SYMPOSIUM

Mechanisms and Methods in Ecoimmunology: Integrating Within-Organism and Between-Organism Processes

C. J. Downs,[1],* J. S. Adelman[†] and G. E. Demas[‡]

*Department of Natural Resources and Environmental Sciences, University of Nevada, 1664 North Virginia Street, MS 168, Reno, NV 89557, USA; [†]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA; [‡]Department of Biology, Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405, USA

From the symposium "Methods and Mechanisms in Ecoimmunology" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

[1]E-mail: cdowns@unr.edu

Synopsis Ecoimmunology utilizes techniques from traditionally laboratory-based disciplines—for example, immunology, genomics, proteomics, neuroendocrinology, and cell biology—to reveal how the immune systems of wild organisms both shape and respond to ecological and evolutionary pressures. Immunological phenotypes are embedded within a mechanistic pathway leading from genotype through physiology to shape higher-order biological phenomena. As such, "mechanisms" in ecoimmunology can refer to both the within-host processes that shape immunological phenotypes, or it can refer the ways in which different immunological phenotypes alter between-organism processes at ecological and evolutionary scales. The mechanistic questions ecoimmunologists can ask, both within-organisms and between-organisms, however, often have been limited by techniques that do not easily transfer to wild, non-model systems. Thus, a major focus in ecoimmunology has been developing and refining the available toolkit. Recently, this toolkit has been expanding at an unprecedented rate, bringing new challenges to choosing techniques and standardizing protocols across studies. By confronting these challenges, we will be able to enhance ecoimmunological inquiries into the physiological basis of life-history trade-offs; the development of low-cost biomarkers for susceptibility to disease; and the investigation of the ecophysiological underpinnings of disease ecology, behavior, and the coevolution of host–parasite systems. The technical advances in, and crossover technologies from, disciplines associated with ecoimmunology and how these advances can help us understand the mechanistic basis of immunological variability in wild species were the focus of the symposium, *Methods and Mechanisms in Ecoimmunology*.

What Is Ecoimmunology?

Ecoimmunology is an emerging discipline that seeks to understand the physiological, ecological, and evolutionary causes and consequences of variation in immune systems (Sheldon and Verhulst 1996; Schulenburg et al. 2009; Martin et al. 2011a; Demas and Nelson 2012b; Zimmerman et al. 2014). To do so, ecoimmunology utilizes techniques from traditionally laboratory-based disciplines—for example, immunology, genomics, proteomics, neuroendocrinology, and cell biology—to reveal how the immune systems of wild organisms both shape and respond to ecological and evolutionary pressures. By focusing on interactions between the immune system and other physiological processes within individuals, as well as variation in immunity across individuals, populations, and species, research in ecoimmunology can illuminate mechanisms underlying higher-order patterns in areas such as the evolution of life history (Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Ricklefs and Wikelski 2002; Lee 2006), disease ecology (Alizon and van Baalen 2008; Boots et al. 2009; Day et al. 2011; Hawley and Altizer 2011; Adelman forthcoming 2015), phenotypic plasticity (Sadd and Schmid-Hempel 2009), and evolutionary constrains (Martin et al. 2008, 2011b; Ardia et al. 2011; Cohen et al. 2012; Robinson and Klein 2012). One theme running through this diversity of topics related to ecoimmunology is the potential to link phenomena from
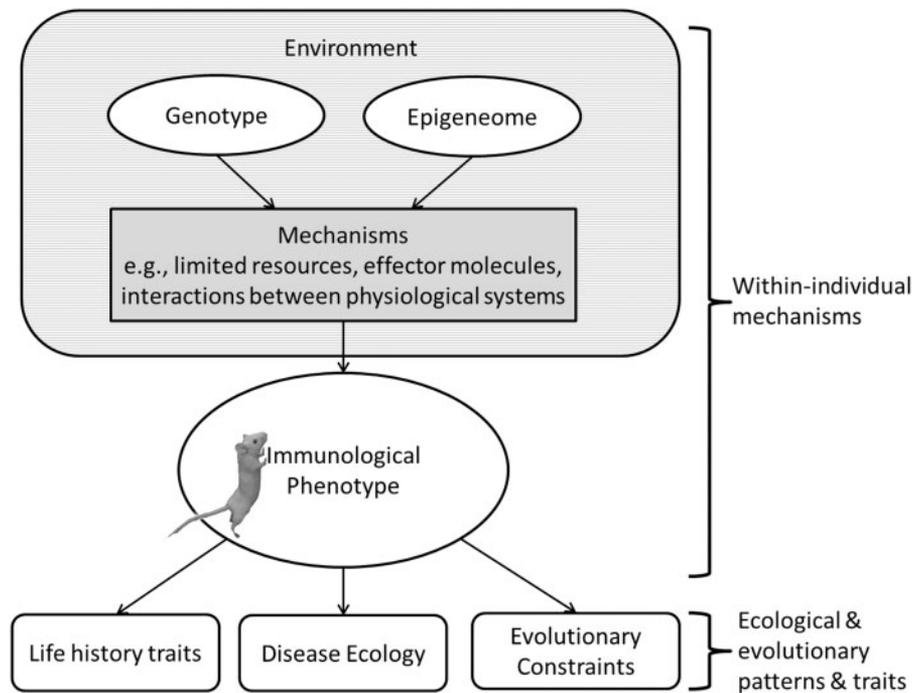
Fig. 1 Immunological traits studied in ecoimmunological studies themselves can be mechanisms that underpin patterns seen in ecology and evolution, thereby leading to a better understanding and to better predictions about these patterns. As phenotypic traits themselves, however, immunological phenotypes can also be better understood by understanding the mechanisms that mediate their expression within the organism.

different levels of biological organization. In this regard, the term "mechanisms" in ecoimmunology can refer to processes within organisms that produce particular immunological phenotypes, or it can refer to the ways in which different immunological phenotypes alter phenomena at ecological and evolutionary scales. Although presented as a dichotomy here, by integrating these different definitions of mechanisms, ecoimmunology has the potential to illuminate how genotypes lead to immunological phenotypes expressed in wild organisms and how these phenotypes relate to patterns at ecological and evolutionary levels (Fig. 1). Here, we first explore ecoimmunological mechanisms at the within-organism level, before discussing how immune phenotypes can themselves act as mechanisms, altering higher-level processes such as the evolution of life histories and the dynamics of infectious diseases.

Within-individual mechanisms underlying ecoimmunological traits

Much work in ecoimmunology has investigated proximate, within-individual mechanisms that drive variation in immunity (Lee 2006; Martin et al. 2008; French et al. 2009; Ashley et al. 2012; Demas and Nelson 2012a; Hasselquist and Nilsson 2012; Robinson and Klein 2012; Graham 2013). Although

the term "mechanisms" carries different meanings across biological disciplines, for the purposes of this review we define within-individual mechanisms broadly as those physiological processes, whether genetic, endocrine, neural, or molecular, with the capacity to modulate immune responses, and thus explain variation in specific immune response within and across individuals, populations, and species.

