specific, inducible immunity occurs at different rates in species with different reproductive paces of life (Fig. 1). Similarly, characterization of natural antibody levels (Matson and others 2005) or bactericidal capacity of blood (Matson and others 2006b) over the nestling period could address whether increased parental investments genuinely improve the quality of offspring, or whether parental effort simply induces a bias in immunological investments from one type to another (that is, immunoredistribution, see below).



**Fig. 1** Predicted development and senescence of immune defense in fast-living and slow-living species. Dotted line represents the age of first reproduction. Note similar paces of development of immune systems due to the random nature of the generation of lymphocytes, but more rapid rate of immunosenescence in fast-living versus slow-living species. Shading represents transition from nonspecific (dark) to specific (light) immune defenses. Fast-living species are predicted to rely more on nonspecific defenses for most of their lives, hence darker shading. Stair-stepped line for slow-living breeders represents potential seasonal variability in rate of immunosenescence as animals make the transition from breeding to nonbreeding states. Larger decrements with each step indicate biasing of investments towards reproduction with increasing age. Asterisks denote breeding events; size indicates breeding effort per event.

Such studies would be valuable for many reasons. First, they may help reconcile whether trade-offs sometimes are not manifest in young animals because (1) their immune systems are not developed to the point that they respond maximally to immune challenges (Holsapple and others 2003), (2) the costs of immune responses outweigh their benefit at certain points during maturation, (3) particular environmental conditions are not so demanding as to mandate trade-offs (Sandland and Minchella 2003), or (4) because they genuinely do not occur. Second, they could help determine whether the absence of steroid hormones effects on the juvenile immune system (that is, in studies of maternal effects) is due to a paucity of immunological substrates on which steroids can act, or if they genuinely are absent. Third, they may help elucidate how experiences during ontogeny affect immunological traits in adulthood. Lack of exposure to antigens in the early postnatal period delays lymphoid development in rodents (Thorbecke 1959). Likewise, neonatal exposure to lipopolysaccharide (LPS), a component of gram-negative bacteria, reduces subsequent fever and pro-inflammatory cytokine production in adult rats (Boisse and others 2004; Ellis and others 2005). To what extent then is immunological variability in wild populations a consequence of differential infection history?

Consideration of immunological ontogeny may reconcile whether differences among populations reflect the pathogenic environment experienced by offspring or physiological trade-offs (Martin, Weil, and Nelson 2006). For example, species that live different distances from the equator or individuals born at different times of the year probably encounter different abundances and diversities of parasites. The state of the adult immune system may reflect these early-life experiences. Changes in abiotic conditions alone can have long-term organizational effects on the adult immune system. Siberian hamsters (*Phodopus sungorus*) exposed prenatally and perinatally to short days (and therefore subjected to more of the pineal-derived hormone, melatonin) produced more inflammation in response to a T-cell antigen in adulthood than did groups of hamsters exposed to long days for the entirety of their development (Weil and others 2006b).

## Immunosenescence

The diversity and effectiveness of immune responses increase as animals mature, but in late adulthood immune defenses tend to decline or at least become dysregulated. Although multiple hypotheses (for example, antagonistic pleiotropy, accumulation of

mutations) may explain such immunosenescence, we focus on the disposable soma theory here (Kirkwood and Rose 1991). Senescence according to the disposable soma theory is a consequence of organisms adjusting investments in reproduction versus self-maintenance in such a way as to maximize fitness at different points in their lives. As individuals age, they should spend fewer resources on maintenance and shunt resources towards reproduction thereby maximizing reproductive success before they die (Zuk and stoehr 2002), especially in cases where reproductive senescence occurs. Consequently, rapid reproduction early in life may compromise reproductive capacity, and perhaps even survival, later in life perhaps via compromises within the immune system.

Evidence for immunosenescence has been documented by ecoimmunologists. In crickets (*Gryllus integer*), bacterial resistance decreases with age both in males and females (Adamo and others 2001), although one type of immune activity (prophenoloxidase activity) decreases only in males. In honeybees (*Apis mellifera*), functional haemocytes, an immune effector cell, are fewer in foragers than in individuals of the nurse caste. Increases in juvenile hormone (JH) are coincident with transition from nurse to foraging caste and induce haemocyte apoptosis and hence immunosenescence. Interestingly, forcing foragers into nursing roles decreases JH and increases haemocyte number. In wild vertebrates in which the immune system is more complex, immunosenescence is less understood, but some examples have been documented. Old collared flycatchers (*Ficedula albicollis*) have weaker humoral immune responses than do birds of either one or three years of age (Cichon and others 2003). Young ruffs (*Philomachus pugnax*) have weaker cell-mediated responses than do mature adults, which in turn have larger responses than do aged individuals (Lozano and Lank 2003).

