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SYMPOSIUM

How Do Migratory Species Stay Healthy Over the Annual Cycle? A Conceptual Model for Immune Function and For Resistance to Disease

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Synopsis Migration has fascinated researchers for years and many active areas of study exist. However, the question of how migratory species stay healthy within the context of their annual cycle remains relatively unexplored. This article addresses this question using Red Knots (*Calidris canutus*) as a model migrant species. We review recent research on immune function in Red Knots and integrate this work with the broader eco-immunological literature to introduce a conceptual model. This model synthesizes earlier ideas about resource allocation and the costs of immunity with recent increases in our knowledge about the vertebrate immune system and then puts these concepts into the context of defense against real pathogens in environments where a myriad of factors change in time and space. We also suggest avenues for further research, which will help to test the model and better link measures of immune function to pressure from pathogens and to optimal defense against disease.

Introduction

Researchers have been fascinated by migration for years and many active areas of study address questions such as how migrants withstand the physiological demands of their travels or how they find their way (Alerstam 1990). However, the question of how migratory species stay healthy, within the broader context of their annual cycle, remains relatively unexplored. This question is especially intriguing since the immune system that protects migrants from pathogens on their travels also comes with costs that must be balanced against other important aspects of migrant life. This article addresses the question of how migratory species stay healthy and uses Red Knots (*Calidris canutus*) as a model. Red Knots are medium-sized shorebirds and are ideal for studying relationships between migration and health because their migratory flyways, physiology, and ecology are well studied (Piersma 2007), and be-cause, recently, much research on Red Knots has

been devoted to clarifying our understanding of immune function over the annual cycle and in different environments (Buehler and Piersma 2008; Buehler et al. 2008a, 2008b, 2008c, 2009a, 2009b, 2009c, 2010). In this article we synthesize earlier ideas about resource allocation and the costs of immunity with recent increases in our knowledge about the vertebrate immune system. We then put these concepts into the context of defense against real pathogens in environments where a myriad of factors change in time and space. Finally, we suggest avenues for further research, which will help to test the model and better link measures of immune function to actual defense against disease.

To understand how migratory species stay healthy over the annual cycle, a good starting point is the basic question: When are the 'toughest' times of the year? Understanding when animals face 'tough times' or 'bottlenecks' allows predictions about when immune function might be decreased due to trade-offs,

or increased due to high pressure from pathogens. Buehler and Piersma (2008) addressed this question by introducing a framework of bottlenecks that constrain Red Knots during their annual cycle. Using the quality of breeding plumage and the timing of molt as indicators, they concluded that nutritional, energetic, temporal (time-limited), and disease-risk bottlenecks vary throughout the year, and that bottlenecks overlap during northward migration and arrival on the breeding grounds in spring, making this period the 'toughest'. Thus, from the perspective of resource limitation, relatively low immune function might be predicted during migration and early breeding. However, from the perspective of pathogen pressure, during migration, migrants travel through a variety of environments harboring potential pathogens, which argues for relatively high levels of defense (Møller and Erritzøe 1998). This apparent paradox highlights the fact that researchers interested in immune function must consider conflicting predictions stemming from the perspective of the allocation of resources on the one hand and the need for defense on the other.

To resolve this problem it is essential to realize that neither immune function nor risks from pathogens are monolithic entities. That immune function is complex and multi-faceted is now well recognized (Adamo 2004; Lee 2006; Matson et al. 2006; Martin et al. 2006b). Different types of immune function have different costs and benefits (Schmid-Hempel and Ebert 2003; Klasing 2004; Lee 2006); thus not only may the strength of an immune response vary in time and space, but so also may the type of immune response initiated. To make this point clear it is important to give a brief introduction to the immune system and the costs of immune function.