Because natural selection does not act upon single traits in isolation, but upon organisms as integrated units, understanding how different immunological phenotypes arise requires an understanding of how the immune system interacts with other physiological systems (Ardia et al. 2011; Martin et al. 2011b; Cohen et al. 2012). Revealing the cellular and molecular processes that underlie such interactions can improve our understanding of how organisms integrate environmental information to shape physiological, immunological, and life-history traits (Zera and Harshman 2001; Ricklefs and Wikelski 2002; Lee 2006).

When considering the mechanisms underlying interactions between the immune system and other physiological systems, Ardia et al. (2011) provided a useful distinction between (1) temporary trade-offs among traits caused by suboptimal investment

due to limited resources and (2) more permanent constraints caused by genetics, epigenetics, and physiological controls. In this section, we first review concepts and methods related to resource-based trade-offs in ecoimmunology. We then discuss other constraints on immune responses, highlighting the emerging idea of physiological regulatory networks and integrators as mediators of trade-offs.

Resource-based trade-offs and allocation versus acquisition

Trade-offs among traits caused by limited resources are typically addressed under the principle of allocation: when traits compete for finite resources, expression of all traits cannot be maximized simultaneously (Stearns 1992). The immune system is expensive in energy and nutrients, and when resources are limited, immune function can trade-off with other costly activities (Klasing and Leshchinsky 1999; Lochmiller and Deerenberg 2000; Norris and Evans 2000; Demas et al. 2012; Iseri and Klasing 2013). For example, a trade-off between immune function and reproduction has been demonstrated in collared flycatchers (*Ficedula albicollis*); those with experimentally increased broods produce fewer antibodies to vaccinations with Newcastle virus, and birds with experimentally decreased broods produced more antibodies (Nordling et al. 1998). Similarly, tree lizards (*Urosaurus ornatus*) reduced investment in reproduction in the face of immune challenges when food was limited, and they were immunosuppressed when investment in reproduction was experimentally increased or when food was limited and they were performing costly reproductive activities (French et al. 2007a, 2007b).

Although the principle of allocation predicts a negative correlation between traits that share a common resource pool (Stearns 1992), often positive correlations are found between traits when this correlation is explored between individuals. Van Noordwijk (1986) proposed an explanation for this paradox. Namely, individuals acquire different quantities of a resource, and individuals with more resources can allocate more resources to both traits (e.g., the "big houses and big cars" paradox). Thus, within an individual for a given level of resources, trade-offs in allocation are expected. Between-individuals, however, if differences in acquisition are more variable than differences in allocation, acquisition can drive observed patterns and result in positive correlations among traits (De Jong and Van Noordwijk 1992). Life-history theory illuminates when differences in allocation and

acquisition should drive patterns of phenotypic relationships, but why do some individuals have more resources available to them, and what specific resources truly limit the expression of given phenotypes? A mechanistic understanding of these trade-offs will provide answers to both of these questions. Indeed, a recent study detailing interactions among fecundity, metabolic rates, food consumption, and body weight in *Drosophila melanogaster* concluded that reduction in food caused by infection-induced anorexia, rather than by changes in allocation from reproduction to immune responses, caused a decrease in fecundity during an immunological challenge (Bashir-Tanoli and Tinsley 2014).

In a similar manner, basic theory makes predictions about interspecific patterns of investment in survival and reproduction, but what drives those relationships and decisions about allocation? A mechanistic understanding of how these trade-offs are mediated will enhance understanding of the relationships between different levels of organization and the patterns seen at these different levels. For example, the hypotheses introduced by Lee (2006) proposed different patterns of investment into immune defenses when comparing individuals within a population, between populations, and between species. Potential mechanisms were also proposed by Lee (2006), and these hypotheses have been the foundation for many studies in ecoimmunology. Downs and Dochtermann (2014) detail similar logic using a trade-off between basal metabolic rate and antibody production as an example and provide a statistical framework for simultaneously estimating correlations within individuals and among individuals; within species and among species; and within individuals, among individuals, and among species. This study not only highlights the need for new and adapted statistical tools to answer complicated questions in ecoimmunology (Buehler et al. 2011; Downs and Dochtermann 2014), but it also highlights the importance of understanding immune mechanisms at multiple levels, again stressing the critical nature of comparative research (and methods) in ecoimmunology.

Evolutionary constraints, physiological regulatory networks, and integrators

In contrast to the temporary trade-offs caused by limited resources, other constraints caused by genetics, epigenetics, or physiological controls can more permanently impact the expression of immune phenotypes (Ardia et al. 2011). Experiments using artificial selection provide some of the most striking

examples of how such processes can alter immune phenotypes in more-permanent ways (Swallow et al. 2009). For example, laboratory mice (*Mus musculus domesticus*) selected for high maximal metabolic rate also exhibited impaired inflammatory responses, even though food was not limited (Downs et al. 2013). Similarly, chickens (*Gallus gallus domesticus*) selected for divergent specific antibody responses also differed in levels of natural antibodies, growth, and egg production (Gross et al. 1980; Siegel et al. 1982; Parmentier et al. 2004). Because these results highlight evolved traits that persist, even when resources are not limited, they illustrate the potential for genetically-based constraints on immune phenotypes.

Recently, several authors proposed that the best way to understand the constraints and plasticity of physiological phenotypes is by understanding the regulatory networks that mediate physiological processes (Martin et al. 2011b; Cohen et al. 2012). In brief, such physiological regulatory networks consist of a system of signaling molecules, grouped into subnetworks, each of which regulates a particular set of physiological processes (e.g., digestion, immune responses, and reproduction) (Cohen et al. 2012). Maintaining organismal fitness requires cross-talk among these subnetworks and integration of information from the external environment, which Martin et al. (2011b) posit to be facilitated by a limited number of molecules, termed integrators, each with myriad connections across the network. Given this proposed architecture, perturbation of one part of a physiological regulatory network can have significant consequences for other parts of the network associated with different physiological systems (Cohen et al. 2012). For example, the level of an individual's response to a pathogen or parasite is determined by genetics, epigenetics, previous exposure to pathogens and parasites, nutritional and physiological state, and environmental conditions. Therefore, to accurately predict the level of immunological responses, researchers must reveal how numerous physiological systems interact to create the expressed phenotype (Schulenburg et al. 2009; Martin et al. 2011b; Cohen et al. 2012). Developing an accurate physiological network requires a detailed knowledge of the molecules involved in numerous physiological processes. Once complete, physiological regulatory networks have the potential to illuminate constraints on the evolution of adaptive immune responses, as well as constraints imposed by acclimating to changes in environmental conditions and emerging diseases (Ardia et al. 2011; Cohen et al. 2012). Expanding our knowledge of signaling molecules involved in immune responses of both model and non-model organisms, as illustrated by Fassbinder-Orth (2014), has great potential for augmenting such knowledge in the context of ecoimmunology.