In chickens, selection for fast growth reduces immune responses in adulthood (Van der Zipp 1983). This observation led to comparative work on immunosenescence among three passerine species. The prediction was that species with low extrinsic mortality and long life would invest more in immune defense over a longer portion of their lives and hence exhibit lower rates of immunosenescence (Hausmann and others 2005). Indeed, short-lived zebra finches (*Taeniopygia gutatta*) showed more rapid cell-mediated immunosenescence than did longer-lived tree swallows (*Tachycineta bicolor*) and Leach's storm petrels (*Oceanodroma leucoroha*). All species, however, experienced some degree of immunosenescence. The authors also emphasized the continued manifestation of immunosenescence in zebra finches

(which were maintained in captivity throughout their lives) indicated that rate of immunosenescence must be in part genetically based.

If immunosenescence has a genetic basis, then data from domesticated species become especially relevant to ecoimmunologists. In humans, the immune system becomes biased towards nonspecific (inflammatory) defenses as specific (adaptive) defenses wane. This pattern occurs partly as a consequence of T cells ceasing to produce the cytokine IL-2, which prevents establishment of T-cell memory, and results in a chronic pro-inflammatory state (known as inflamm-aging) (Franceschi and others 1999; De Martinis and others 2005). Although intriguing, it is impossible to know whether immunosenescence observed in humans occurs in a similar fashion in wild animals. For instance, food availability impinges on immune activity and thus the rate of immunosenescence. In many cases, food restriction (FR) reduces immune responses (Lochmiller and others 1993; Demas and Nelson 1998; Alonso-Alvarez and Tella 2001). However, modest FR, which is probably common for wild animals, can retard immunosenescence in domesticated species. Indeed, food-restricted rodents in the laboratory tend to outlive those fed *ad libitum*, in part because their immune systems senesce at lower rates (Fernandes and others 1997). Mild FR in these cases tends to enhance nonspecific immune activity but compromise immunological memory (constitutive, specific) (Nakamura and others 1990). Altogether these factors highlight that studies of immunosenescence in the field are likely to be difficult due to condition dependence. Such investigations would be valuable, however, and may explain many unexpected or counter-intuitive results in other studies (Reznick and Ghalambor 2005).

In spite of impediments to some studies of immunosenescence, others have been fruitful, particularly those considering terminal investment. If the soma is indeed disposable, at some point in an animal's life it should invest all its available resources in reproduction (Clutton-Brock 1984). The best example of this strategy comes from work on snails (*Biomphalaria glabrata*), which exhibit a burst of reproduction when infected with (or simply exposed to) the parasite, *Schistosoma mansoni* (Minchella and Loverde 1981). This strategy is adaptive because infection by *S. mansoni* results in castration, thus condensing lifetime reproductive potential into a short window of time. Recent work on birds has detected similar terminal investments. Female house sparrows were more likely to lay replacement clutches and lay larger second versus first clutches after nonspecific immune activity (LPS) was induced.

Further, offspring grew faster when their mothers were immune challenged (Bonneaud and others 2003, 2004). Investment priorities in self versus offspring also appear to change with age. Old, but not young, blue-footed boobies (*Sula nebouxii*), for example, increase reproductive output when immune activity is induced by the same nonspecific immune challenge (Velando and others 2006). Terminal investments are not limited to birds. Identical nonspecific immune challenge also delays photoperiod-mediated seasonal regression of the reproductive tract in Siberian hamsters (Weil and others 2006a). Typically, these seasonal breeders regress their reproductive systems as day lengths shorten. Maintenance of some reproductive integrity over a longer portion of the year may promote short-term fitness, but compromise survival.