The vertebrate immune system and its costs

A good way to understand the immune system is to follow the path of a hypothetical pathogen (Fig. 1; sequence of events from Murphy et al. [2008]). This path begins outside the body where the invader must first overcome the body's physical (i.e., skin), chemical (i.e., pH changes in the gut) and behavioral (i.e., preening) barriers. Once a pathogen enters the body it encounters 'constitutive' (always present) (see glossary for definitions of words in italics) aspects of the immune system (Schmid-Hempel and Ebert 2003). These include soluble blood proteins, non-specific (natural) antibodies, and surveillance cells of the immune system such as phagocytes (macrophages and granulocytes) and lymphocytes (natural killer

cells; arrows labeled 1 in Fig. 1). These cells engulf invaders and release soluble mediators (cytokines) that attract more phagocytes and dendritic cells to the site of infection. For many pathogens the path ends here, and these non-specific cells and soluble proteins can clear the infection within a few hours. However, if the pathogen is persistent, macrophages release pro-inflammatory cytokines initiating 'induced' aspects of immune function (Schmid-Hempel and Ebert 2003). These include non-specific inflammation and the acute-phase response (APR: arrow 2 in Fig. 1) producing symptoms such as lethargy (low energy), anorexia (loss of appetite), and fever. Concurrently, the liver produces acute-phase proteins and diverts amino acids away from normal processes (i.e., growth or reproduction). While non-specific defenses control the infection, antigen-presenting cells (i.e., dendritic cells), which have engulfed the pathogen, are migrating to the lymph nodes or spleen. These cells present pathogen peptides on major histocompatibility complex (MHC) receptors to T-cells for specific recognition (arrow 3 in Fig. 1). This initiates 'acquired immune function' and over the next few days T- and B-cells that specifically recognize the pathogen will proliferate. Depending on the type of pathogen first recognized by the antigen presenting cells, cytokines are released that determine the character of the acquired immune response. Cytokines (and the cytokine milieu) are instrumental in the development and maturation of helper-T-cells. In mammals, at least four subsets of helper-T-cells have been discovered, and each subset seems targeted against different types of invaders (Reiner 2007): type-1-helper-T-cells (Th1) mediate cytotoxic T-cell responses against intracellular pathogens such as viruses, type-2-helper-T-cells activate B-cells and drive antibody-based responses against extracellular pathogens such as parasitic worms, type-17-helper-T-cells (Th17) mediate acute inflammation at epithelial surfaces against extracellular bacteria and prokaryotes, finally regulatory helper-T-cells (T_{reg}) provide counter regulation; arrows 4 and 5 in Fig. 1). These aspects of the acquired immune response (i.e., cytotoxic T-cells, specific antibodies, inflammatory or regulatory cytokines) will feed back into aspects of 'innate immunity' to greatly increase the efficiency of the response by specifically marking the pathogen for destruction. After the infection has been cleared, memory cells (both T and B types) remain, providing a swift and specific response in the event that the same pathogen is encountered again (Murphy et al. 2008).