Although our understanding of physiological regulatory networks is far from complete, integrators provide a useful framework for thinking about mechanisms that mediate immune responses. Much research has focused on endocrine hormones as potential integrators of the vertebrate system because endocrine hormones mediate communication between different systems of the body, are involved in regulations of physiology and behavior, and are particularly likely to shape interactions across physiological systems and overall immune phenotypes (Besedovsky and del Rey 1996; Martin et al. 2008; Demas et al. 2011a). Sex hormones (e.g., estradiol, testosterone, and progesterone), leptin, glucocorticoids (cortisol and corticosterone), and melatonin have received the most attention as vertebrate integrators. Because each of these hormones deals with functions of life history and homeostasis (e.g., sex hormones and reproduction, leptin and energy balance, and glucocorticoids and stress), numerous studies have sought to understand how they may coordinate immune changes during various periods of an animal's life (Ahmed et al. 1985; Schuurs and Verheul 1990; Hotchkiss and Nelson 2002; Martin et al. 2008; French et al. 2011; Demas and Nelson 2012a). As interactions among these hormones and the immune system have been reviewed extensively elsewhere (Ahmed et al. 1985; Schuurs and Verheul 1990; Hotchkiss and Nelson 2002; Martin et al. 2008; French et al. 2011; Demas and Nelson 2012a), we do not treat this topic at length here. However, it is important to note that although many of these hormones were first investigated in a particular animal class there is mounting evidence that similar mechanisms exist across animal classes. For example, while the initial focus of research on leptin was in mammals, a sequence for leptin was recently found in a bird, the Peregrine falcon (*Falco peregrinus*) (Prokop et al. 2014). Additionally, most work on hormonal responses to stress has focused on the role of glucocorticoids in vertebrates, but there is mounting evidence that signaling molecules associated with the stress responses in invertebrates are also involved in regulations of immune responses (Adamo 2014). Such studies suggest that while the precise identity of integrators may vary across taxa, the principle of integrators may hold widespread relevance.

Molecules that act exclusively intracellularly, and do not circulate systemically like hormones, can also induce pleiotropic effects and act as integrators. For

example, hypoxia inducible factors (HIFs) are transcriptional factors upregulated when cellular oxygen levels fall; they can affect numerous physiological systems (Bracken et al. 2003). When upregulated, HIF in turn upregulates genes associated with inflammation, antibody production, erythrocyte production, iron transport, angiogenesis, and energy metabolism (Bracken et al. 2003; Baze et al. 2010; Majmundar et al. 2010). Similarly, NF-$\kappa\beta$ is involved in the regulation of innate immunity and inflammatory gene expression, and is also induced by hypoxia (Hayden and Ghosh 2008; Cummins et al. 2010). As such, intracellular regulatory factors such as HIF and NF-$\kappa\beta$ help explain mechanistic links between physiological systems.

Although some interactions among systems within the physiological regulatory network are highly constrained because of pleiotropic genetic effects, it is important to remember that interactions between these systems are not always static. Rather, the structure of interactions between systems can change over time, for example during development and with acclimation to new environmental conditions (Ardia et al. 2011). A better understanding of how these systems interact within organisms at different time-scales (within a lifetime versus on an evolutionary time-scale) will improve predictions and models about how changes in one system will affect other systems and how organisms acclimate to changing environments. As discussed earlier, selection acts on the whole organisms rather than on single traits. As such, interactions between physiological systems that lead to trade-offs within an individual's lifetime may be altered by selection so that these trade-offs are absent on an evolutionary time-scale (Downs et al. 2012). For example, mice selected for high voluntary wheel-running have elevated circulating baseline corticosterone levels (Girard and Garland 2002; Malisch et al. 2007, 2008). Despite the well-documented link between chronic exposure to elevated corticosterone and suppressed immune function (Buchanan 2000; Sapolsky et al. 2000), these mice did not have reduced inflammatory response, suggesting that a compensatory mechanism had evolved that broke the relationship between these systems (Downs et al. 2012). Similarly, all trade-offs causes by limited resources occur at the within-individual level and are thus temporary (Downs and Dochtermann 2014). Further elucidation of the structure and plasticity of physiological regulatory networks will undoubtedly help researchers understand how such trade-offs among immune and other traits can vary so widely across levels of biological organization.

Immunological traits as mechanisms

As described in the previous sections, when investigating immunological phenotypes genetic, epigenetics, environmental influences, and interactions among physiological systems are mechanisms (Fig. 1). When describing patterns at scales above the individual, these mechanisms are still important; however, for higher-order patterns the phenotypes expressed by the individual can themselves be considered mechanisms. As such, understanding immunological traits helps improve predictions and explanations in fields such as life-history theory, disease ecology, and organismal ecology.

Ecoimmunology as a mechanism in life-history theory

Are mechanisms important for understanding the evolution of life-history traits and life-history trade-offs? One school of thought argues that life-history theory only involves predictions about the main features of life cycles, but not the mechanisms that drive these trade-offs (Stearns 2011). For example, theory predicts that long-lived species should favor current survival and future reproduction over current reproduction (Williams 1966). Within this perspective, knowledge of mechanisms is unnecessary for understanding variation in life-history strategies because basic theory successfully predicts the main features of life cycles (Flatt et al. 2011). Proponents of this view argue further that most studies of the mechanisms underlying life histories have not improved the predictive power of theory, with the possible exception of antagonistic pleiotropy (Stearns 2011). The alternative perspective is that theory also includes predictions about the mechanisms that mediate relationships among main features of life cycles (Flatt et al. 2011). In this second perspective, physiological mechanisms play an essential role in regulating and constraining the evolution of life-history trade-offs (Sibly and Calow 1986; Zera and Harshman 2001; Ricklefs and Wikelski 2002). Although progress may be in early stages, understanding the mechanisms that underlie life-history traits will improve predictions about extant variation in, and evolution of, life-history strategies (Flatt et al. 2011).

Within this second framework, ecoimmunology can help explain existing patterns in life-history strategies and trade-offs that arise between traits and immune function (e.g., Sheldon and Verhulst 1996; Lochmiller and Deerenberg 2000; Lee 2006). One recent attempt to explain how physiological traits mediate life-history traits is the life-history/physiological nexus or pace-of-life hypothesis (Ricklefs

and Wikelski 2002). This hypothesis extends previous theoretical work incorporating physiological mechanisms into life-history theory, and posits that immune defenses, endocrine signaling, and metabolism are key physiological processes that shape life histories (Sibly and Calow 1986; Zera and Harshman 2001; Reznick et al. 2002; Ricklefs and Wikelski 2002). The pace-of-life hypothesis makes testable predictions about how physiological traits should relate to each other and with life histories. For example, the pace-of-life hypothesis posits that slow-paced species should have lower metabolic rates, lower inflammatory immune responses, and higher constitutive immune responses than do fast-paced species (Ricklefs and Wikelski 2002; Lee 2006).

Sandmeier and Tracy (2014) argue that the pace-of-life hypothesis is based on endothermic animals, which may bias its predictions about immune function. By explicitly including considerations of body temperature and metabolic rate, two important aspects of ectotherms' physiology, they extend the pace-of-life hypothesis to make predictions about how patterns of investment in immune function differ between ectotherms and endotherms (Sandmeier and Tracy 2014). This work demonstrates how studies of physiological phenotypes as mechanism of higher-order phenomenon can actually lead to advances in theory.