Based on the above studies, it is obvious that much progress in immunosenescence has already been made. It is likely, though, that incorporation of the IDCM will lead to further gains in the field. For instance, which aspect of the immune system degrades first in wild taxa: specific or nonspecific, and why? Such consideration may explain why organisms elevate some immune responses during breeding versus nonbreeding seasons (Fig. 1). Is this because of the relative costs of different types of defense, and more importantly, do biases towards more nonspecific defenses in winter or summer occur with increasing age? Generally, immune activity is elevated in short versus long days. T-cell-mediated cutaneous inflammation, which can be classified as inducible, but either specific or nonspecific depending on the substance used to induce it (Martin, Hasselquist, and Wikelski 2006), is increased in temperate-dwelling rodents and passerines in short versus long days (Bilbo and others 2002; Martin and others 2004; Pyter and others 2005). However, other inducible, specific defenses, namely humoral immune activity, are elevated during long days not short days both in birds (Hasselquist and others 1999) and small mammals (Hadley and others 2002). The IDCM may also help identify the direct causes of immunosenescence. Do animals begin to rely more on constitutive, specific defenses (immunological memory) as they age? Why then does "inflamm-aging" occur in humans (De Martinis and others 2005)? Is this because oxidative damage over an animal's lifetime leads to heightened sensitivity of self-antigens and hence autoimmunity (Cichon and others 2003), or because immunological space simply fills up (Franceschi and others 1999)?

Such questions lead to predictions about differential rates of immune senescence among populations or species. For instance, pace of reproductive life probably impinges on rate of immune senescence (Fig. 1). Also, because early in development philopatric animals

probably experience most of the pathogenic environment they will encounter in their lifetimes, they likely rely more on specific, constitutive defenses. Conversely, migratory species and species living in seasonal environments probably experience a larger and more dynamic pathogenic pool over the year and throughout their lives overall. Thus, their specific, constitutive defenses may wane and inflammatory defenses be enhanced (to the extent possible) as they age. Finally, organisms that live on islands would experience specific, constitutive immunosenescence as a function of distance from the mainland, as pathogenic diversity is likely to decrease with distance. Of course, all these speculations are contingent upon immunological memory being maintained over a significant portion of an individual's life. This possibility has also been relatively unstudied, but may be extensively variable among populations or species.

### Immunoredistribution

One of the best examples that oversimplification in ecoimmunology has retarded progress involves the dichotomy of immunosuppression versus immunoredistribution (Dhabhar and others 1995, 1996; Dhabhar and McEwen 1997a). Two classes of steroid hormones, the androgens and the glucocorticoids, have extensive effects on the immune system. In ecoimmunology, these hormones are conventionally considered immunosuppressive. Indeed, the immunocompetence handicap hypothesis (Folstad and Karter 1992), one of the pillars of ecoimmunology, suggests that honesty of sexually selected traits is enforced via the exclusively suppressive effects of testosterone on the immune system. Unarguably, many laboratory and clinical studies have demonstrated that females or castrated males have stronger immune responses and reduced susceptibility to infection than do intact males (Klein 2000). Additionally, seasonally breeding animals tend to have enhanced immune function, somewhat irrespective of the immune component measured (but see above), during the nonbreeding season when androgen concentrations are low (Wingfield and others 1990; Nelson and Demas 1996). The exclusiveness of the immunocompetence handicap hypothesis, however, does not account for inconsistencies among studies or the known complex effects of androgens on immune activity (Hasselquist and others 1999; Owen-Ashley and others 2004; Roberts and others 2004; Greenman and others 2005). Even alternative hypotheses take the all-or-none approach in explaining the effects of testosterone on immune activity. For example, speculation that testosterone-induced immunosuppression evolved as a mechanism to prevent damage to

developing spermatozoa (which may be antigenic) (Wingfield and others 1997) seems valid. A better strategy for an individual, however, would be local immunosuppression by testosterone (at the level of the gonads) coupled with insensitivity in other tissues. In this way, infection is prevented and sperm are protected.

Such a hypothesis proposing more intricate effects of androgens on the immune system has been offered (Braude and others 1999), but it has yet to be rigorously tested. Because testosterone concentrations are typically elevated in response to social stimuli, including those occurring during inter-male aggressive encounters, courtship, or sexual displays (Bronson and Desjardins 1982; Wingfield and others 1990), as well as during reproduction, one might expect that testosterone would help distribute immune resources to locations likely to be wounded (for example, skin) or to experience transfer of ectoparasites or sexually transmitted pathogens (for example, mucosal tissues lining the reproductive tract). Simultaneously, its effects may be tightly and differentially regulated at the level of the tissue, allowing androgens to be suppressive in some cases but perhaps enhancing in others (Soma 2006). Such outcomes could be directly mediated by testosterone or indirectly through the effects of testosterone on corticosteroids (Evans and others 2000). Specifically, elevated testosterone could induce redistribution of immune cells indirectly, via enhanced glucocorticoid release, or by affecting behaviors that feed back to increase glucocorticoid signaling (Braude and others 1999).