All of the aspects of immune function described above have costs as well as benefits. At the ecological
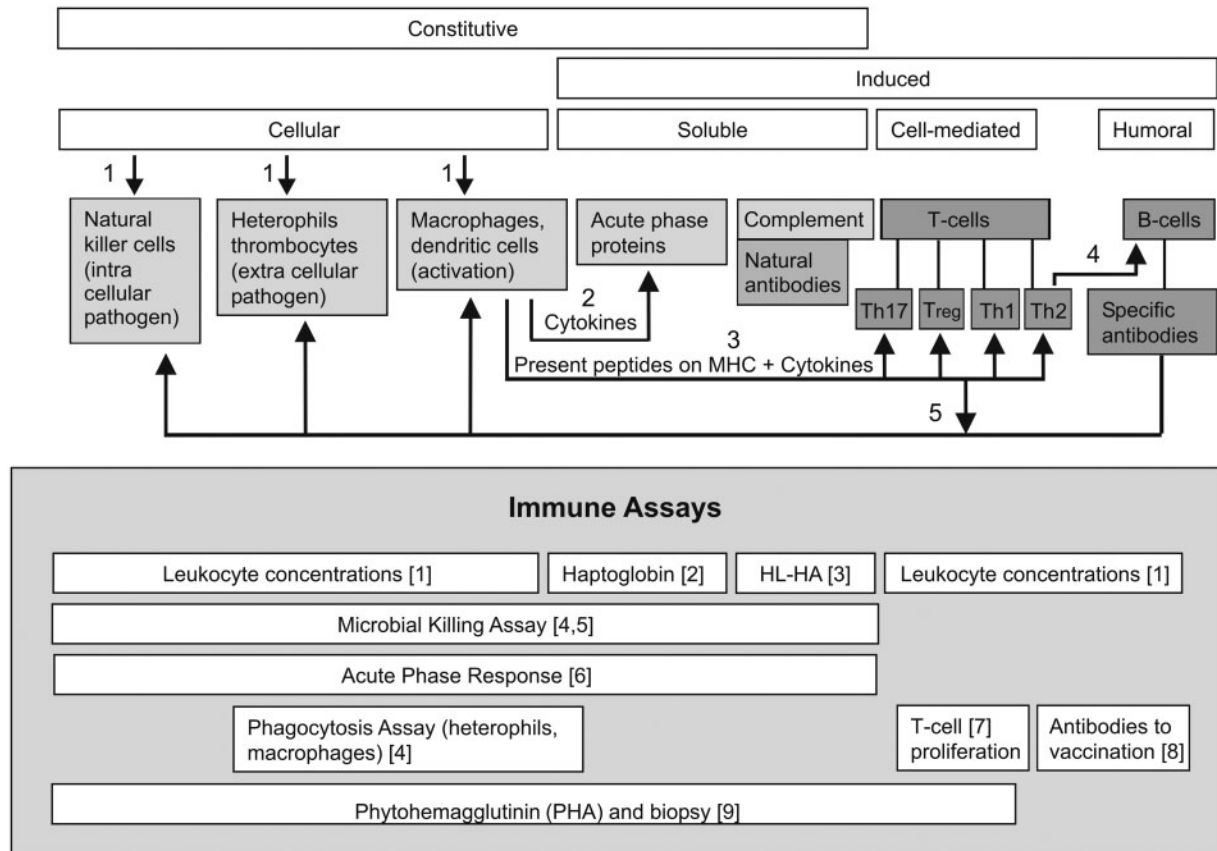
Fig. 1 A simplified representation of the immune system. The cells and soluble mediators of immunity are shown below the boxed categories; shading represents the specificity of each mediator. Non-specific mediators of immunity (innate immunity) are shaded light grey and specific mediators of immunity (acquired immunity) are shaded dark grey. Natural antibodies have relatively broad specificity and are represented by intermediate shading. Thick lines with arrows show the approximate path of a pathogen through the immune system during an immune response (see text). Boxed categories refer to constitutive immunity, which is constantly maintained and induced immunity, which is triggered by a challenge. Cellular and soluble components refer to mediators that are either cells or are found in the body fluids. The terms cell-mediated and humoral immunity are by convention restricted to immunity mediated by T-, B-cells or antibodies. Commonly used assays to measure immune function are represented by boxes positioned below the aspects of immunity they quantify. (1) Campbell and Ellis (2007), (2) haptoglobin-like activity rather than the actual haptoglobin protein is measured (Matson 2006), (3) HL-HA: hemolysis-hemagglutination (Matson et al. 2005), (4) Millet et al. (2007), (5) Tieleman et al. (2005), (6) Bonneaud et al. (2003), (7) Bentley et al. (1998), (8) Hasselquist et al. (1999), (9) Martin et al. (2006a).

time scale, the maintenance and use of the immune system comes with 'resource costs,' paid in nutrients, energy or time, that are important for both immune function and for other aspects of the host's life (Zuk and Stoehr 2002; Schmid-Hempel 2003). For example, nutrients and energy used in an APR are no longer available for migration or reproduction. In the same way, time lost when an animal experiences lethargy as part of an APR can no longer be used for migration or reproduction (Owen-Ashley and Wingfield 2007; Adelman and Martin 2009). Such trade-offs have been found in sparrows, which show reduced mating behavior and parental care after an APR is induced (Bonneaud et al. 2003; Owen-Ashley et al. 2006; Owen-Ashley and

Wingfield 2006). Additionally, 'immunopathology costs,' paid in collateral damage to the host, arise when the immune system causes damage to the host as well as to invaders (Råberg et al. 1998; Graham et al. 2005; Reiner 2007). The risk of immunopathology may increase during heavy physical exercise, when muscle damage and heat-shock proteins stimulate the immune system in the same way as damage caused by infection, causing a response directed against the host (Råberg et al. 1998). This raises the question of whether migrants are more likely to tolerate pathogens, rather than to resist them, during periods of migration (Råberg et al. 2009), but a full discussion of this idea is beyond the scope of the present article.