If predictions from the pace-of-life hypothesis are supported by research, then this mechanistic understanding of life histories will improve the ability to predict how organisms and populations will respond to environmental changes, both natural and anthropogenic (e.g., Martin et al. 2010). An improved understanding of such mechanisms will also help scientists make more specific predictions about the timing of events in an individual's life cycle and allow individual variation to be better integrated into theory. Because models of population dynamics are based on estimates of life-history traits (e.g., probability of annual survival and annual reproductive success), understanding these mechanisms, and their variation, at the level of the individual will also improve models of population dynamics and inform conservation and management plans.

Common approaches to test the links between immune function and existing patterns of life histories include combinations of experimentally altering investment in life-history traits (e.g., Gustafsson et al. 1994; Nordling et al. 1998; Hõrak et al. 1999; Ardia et al. 2003), experimentally inducing an immune challenge to individuals with different life-history patterns (e.g., Moreno et al. 2001; Bonneaud et al. 2003; Sanz et al. 2004), or correlating life-history traits with immune capacity using simple immune assays (e.g., Moreno et al. 2005; Martin et al. 2007; Lee et al. 2008). When exploring interspecific patterns of life-history traits and immune function, the latter approach often involves looking at averages of traits for species instead of accounting for intraspecific variation, making it difficult to draw conclusions about how individual variation in immune capacity affects investment in life-history patterns (Downs and Dochtermann 2014). When investigating patterns across individuals within a species, immune capacity often is correlated with a trait that is a proxy for survival, reproduction, or both, and rarely are true values of fitness obtained (but see Nussey et al. 2014). Experiments in which investment in a life-history trait is manipulated, inform hypotheses about how investment in reproduction influences investment in immune responses. They do not necessarily inform hypotheses about how investment in immune function alter investment in reproduction because life-history theory suggests that some traits should be better protected than others because within a life-history strategy some traits are given higher priority (Stearns 1992). Although these approaches have provided invaluable information of physiological trade-offs with immune function, and have helped to begin to tease apart the complicated interactions among physiological systems, including the immune system, there are limitations when interpreting data from ecoimmunological studies regarding how immune phenotypes inform life-history theory.

A common problem with integrating immune phenotypes into life-history theory is that the immune system is complex and interacts with other physiological systems (Lee 2006; Martin et al. 2006; Matson et al. 2006; Demas et al. 2011a; Demas and Nelson 2012a). Conclusions about immunocompetence often are drawn from simple assays that do not necessarily correspond with how an individual responds when challenged by a parasite or pathogen, the history of exposure to parasite and pathogen for individuals is often unknown, and it is difficult to take repeated measure from individuals (Keil et al. 2001; Adamo 2004; Viney et al. 2005; Pedersen and Babayan 2011). Interpretations of field experiments will improve as connections among physiological systems are explored further (Martin et al. 2011b; Cohen et al. 2012), as simple immune assays are correlated with outcomes of infections and fitness characteristics (Viney et al. 2005; Demas et al. 2011b; Graham et al. 2011; Pedersen and Babayan 2011), and as new techniques are introduced that allow

researchers to better quantify the immune system of individuals in natural systems (Boughton et al. 2011; Demas et al. 2011b). These are not mutually exclusive problems, as new techniques will help develop understanding of physiological regulatory networks (see above) and inform the relationship between simple immune assays, immune responses to challenges, and fitness characteristics.

One of the best ways to understand how selection acts on immune phenotypes in relationship to life-history traits is to explore the true fitness consequences of different immune responses (Graham et al. 2011). Such studies are difficult, but with thoughtful consideration, researchers can identify powerful study organisms in natural settings, such as the free-living Soay sheep (*Ovis aries*) on the outer Hebrides islands of Scotland (Graham et al. 2010; Brown et al. 2013; Nussey et al. 2014). A recent study of antibody production in these animals revealed complex relationships between different types of antibodies with survival overwinter, parasite load, and body mass (Nussey et al. 2014). By adapting tools developed for veterinary research, this study showed that the immune response in wild animals is complex, and has important effects on the health and life histories of wild animals (Garnier and Graham 2014; Nussey et al. 2014).

To truly understand how immune phenotypes correlate with life-history traits and how life-history traits shape immune phenotypes we also need to tease apart acclimated, plastic responses from evolved responses (Schmid-Hempel 2003). Evidence from domestic and laboratory animals indicate that immune responses are heritable and predictive of survival and reproductive capacity (Schmid-Hempel 2003). For example, antibody titers both can be altered by artificial selection and predict survival in chickens (Siegel and Gross 1980; Sun et al. 2011). However, immune responses in the wild are highly plastic and depend on availability of resources, stage of the annual cycle, parasite loads in the environment, and history of exposure to parasites, among other factors (Lee 2006; Buehler et al. 2008; Love et al. 2008; Pedersen and Babayan 2011; Horrocks et al. 2012; Bashir-Tanoli and Tinsley 2014). Experiments across multiple generations in the wild must be conducted to assess the quantitative genetics underlying immune responses and the heritability of these traits.

Ecoimmunology as a mechanism in disease ecology

In addition to helping link physiology and the evolution of life history, understanding how, and why, immune defenses vary will reveal important mechanisms underlying the dynamics of disease both at ecological and evolutionary time-scales (Adelman et al. 2014). Although classic epidemiological models assume random variation in organisms' responses to infection (Anderson and May 1979), recent work has shown that incorporating more realistic details of such heterogeneity can improve predictions of the spread and persistence of pathogens. For example, in many infectious diseases, individual variation in infectiousness follows a highly skewed distribution, with very few individuals causing the majority of new cases (Woolhouse et al. 1997). In a study of one such disease, severe acute respiratory syndrome, Lloyd-Smith et al. (2005) illustrated that models incorporating this heterogeneity predicted rarer, more rapidly-spreading epidemics and argued for use of different types of public health interventions than did models assuming more normally-distributed variation in infectiousness. In addition, models of host–pathogen coevolution have shown that the precise nature of trade-offs between immune defenses and other life-history traits can greatly alter the evolution of a pathogen's virulence, or its propensity to harm infected hosts (Best et al. 2009). Recent advances in theoretical modeling also have enabled increased integration of within-organism processes (e.g., immune-mediated reductions in pathogens' replication) and between-organism processes (e.g., transmission of pathogens) (Alizon and van Baalen 2008; Mideo et al. 2008; Day et al. 2011). Although accurately parameterizing such models can prove challenging, theoreticians and empiricists have begun to forge partnerships across disciplines, producing models that incorporate immunological details to predict the dynamics of infectious diseases more accurately (Alizon and van Baalen 2008; Day et al. 2011; Hawley and Altizer 2011; Klein et al. 2014). For example, by modeling both host immune defenses and within-host evolution of the hepatitis C virus, Luciani and Alizon (2009) showed that cross-reactivity of immune responses can be a crucial determinant of the chronicity of infection and the probability of transmission. Similarly, understanding the mechanisms that affect survival following infection will help improve management decisions when new diseases emerge. For example, a literature review of the survival of different amphibian species infected with *Batrachochytrium dendrobatidis* (Bd) underscores the complicated interactions between genetics and environment that drive disease outcomes. New genomics approaches will help unlock these interactions by identifying key genomic traits that enhance

survival after infection with Bd and thus focus conservation priorities on those species and regions most likely to be saved (Longo et al. 2014).