As with testosterone, the effects of glucocorticoids on the immune system are complex and dynamic. There is abundant empirical evidence that chronically high concentrations of glucocorticoids are associated with immunosuppression and disease (Sapolsky and others 2000). These observations led to hypotheses that glucocorticoids serve as "brakes" on the immune system, having evolved in part to prevent runaway inflammation and immune-mediated pathology (Råberg and others 1998). As noted above for testosterone though, it is not intuitively obvious why glucocorticoids would obligatorily induce immunosuppression, or more specifically why some species would not have become less sensitive to the effects of chronically high glucocorticoids. Evidence from laboratory strains rodents and free-living house sparrows indicates that differential glucocorticoid sensitivity of the immune system exists across taxa (Dhabhar and others 1997; Martin and others 2005). Further, production of corticosteroids, in addition to other aspects of the stress response, is often associated with inhibition of a number of processes, such as

digestion and aspects of reproduction (Sapolsky and others 2000), activities that are typically employed only when conditions are not life-threatening (such as during a severe storm). Because immune activity is by and large costly (Lochmiller and Deerenberg 2000), it is generally believed to be expendable during short-term stress. Immunosuppression in response to elevation of corticosteroids was thought to be an energy-saving strategy that shunted resources into surviving stressful situations (Maier and others 1994).

Mounting evidence indicates, however, that some corticosteroid-mediated mechanisms of immunosuppression, namely apoptosis of leukocytes, are energetically costly (Dhabhar and McEwen 1997a). Thus, it is difficult to explain how immunosuppression, a potentially active process, could free up resources in the short term for processes favoring escape of predators or survival of inclement weather. One hypothesis proposes that immunosuppression may prevent immune-mediated pathology during times of stress (Råberg and others 1998; Braude and others 1999). In other words, secondary effects of physical stressors may tip the delicate balance organisms face between maintaining strong immune defences and preventing immune-mediated self-destruction. A second nonexclusive possibility, which currently has greater empirical support, is that corticosteroid-mediated immunosuppression is immunoredistribution in disguise (Dhabhar and others 1995).

Prolonged or chronic stressors are generally immunosuppressive, but acute stressors are immunoenhancing. Acute stressors result in a trafficking of immune cells to "front-line" areas of defense including the spleen, lymph nodes, gut, and skin (Dhabhar and others 1996). For example, rats restrained for 2 h prior to immune challenge (which raises levels of corticosteroids) had elevated immune responses in the skin relative to unrestrained rats or rats that had been restrained daily for several weeks prior to the challenge (Dhabhar and McEwen 1997b). Moreover, degree of redistribution appears to be contingent on environmental conditions. In Siberian hamsters, leukocytes disappeared from circulation more rapidly in response to acute stress and T-cell-mediated inflammation in the skin was more robust (presumably because of immune cells leaving blood and entering skin) in animals kept under short-day versus long-day conditions (Bilbo and others 2002). This set of findings highlights the importance of considering physiological variables in their proper context. The same stressor (2 h of restraint) results in different effects on the immune system depending on the number of exposures and even the time of year. More importantly, had only one aspect of the immune

system been measured (for example, circulating leukocytes), reallocation within the immune system would have been missed.

## Summary

Although continued technological improvements will lead to increased understanding of the immune system in natural contexts, currently available tools have provided much knowledge already and will continue to be valuable in the future. This value may be further enhanced via active incorporation of basic immunology into ecoimmunological studies. In addition, continued expansion of the field of ecoimmunology, particularly involving work on the development, senescence, and redistribution of immune defenses, may provide novel and important insight into long-unresolved issues in ecology and evolutionary biology. The immunocompetence handicap hypothesis (Folstad and Karter 1992) provided a mechanism for a cornerstone of research into sexual selection (Hamilton and Zuk 1982). Future research in ecoimmunology will hopefully provide additional successes.

## Acknowledgments

The authors thank Kristen Navara for comments on a previous draft. Preparation of this manuscript and studies cited from our laboratory have been supported by NIH grants MH57535 and MH66144 and NSF grant IBN 04-16897.

*Conflict of interest:* None declared.

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