Negative relationships between components of immune function and other traits relevant to host fitness, may, over longer timescales, become fixed as a negative genetic covariance (Stearns 1992). This can be thought of as an 'evolutionary cost' (Zuk and Stoehr 2002; Schmid-Hempel 2003). For example, that turkeys (*Meleagris gallopavo*) selected for higher body mass and egg production show reduced immune function (Nestor et al. 1996), suggests that the immune system may evolve at the expense of some other trait, or vice versa.

Within these general costs, each aspect of immune function requires different resources in different quantities and carries different risks of immunopathology. As a result, different aspects of immune function may be used during different times of the year or in different environments. For example, indices of immune function, measured monthly in Red Knots and analyzed using principle-component analysis, indicated that aspects of immune function co-varied (grouped together) in similar ways among-individuals as well as over time (Buehler et al. 2008b). Interpretation of these groups of immune indices from a cost-benefit perspective suggested that some aspects of immune function (those associated with non-specific phagocytosis and inflammation) were more costly than others (those associated with aspects of acquired immunity). Over the annual cycle, the costly aspects seemed to be used only when their benefits outweighed their costs (during change in mass in preparation for migration and breeding) and may have been down-regulated when their costs outweighed their benefits (during molt).

In the same study, temperature was manipulated to determine the effect of increased expenditure of energy on constitutive immune function, but little effect was found under conditions of unlimited resources (Buehler et al. 2008b). Access to food was then manipulated, but as with energy expenditure, little effect was found on constitutive immune function (Buehler et al. 2009a). However, when birds were challenged with lipopolysaccharide (LPS) to induce an APR, knots enduring limited access to food adjusted aspects of the APR. These studies suggest that even under conditions of increased energy expenditure or limited resources, a baseline level of constitutive immune function is maintained, while birds save energy on more costly aspects of immune defense such as the APR (Klasing 2004).

Finally, more costly aspects of immune function, associated with non-specific phagocytosis and inflammation, were found to be lower in knots living in captivity than those in the wild (Buehler et al. 2008c). If birds are released from trade-offs in the benign conditions of captivity, then this result is surprising from the perspective of resource allocation. However, from a pathogen-risk perspective, in captivity where cleaning regimes are likely to decrease pressure from pathogens (at least for Red Knots), the costs of certain aspects of immunity might outweigh their benefits.

The work on knots focused mainly on aspects of constitutive immune function; however, studies on other animals illustrate the costs associated with other aspects of immunity (see Supplementary Tables S1–S3 for a literature review that is useful for identifying patterns and inconsistencies, but is not exhaustive). Aspects of induced but non-specific immune function such as the APR and inflammation have been studied using injections of LPS and phytohemagglutinin (PHA). In sparrows, males reduced territorial aggression and song after treatment with LPS (Owen-Ashley and Wingfield 2006; Owen-Ashley et al. 2006) and females reduced their feeding of chicks or even abandoned their young (Bonneaud et al. 2003). This suggests trade-offs or constraint, a result that is not surprising given the APR's requirements for nutrients, energy and time (Klasing 2004). Inflammation induced by injecting PHA is also negatively related to reproduction in experimental studies of birds (Ilmonen et al. 2003; Martin et al. 2004; Greenman et al. 2005) suggesting trade-offs or constraint. This response also appears to be condition-dependent, resource-dependent, and energetically costly (Gonzalez et al. 1999; Alonso-Alvarez and Tella 2001; Lifjeld et al. 2002; Ilmonen et al. 2003; Martin et al. 2003; Navarro et al. 2003; Owen and Moore 2008), but it is not traded-off when physical workload (not related to reproduction) is increased (Soler et al. 1999; Hasselquist et al. 2007). Using PHA to induce swelling of the wing web in birds has also been used as a measure of acquired immunity mediated by Th1-cells; however, this assay likely represents aspects of non-specific inflammation as well as Th1-cell-mediated responses (Martin et al. 2006a). More specific measures of Th1 responses have been carried out in humans, in whom Th1-related cytokines are lower during pregnancy (Wegmann et al. 1993), again suggesting trade-offs or constraints. In contrast, Th2-related cytokines are higher during pregnancy (Wegmann et al. 1993). In birds Th2-related responses, measured by inducing specific antibodies, do not appear to be dependent upon condition, nor are they energetically very costly (Gonzalez et al. 1999; Hasselquist et al. 2007). The same is true for various aspects of constitutive immune function (Soler et al. 1999; Ilmonen et al. 2003; Tieleman et al. 2008; Buehler et al. 2009a).