On area of particular importance in linking ecoimmunology and disease ecology is elucidating the mechanisms that underlie two distinct classes of responses to infection: resistance and tolerance. Resistance involves reducing pathogen numbers (i.e., a host's ability to kill invading organisms), whereas tolerance involves minimizing fitness losses per unit pathogen (Caldwell et al. 1958; Simms 2000; Råberg et al. 2009). Although tolerant hosts may not substantially reduce the numbers of an invading pathogen, they can maintain a higher degree of fitness in the face of infection. Given that hosts that are tolerant or resistant can harbor very different levels of pathogens, survive for different lengths of time, or both, theoretical studies have shown that tolerance and resistance can have complex, sometimes opposite, consequences for the prevalence of diseases and for the evolution both of pathogens and hosts (Boots et al. 2009). For instance, in a classic model of the evolution of hosts, Roy and Kirchner (2000) showed that if resistance begins spreading through a host population, prevalence of the pathogen will decrease, which in turn reduces the fitness advantages of maintaining resistance. By contrast, if tolerance spreads through a host's population, the prevalence of the pathogen will increase, further increasing the fitness advantage of tolerance, potentially driving this trait to fixation. Although this example illustrates that tolerance and resistance can set hosts' populations on very different evolutionary trajectories, to fully understand the causes and consequences of tolerance and resistance, we must understand the physiological mechanisms that underlie differences in tolerance and resistance.

Although botanists have long-studied tolerance from a phenomenological perspective (Caldwell et al. 1958), immunologists have focused almost exclusively on mechanisms of resistance. As such, little work has addressed the possible physiological mechanisms that underlie tolerance. Recently, however, several authors have proposed that reductions in certain types of immune responses may facilitate tolerance (Råberg et al. 2009; Sears et al. 2011). Specifically, in vertebrates, inflammatory responses effectively kill a broad array of pathogens, and also induce significant damage to a host's own tissues, which could lead to a trade-off between resistance and tolerance. Long et al. (2008) showed support for this idea, as mice, in which a key pro-inflammatory signal had been blocked, showed reduced pathology (loss of mass; anemia), but higher internal levels of pathogen, during infection with rodent malaria (i.e., greater tolerance). Similarly, during infection with *Mycoplasma gallisepticum*, house finches with lower levels of pro-inflammatory signaling exhibited less severe pathology (loss of mass; conjunctivitis) for a given level of pathogen (again, greater tolerance) (Adelman et al. 2013). Although these examples suggest inflammatory signaling may help shape resistant versus tolerant phenotypes, the tools to assess markers of inflammation currently are limited in non-model organisms, hindering comparative work on the topic. Recent work by Fassbinder-Orth (2014), however, illustrates how researchers can adapt techniques based on gene-expression to measure myriad immune signals in non-models. With tools such as these in hand, ecoimmunologists will be able to probe inflammatory responses more accurately and address whether these responses truly underlie differences in resistance and tolerance.

Expanding the ecoimmunologist's toolkit

In order to expand our knowledge of the mechanisms that link immune systems with other physiological processes and with population-level phenomena, ecoimmunologists must continue to diversify and refine laboratory and field techniques. Although adapting techniques from more traditional, laboratory-based disciplines has been an important focus in ecoimmunology to date, progress in this area faces a number of challenges. For example, the development of species-specific reagents, antibodies, and probes can be expensive and time-consuming, and often requires volumes of samples not easily attainable in many species. One reasonably effective approach to this limitation has been to acquire commercially available reagents for closely (or sometimes not-so-closely) related model systems and adapt them for use in one's own study organism (e.g., by altering dilutions or sequences). Although this approach has allowed for slow but steady progress in a number of species, including addressing relationships between immune phenotypes and organismal fitness in wild mammals (Graham et al. 2010), it continues to hamper meaningful cross-species comparisons. That is, the lack of species-specific reagents does not allow one to determine with sufficient confidence whether observed differences across species reflect true biological differences or merely technical differences (e.g., how well the heterologous labeling antibodies bind across different species). These and other technical limitations have tempered the impressive growth in the field of ecoimmunology.

Despite these limiting forces, the current ecoimmunology toolkit has the potential to expand at an unprecedented rate, yielding new challenges to choosing techniques and standardizing protocols across studies (Boughton et al. 2011; Demas et al. 2011b). The costs of powerful new laboratory and field technologies have fallen dramatically in the past decade, opening the potential for ecoimmunologists and integrative biologists to uncover new physiological and ecological mechanisms driving their study systems. For example, the adaptation of traditional enzyme-linked immunosorbent assay (ELISA) techniques to novel systems can open up a range of questions about the dynamics of diseases in wild systems (Garnier and Graham 2014). In addition, the development of multiplex technologies for gene expression enables quantification of a wide range (and large number) of cytokines, chemokines, and immunologically-relevant markers in very small quantities of biological fluids (Bonneaud et al. 2011; Fassbinder-Orth 2014). Similarly, passive integrated transponders (PIT tags) can be affixed to wild animals from crickets to panthers, and read at any number of radio frequency identification stations, enabling both the monitoring of whole-organism responses to infection, including fever and reduced locomotion, as well as changes in inter-individual rates of contact (Bonter and Bridge 2011; Bridge and Bonter 2011; Adelman et al. 2014).

As these technologies have been developing at an accelerated pace, the questions ecoimmunologists hope to answer have also expanded. Increasingly, empirical studies and review papers seek direct, mechanistic links among individual variations in immune responses, other life-history traits, defenses against parasites, spread of disease, and, ultimately, fitness (Adelman et al. 2010; French et al. 2011; Graham et al. 2011). In addition, as cross-species reagents become more reliable and powerful, ecoimmunology can provide further insights into the complexities of pathogens that spread among multiple species of hosts (Previtali et al. 2012). Without tools that reliably link immune responses with the outcome of infection across species, we remain highly limited in our understanding of why species infected with the same pathogen differ so greatly in their resistance and tolerance, and what these differences mean for the persistence and evolution of pathogens (Kurtenbach et al. 2002; Rigaud et al. 2010). Answering such questions could cut across levels of biological organization, informing not only our understanding of how host–pathogen interactions influence ecological communities, but how, and when, diseases jump across species—an issue

of critical importance to the health both of humans and wildlife (Daszak et al. 2000; Keesing et al. 2010; Jones et al. 2008). Although the new technologies discussed here have allowed for substantial improvements in both breadth and depth within the still infant field of ecoimmunology; sustained progress in this area, however, will be necessary in order for the field to continue to mature.