These studies support the idea that different aspects of immune function have different costs, and that different types of immunity may be used under different circumstances. When different aspects of immune function are used, depends upon the resources available to the animal and the need for defense (i.e., the level and type of pathogen threat). When we place this simple observation into the context of what we are learning about the vertebrate immune system, it becomes possible to more precisely define ideas about the costs and benefits of different aspects of immune function. Synthesizing results from the eco-immunological studies described above with ideas from immunology (Klasing 2004; Reiner 2007; Clark 2008; Murphy et al. 2008), we suggest the following framework for the costs of immunity: aspects of constitutive immunity are a first line of defense and appear to be maintained at a baseline level, despite fluctuations in energy balance; however, these indices may vary with pathogen pressure or 'current infection.' The induced APR and non-specific inflammation are essential defenses against novel pathogens when aspects of constitutive immunity are not enough; however, these responses should be tightly regulated because they are costly in terms of nutrients, energy and time, and carry high risks for immunopathology. Cytotoxic-T-cell-mediated immunity (Th1), as well as acute inflammatory responses (Th17) are essential for pathogen-specific defense against intracellular invaders and extracellular invaders at epithelial surfaces, but may be costly in terms of nutrients, energy, and immunopathology. Immunity associated with specific antibody defense against extracellular pathogens (Th2) has relatively low cost for maintenance and use. All acquired helper-T-cell responses have relatively high costs during development (when B- and T-cell repertoires are generated) and have long latency periods (Klasing 2004).

Illustrating the concept of immune potential in red knots

To consolidate ideas about resource allocation and pathogen pressure introduced by Buehler and Piersma (2008), with ideas about immune costs and benefits discussed above, we now introduce a model linking resources limited during bottlenecks with resources needed to pay immune costs. The assumptions of the model are presented in Table 1 following the style used by Scheiner and Willig (2008).

The costs of immune function discussed above can be roughly matched to the bottlenecks introduced by Buehler and Piersma (2008). Nutritional and

Table 1 Assumptions made by the actual defense model which puts ideas about allocation of resources and the costs of immunity into the broader context of defense against real pathogens in environments where a myriad of factors change in time and space

1. Organisms stay healthy by having a system of defense and maintenance that is variable in time and space.
2. Diversity in the system depends on genetic and environmental factors.
3. Pathogens and commensal microbes exert selection pressure on the system.
4. Microbes are distributed heterogeneously over space and time.
5. The system has costs as well as benefits and is therefore constrained by genetic and environmental factors.
6. Resources are finite and are distributed heterogeneously over space and time.
7. Other expenses which compete with defense for resources are also distributed heterogeneously over space and time.

We follow the style used by Scheiner and Willig (2008).

energetic bottlenecks link to resource costs paid in nutrients and energy, while temporal bottlenecks correspond to resource costs paid in time. Furthermore, because extreme energy expenditure can increase the risk of immunopathology (Råberg et al. 1998), energetic bottlenecks can also be linked to immunopathological costs. During a bottleneck, nutrients, energy, and time are limited and only what is leftover can be used to pay the costs of immunity. Any surplus thus represents the inverse strength of a bottleneck and can be considered 'capital' that can be used for investment in immunity. Because capital is saved, and the costs of immune function are paid in similar currencies, we can now more precisely define the concept of immune potential. 'Immune potential' refers to the aspects of immune function at an animal's disposal given its circumstances at a particular time and place. Immune potential is constrained by bottlenecks, which limit the amount of available capital to invest in any given aspect of immune function. Because bottlenecks vary in time and space, the amount of capital available for investment in immune function, and thus immune potential, will also vary in time and space.

We give three examples using Red Knots to illustrate the idea of immune potential. First, looking at variation in immune potential over time during the annual cycle, time, and energy are most limited during breeding and migration (Buehler and Piersma 2008). Thus, during those periods immune potential for aspects of immune function such as the APR might be dampened since the costs of the lethargy and anorexia might compromise reproduction or delay migration. Furthermore, immune potential

for non-specific inflammation might also be damp-ened, thereby decreasing the risk of immunopathol-ogy during flight. Second, using the contrasting annual cycles of *C. c. islandica* and *C. c. rufa* to ex-amine variation in immune potential between sub-species, one might predict that *C. c. islandica* (that migrates the shortest distance) would have the most capital to invest and *C. c. rufa* (that migrates the longest distance) the least. Taking the resource of time as an example, this is true. *C. c. rufa* spend nearly 8 months of the year either migrating or reproducing (Buehler and Piersma 2008); thus they have very little time to spare for the lethargy that accompanies an APR. However, if we take the re-source of nutrients as an example, *C. c. islandica*, wintering in the North Temperate Zone, run the risk of high-energy expenditure combined with lim-ited food resources if their feeding areas freeze over during severe winter weather (Vézina et al. 2006), while when *C. c. rufa* finally arrive in their south temperate wintering areas food resources are abun-dant (van Gils et al. 2005). Thus, aspects of immune function requiring nutrients and energy (i.e., the APR) may be more limited on the wintering grounds in *C. c. islandica* than in *C. c. rufa*. Third, it is im-portant to remember that immune potential can vary in space as well as in time. Regardless of its subspe-cies, a Red Knot wintering in a habitat of high qual-ity is more likely to have the potential for the full compliment of immune responses, whereas another individual of the same subspecies wintering in a hab-itat of poor quality may have limited immune po-tential for more costly aspects of immune function.

## Generalizing the model: required response and actual defense

Having linked bottlenecks that fluctuate in time and space with limited resources used as capital for in-vestment in immune function, it is now time to place this perspective on allocation of resources into the broader context of pressure from pathogens.

Our discussion of the costs of immunity has not yet highlighted the fact that the benefits of different aspects of the immune function are dependent on the pathogen at hand. Threats from pathogens come in a myriad of forms, and the actual pathogen load carried by a host organism depends not only on host susceptibly (including the hosts immune poten-tial), but also on the level of exposure to pathogens (a parameter which, like immune potential, varies in space and time) and the virulence of the pathogen (Benskin et al. 2009). Exploring all the nuances of pathogen exposure, virulence, and type is beyond the

scope of this article, therefore, we classify pathogens into four simple categories; first intracellular or ex-tracellular, and then known (previously encountered pathogens against which the host already possesses memory cells) or unknown (no previous exposure). When an unknown pathogen invades, constitutive and non-specific immunity are the first and only defenses. If the attack is very strong and spreading (high virulence), a systematic innate response will be induced (i.e., the APR and inflammation). After a few days acquired immunity mediated either by Th1 (cytotoxic T-cell), Th2 (antibody) or Th17 (acute epithelial inflammation) will come into play, depending on the nature of the pathogen (i.e., intracellular, extracellular bacterial, prokaryote, or parasitic). Thus, all aspects of immune function, re-gardless of cost, are necessary under certain circum-stances and the 'required response' to a challenge (given unlimited immune potential) will depend on the nature of the pathogen. However, as just discuss-ed, immune potential is rarely unlimited; thus, an animal's 'actual defense' is defined as the best possi-ble response against the particular pathogen (re-quired response), but is constrained by the animal's immune potential. This idea is summarized in Fig. 2 and terms are defined in Table 2.

To illustrate the model we first give an example using Red Knots and then to illustrate the model's potential for generalization, we consider two hypo-thetical shorebird species, one migrant and one non-migrant. In these examples, variation in avail-able capital and pathogen pressure is not based on empirical data but is simplified to highlight the flex-ibility of the model to different circumstances. By approaching immune function from this perspective we hope to highlight the fact that an animal's actual defense is a balance between the capital it has to invest in the costs of immunity, and the benefits of different aspects of immunity based on the pathogen threat.