Conclusions

A key goal of the 2014 SICB symposium on *Mechanisms and Methods in Ecological Immunology* was to confront the challenges discussed above, as well as others, with the important goal of enhancing ecoimmune investigations into the physiological basis of life-history trade-offs; the development of low-cost biomarkers for susceptibility to disease; and the investigation of the ecophysiological underpinnings of disease ecology, behavior, and the coevolution of host–parasite systems. Presentations in the symposium addressed the technical advances in, and crossover technologies from, disciplines associated with ecoimmunology, for example, genomics (Longo et al. 2014), neuroendocrine regulation (Adamo 2014), remote biomonitoring (Adelman et al. 2014), ELISAs for non-model species (Garnier and Graham 2014), and microsphere ELISAs (Fassbinder-Orth 2014) and how these advances can help us understand the mechanistic basis of immunological variability in wild species. There was also a focus on new statistical modeling techniques that will help address hypotheses at multiple levels of organization simultaneously (Downs and Dochtermann 2014). Given the rate at which new technologies are becoming available to all integrative biologists, not just ecoimmunologists, we will continue to face significant challenges of incorporating new methods. Through continued dialog and collaboration, however, we can ensure that the field of ecoimmunology is ready to confront these challenges.

Acknowledgments

Funding

References

Adamo SA. 2004. How should behavioural ecologists interpret measurements of immunity? Anim Behav 68:1443–9.

Adamo SA. 2014. The effects of stress hormones on immune function may be vital for the adaptive reconfiguration of the immune system during fight-or-flight behavior. Integr Comp Biol 54:419–26.

Adelman JS. Forthcoming 2015. Immune systems: linking organisms, populations, and evolution through disease. In: Martin LB, Ghalambor CK, Woods HA, editors. Integrative organismal biology. Hoboken (NJ): Wiley.

Adelman JS, Cordoba-Cordoba S, Spoelstra K, Wikelski M, Hau M. 2010. Radiotelemetry reveals variation in fever and sickness behaviours with latitude in a free-living passerine. Funct Ecol 24:813–23.

Adelman JS, Kirkpatrick L, Grodio JL, Hawley DM. 2013. House finch populations differ in early inflammatory signaling and pathogen tolerance at the peak of *Mycoplasma gallisepticum* infection. Am Nat 181:674–89.

Adelman JS, Moyers SC, Hawley DM. 2014. Using remote biomonitoring to understand heterogeneity in immune-responses and disease-dynamics in small, free-living animals. Integr Comp Biol 54:377–86.

Ahmed SA, Penhale WJ, Talal N. 1985. Sex hormones, immune responses, and autoimmune diseases: mechanisms of sex hormone action. Am J Pathol 121:531–51.

Alizon S, van Baalen M. 2008. Acute or chronic? within-host models with immune dynamics, infection outcome, and parasite evolution. Am Nat 172:E244–56.

Anderson RM, May RM. 1979. Population biology of infectious diseases: Part I. Nature 280:361–7.

Ardia DR, Parmentier HK, Vogel LA. 2011. The role of constraints and limitation in driving individual variation in immune response. Funct Ecol 25:61–73.

Ardia DR, Schat KA, Winkler DW. 2003. Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*). Proc R Soc Lond Ser B Biol Sci 270:1679–83.

Ashley NT, Weil ZM, Nelson RJ. 2012. Inflammation: mechanisms, costs, and natural variation. Annu Rev Ecol Evol Syst 43:385–406.

Bashir-Tanoli S, Tinsley MC. 2014. Immune response costs are associated with changes in resource acquisition and not resource reallocation. Funct Ecol 28:1011–9.

Baze MM, Schlauch K, Hayes JP. 2010. Gene expression of the liver in response to chronic hypoxia. Physiol Genomics 41:275–88.

Besedovsky HO, del Rey A. 1996. Immune–neuro–endocrine interactions: facts and hypotheses. Endocr Rev 17:64–102.

Best A, White A, Boots M. 2009. The implications of coevolutionary dynamics to host–parasite interactions. Am Nat 173:779–91.

Bonneaud C, Balenger SL, Russell AF, Zhang JW, Hill GE, Edwards SV. 2011. Rapid evolution of disease resistance is accompanied by functional changes in gene expression in a wild bird. Proc Natl Acad Sci USA 108:7866–71.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, Sorci G. 2003. Assessing the cost of mounting an immune response. Am Nat 161:367–79.

Bonter DN, Bridge ES. 2011. Applications of radio frequency identification (RFID) in ornithological research: a review. J Field Ornithol 82:1–10.

Boots M, Best A, Miller MR, White A. 2009. The role of ecological feedbacks in the evolution of host defence: what does theory tell us? Philos Trans R Soc B Biol Sci 364:27–36.

Boughton RK, Joop G, Armitage SAO. 2011. Outdoor immunology: methodological considerations for ecologists. Funct Ecol 25:81–100.

Bracken CP, Whitelaw ML, Peet DJ. 2003. The hypoxia-inducible factors: key transcriptional regulators of hypoxic responses. Cell Mol Life Sci 60:1376–93.

Bridge ES, Bonter DN. 2011. A low-cost radio frequency identification device for ornithological research. J Field Ornithol 82:52–9.

Brown EA, Pilkington JG, Nussey DH, Watt KA, Hayward AD, Tucker R, Graham AL, Paterson S, Beraldi D, Pemberton JM, et al. 2013. Detecting genes for variation in parasite burden and immunological traits in a wild population: testing the candidate gene approach. Mol Ecol 22:757–73.

Buchanan KL. 2000. Stress and the evolution of condition-dependent signals. Trends Ecol Evol 15:156–60.

Buehler DM, Piersma T, Matson K, Tieleman BI. 2008. Seasonal redistribution of immune function in a migrant shorebird: annual-cycle effects override adjustments to thermal regime. Am Nat 172:783–96.

Buehler DM, Versteegh MA, Matson KD, Tieleman BI. 2011. One problem, many solutions: simple statistical approaches help unravel the complexity of the immune system in an ecological context. PLoS One 6:e18592.

Caldwell RM, Schafer JF, Compton LE, Patterson FL. 1958. Tolerance to cereal leaf rusts. Science 128:714–5.

Cohen AA, Martin LB, Wingfield JC, McWilliams SR, Dunne JA. 2012. Physiological regulatory networks: ecological roles and evolutionary constraints. Trends Ecol Evol 27:428–35.

Cummins EP, Oliver KM, Lenihan CR, Fitzpatrick SF, Bruning U, Scholz CC, Slattery C, Leonard MO, McLoughlin P, Taylor CT. 2010. NF-kappa B links CO_2 sensing to innate immunity and inflammation in mammalian cells. J Immunol 185:4439–45.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science 287:443–9.

Day T, Alizon S, Mideo N. 2011. Bridging scales in the evolution of infectious disease life histories: theory. Evolution 65:3448–61.

De Jong G, Van Noordwijk AJ. 1992. Acquisition and allocation of resources: genetic (co)variances, selection, and life histories. Am Nat 139:749–70.

Demas GE, Adamo SA, French SS. 2011a. Neuroendocrine-immune crosstalk in vertebrates and invertebrates: implications for host defence. Funct Ecol 25:29–39.

Demas GE, Greives TJ, Chester EM, French SS. 2012. The energetics of immunity:mechanisms mediating trade-offs in eco-immunology. In: Demas GE, Nelson RJ, editors. Ecoimmunology. New York (NY): Oxford University Press. p. 257–96.

Demas GE, Nelson RJ, editors. 2012a. Ecoimmunology. New York (NY): Oxford University Press.

Demas GE, Nelson RJ. 2012b. Introduction to ecoimmunology. In: Demas GE, Nelson RJ, editors. Ecoimmunology. New York (NY): Oxford University Press. p. 3–6.

Demas GE, Zysling DA, Beechler BR, Muehlenbein MP, French SS. 2011b. Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. J Anim Ecol 80:710–30.

Downs CJ, Brown JL, Wone B, Donovan ER, Hunter K, Hayes JP. 2013. Selection for increased mass-independent maximal metabolic rate suppresses innate but not adaptive immune function. Proc R Soc B Biol Sci 280:20122636.

Downs CJ, Dochtermann NA. 2014. Testing hypotheses in ecoimmunology using mixed models: disentangling hierarchical correlations. Integr Comp Biol 54:407–18.

Downs CJ, Schutz H, Meek TH, Dlugosz EM, Acosta W, de Wolski KS, Malisch JL, Hayes JP, Garland T. 2012. Within-lifetime trade-offs but evoutionary freedom for hormonal and immunological traits: evidence from mice bred for high voluntary exercise. J Exp Biol 215:1651–61.

Fassbinder-Orth CA. 2014. Methods for quantifying gene expression in ecoimmunology: from qPCR to RNA-Seq. Integr Comp Biol 54:396–406.

Flatt T, Heyland A, Stearns SC. 2011. What mechanistic insighs can or cannot contribute to life history evolution: an exchange between Stearns, Heyland, and Flatt. In: Flatt T, Heyland A, editors. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. New York (NY): Oxford University Press. p. 375–9.

French SS, Dearing MD, Demas GE. 2011. Leptin as a physiological mediator of energetic trade-offs in ecoimmunology: implications for disease. Integr Comp Biol 51:505–13.

French SS, DeNardo DF, Moore MC. 2007a. Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? Am Nat 170:79–89.

French SS, Johnston GIH, Moore MC. 2007b. Immune activity suppresses reproduction in food-limited female tree lizards Urosaurus ornatus. Funct Ecol 21:1115–22.

French SS, Moore MC, Demas GE. 2009. Ecological immunology: the organism in context. Integr Comp Biol 49:246–53.

Garnier R, Graham AL. 2014. Insights from parasite-specific serological tools in eco-immunology. Integr Comp Biol 54:363–76.

Girard I, Garland T. 2002. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J Appl Physiol 92:1553–61.

Graham AL. 2013. Optimal immunity meets natural variation: the evolutionary biology of host defence. Parasite Immunol 35:315–7.

Graham AL, Hayward AD, Watt KA, Pilkington JG, Pemberton JM, Nussey DH. 2010. Fitness correlates of heritable variation in antibody responsiveness in a wild mammal. Science 330:662–5.

Graham AL, Shuker DM, Pollitt LC, Auld S, Wilson AJ, Little TJ. 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. Funct Ecol 25:5–17.

Gross WG, Siegel PB, Hall RW, Domermuth CH, Duboise RT. 1980. Production and persistence of antibodies in chickens to sheep erythrocytes 2. Resistance to infectious-diseases. Poult Sci 59:205–10.

Gustafsson L, Nordling D, Andersson MS, Sheldon BC, Qvarnstrom A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. Phil Trans R Soc Lond Ser B Biol Sci 346:323–31.

Hasselquist D, Nilsson JA. 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? Anim Behav 83:1303–12.

Hawley DM, Altizer SM. 2011. Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. Funct Ecol 25:48–60.

Hayden MS, Ghosh S. 2008. Shared principles in NF-$\kappa\beta$ signaling. Cell 132:344–62.

Hõrak P, Tegelmann L, Ots I, Moller AP. 1999. Immune function and survival of great tit nestlings in relation to growth conditions. Oecologia 121:316–22.

Horrocks NPC, Hegemann A, Matson KD, Hine K, Jaquier S, Shobrak M, Williams JB, Tinbergen JM, Tieleman BI. 2012. Immune indexes of larks from desert and temperate regions show weak associations with life history but stronger links to environmental variation in microbial abundance. Physiol Biochem Zool 85:504–15.

Hotchkiss AK, Nelson RJ. 2002. Melatonin and immune function: hype or hypothesis? Crit Rev Immunol 22:351–71.

Iseri VJ, Klasing KC. 2013. Dynamics of the systemic components of the chicken (Gallus gallus domesticus) immune system following activation by Escherichia coli; implications for the costs of immunity. Dev Comp Immunol 40:248–57.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. 2008. Global trends in emerging infectious diseases. Nature 451:990–4.

Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, et al. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468:647–52.

Keil D, Luebke RW, Pruett SB. 2001. Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. J Immunol 167:4543–52.

Klasing KC, Leshchinsky TV. 1999. Functions, costs, and benefits of the immune system during development and growth. Johannesburg: BirdLife South Africa.

Klein EY, Graham AL, Llinas M, Levin S. 2014. Cross-reactive immune responses as primary drivers of malaria chronicity. Infect Immun 82:140–51.

Kurtenbach K, De Michelis S, Etti S, Schafer SM, Sewell HS, Brade V, Kraiczy P. 2002. Host association of *Borrelia burgdorferi* sensu lato—the key role of host complement. Trends Microbiol 10:74–9.

Lee KA. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integr Comp Biol 46:1000–15.

Lee KA, Wikelski M, Robinson WD, Robinson TR, Klasing KC. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. J Anim Ecol 77:356–63.

Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. 2005. Superspreading and the effect of individual variation on disease emergence. Nature 438:355–9.

Lochmiller RL, Deerenberg C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.

Long GH, Chan BHK, Allen JE, Read AF, Graham AL. 2008. Blockade of TNF receptor 1 reduces disease severity but increases parasite transmission during *Plasmodium chabaudi chabaudi* infection. Int J Parasitol 38:1073–81.

Longo AV, Burrowes PA, Zamudio KR. 2014. Genomic studies of disease-outcome in host–pathogen dynamics. Integr Comp Biol 54:427–38.

Love OP, Salvante KG, Dale J, Williams TD. 2008. Sex-specific variability in the immune system across life-history stages. Am Nat 172:E99–112.

Luciani F, Alizon S. 2009. The evolutionary dynamics of a rapidly mutating virus within and between hosts: the case of hepatitis C virus. PLoS Comput Biol 5:e1000565.

Majmundar AJ, Wong WJ, Simon MC. 2010. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40:294–309.

Malisch J, Breuner C, Gomes F, Chappell M, Garland T. 2008. Circadian pattern of total and free corticosterone concentrations, corticosteroid-binding globulin, and physical activity in mice selectively bred for high voluntary wheel-running behavior. Gen Comp Endocrinol 156:210–7.

Malisch JL, Saltzman W, Gomes FR, Rezende EL, Jeske DR, Garland T. 2007. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. Physiol Biochem Zool 80:146–56.

Martin LB, Hawley DM, Ardia DR. 2011a. An introduction to ecological immunology. Funct Ecol 25:1–4.

Martin LB, Hopkins WA, Mydlarz LD, Rohr JR. 2010. The effects of anthropogenic global changes on immune functions and disease resistance. Ann N Y Acad Sci 1195:129–48.

Martin LB, Liebl AL, Trotter JH, Richards CL, McCoy K, McCoy MW. 2011b. Integrator networks: illuminating the black box linking genotype and phenotype. Integr Comp Biol 51:514–27.

Martin LB, Weil ZM, Nelson RJ. 2006. Refining approaches and diversifying directions in ecoimmunology. Integr Comp Biol 46:1030–9.

Martin LB, Weil ZM, Nelson RJ. 2007. Immune defense and reproductive pace of life in Peromyscus mice. Ecology 88:2516–28.

Martin LB, Weil ZM, Nelson RJ. 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Phil Trans R Soc B Biol Sci 363:321–39.

Matson KD, Cohen AA, Klasing KC, Ricklefs RE, Scheuerlein A. 2006. No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. Proc R Soc B Biol Sci 273:815–22.

Mideo N, Alizon S, Day T. 2008. Linking within- and between-host dynamics in the evolutionary epidemiology of infectious diseases. Trends Ecol Evol 23:511–7.

Moreno J, Merino S, Sanz JJ, Arriero E, Morales J, Tomas G. 2005. Nestling cell-mediated immune response, body mass and hatching date as predictors of local recruitment in the pied flycatcher *Ficedula hypoleuca*. J Avian Biol 36:251–60.

Moreno J, Sanz JJ, Merino S, Arriero E. 2001. Daily energy expenditure and cell-mediated immunity in pied flycatchers while feeding nestlings: interaction with moult. Oecologia 129:492–7.

Nordling D, Andersson M, Zohari S, Gustafsson L. 1998. Reproductive effort reduces specific immune response and parasite resistance. Proc R Soc B Biol Sci 265:1291–8.

Norris K, Evans MR. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav Ecol 11:19–26.

Nussey DH, Watt KA, Clark A, Pilkington JG, Pemberton JM, Graham AL, McNeilly TN. 2014. Multivariate immune defences and fitness in the wild: complex but ecologically important associations among plasma antibodies, health and survival. Proc R Soc B Biol Sci 281:20132931.

Parmentier HK, Lammers A, Hoekman JJ, Reilingh GD, Zaanen ITA, Savelkoul HFJ. 2004. Different levels of natural antibodies in chickens divergently selected for specific antibody responses. Dev Comp Immunol 28:39–49.

Pedersen AB, Babayan SA. 2011. Wild immunology. Mol Ecol 20:872–80.

Previtali MA, Ostfeld RS, Keesing F, Jolles AE, Hanselmann R, Martin LB. 2012. Relationship between pace of life and immune responses in wild rodents. Oikos 121:1483–92.

Prokop JW, Schmidt C, Gasper D, Duff RJ, Milsted A, Ohkubo T, Ball HC, Shawkey MD, Mays HL Jr, Cogburn LA. 2014. Discovery of the elusive leptin in birds: identification of several 'missing links' in the evolution of leptin and its receptor. PLoS One 9:e92751.

Råberg L, Graham AL, Read AF. 2009. Decomposing health: tolerance and resistance to parasites in animals. Phil Trans R Soc B Biol Sci 364:37–49.

Reznick D, Bryant MJ, Bashey F. 2002. r- and K-selection revisited: the role of population regulation in life-history evolution. Ecology 83:1509–20.

Ricklefs RE, Wikelski M. 2002. The physiology/life-history nexus. Trends Ecol Evol 17:462–8.

Rigaud T, Perrot-Minnot MJ, Brown MJF. 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proc R Soc B Biol Sci 277:3693–702.

Robinson DP, Klein SL. 2012. Sex differences in immune responses to viruses. In: Demas GE, Nelson RJ, editors. Ecoimmunology. New York (NY): Oxford University Press. p. 497–529.

Roy BA, Kirchner JW. 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54:51–63.

Sadd BM, Schmid-Hempel P. 2009. PERSPECTIVE: principles of ecological immunology. Evol Appl 2:113–21.

Sandmeier FC, Tracy RC. 2014. The metabolic pace-of-life model: incorporating ectothermic organisms into the theory of vertebrate ecoimmunology. Integr Comp Biol 54:387–95.

Sanz JJ, Moreno J, Merino S, Tomas G. 2004. A trade-off between two resource-demanding functions: post-nuptial moult and immunity during reproduction in male pied flycatchers. J Anim Ecol 73:441–7.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89.

Schmid-Hempel P. 2003. Variation in immune defence as a question of evolutionary ecology. Proc R Soc Lond Ser B Biol Sci 270:357–66.

Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT. 2009. Ecological immunology. Phil Trans R Soc B Biol Sci 364:3–14.

Schuurs A, Verheul HAM. 1990. Effects of gender and sex steroids on the immune response. J Steroid Biochem Mol Biol 35:157–72.

Sears BF, Rohr JR, Allen JE, Martin LB. 2011. The economy of inflammation: when is less more? Trends Parasitol 27:382–7.

Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–21.

Sibly RM, Calow P. 1986. Physiological ecology of animals: an evolutionary approach. Oxford: Blackwell Scientific Publications.

Siegel PB, Gross WB. 1980. Production and persistence of antibodies in chickens to sheep erythrocytes 1. Directional selection. Poult Sci 59:1–5.

Siegel PB, Gross WB, Cherry JA. 1982. Correlated responses of chickens to selection for production of antibodies to sheep erythrocytes. Anim Blood Groups Biochem Genet 13:291–7.

Simms EL. 2000. Defining tolerance as a norm of reaction. Evol Ecol 14:563–70.

Stearns SC. 1992. The evolution of life histories. New York (NY): Oxford University Press.

Stearns SC. 2011. Does impressive progress on understanding mechanisms advance life history theory? In: Flatt T, Heyland A, editors. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. New York (NY): Oxford University Press. p. 365–74.

Sun Y, Parmentier HK, Frankena K, van der Poel JJ. 2011. Natural antibody isotypes as predictors of survival in laying hens. Poult Sci 90:2263–74.

Swallow JG, Hayes JP, Koteja P, Garland T Jr. 2009. Selection experiments and experimental evolution of performance and physiology. In: Garland T Jr, Rose MR, editors. Experimental evolution: concepts, methods, and applications of selection experiments. Berkeley (CA): University of California Press. p. 752.

Van Noordwijk AJ, De Jong G. 1986. Acquisition and allocation of resources - their influence on variation in life-history tactics. Am Nat 128:137–42.

Viney ME, Riley EM, Buchanan KL. 2005. Optimal immune responses: immunocompetence revisited. Trends Ecol Evol 20:665–9.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat 100:687–90.

Woolhouse MEJ, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, Hagan P, Hii JLK, Ndhlovu PD, Quinnell RJ, et al. 1997. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc Natl Acad Sci USA 94:338–42.

Zera AJ, Harshman LG. 2001. The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95–126.

Zimmerman LM, Bowden RM, Vogel LA. 2014. A vertebrate cytokine primer for eco-immunologists. Funct Ecol published online (doi:10.1111/1365-2435.12273).