Consider a Red Knot of the *C. c. rufa* subspecies during stopover in Delaware Bay, USA. This stopover is the final refueling area for knots of this subspecies before arrival on the breeding grounds. In terms of available capital (working from the bottom up in Fig. 2) time is very limited because this bird must refuel and reach the breeding grounds within a few weeks in order to successfully breed. Further-more, upon arrival, when the bird is likely exhausted after having flown more than 8000 km from stopover areas in South America, nutrients, and energy might also be limited (Buehler and Piersma 2008). In terms of pathogen pressure (working from top down in Fig. 2) avian influenza is a threat for shorebirds
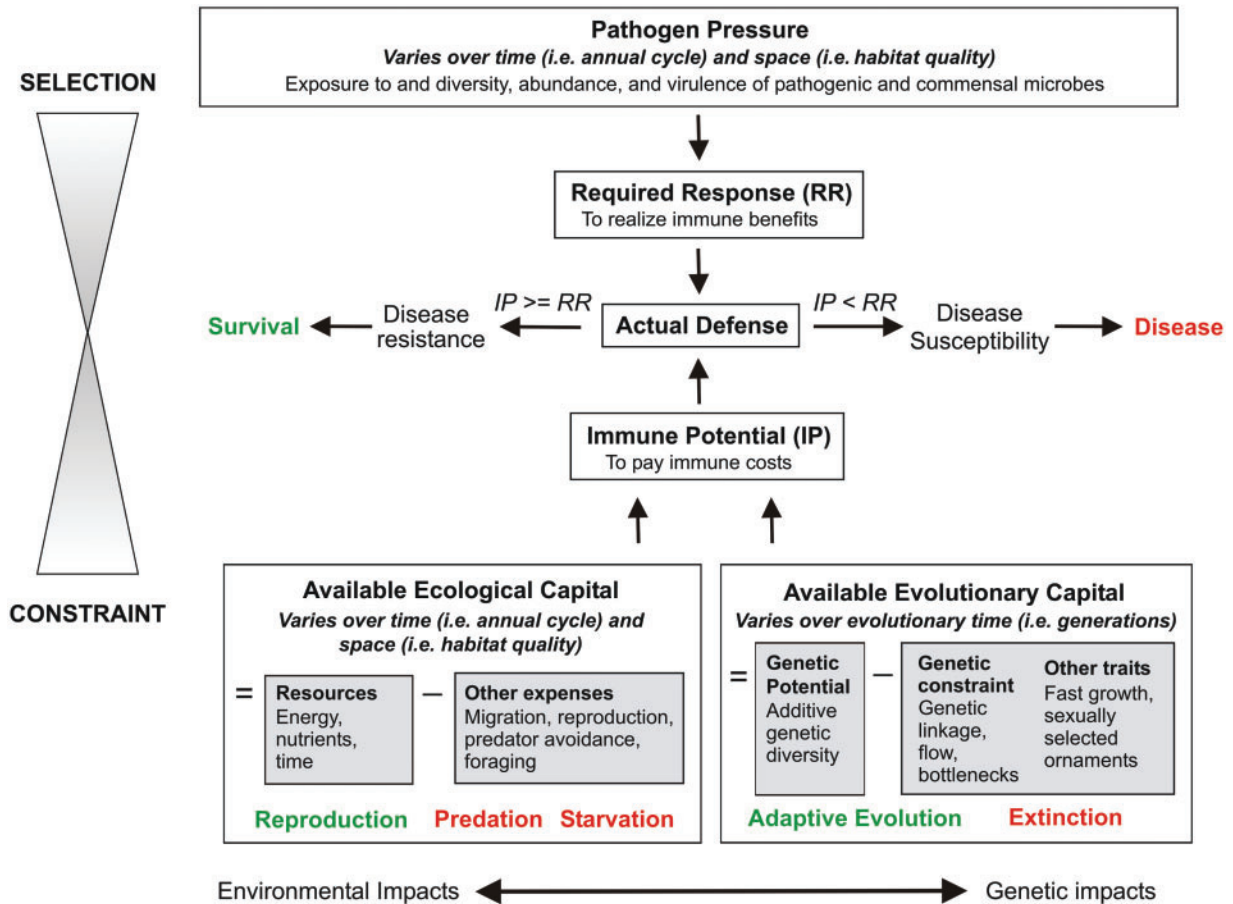
Fig. 2 A generalization of the actual defense model, highlighting the fact that an animal's 'actual defense' is a balance between the capital it has to invest in the costs of immunity on the one hand ('immune potential'), and the pathogen threats it faces on the other hand ('required response'). Definitions for the terms used in the model are in Table 2. Put simply, if an animal's immune potential is greater than or equal to the immune response required to fight a given pathogen, then disease resistance and survival is the outcome. Conversely, if an animal's immune potential is less than the required response, then disease and possible mortality ensue. The model can be related to fitness in a broader sense since disease may result in mortality or reduced fitness due to lethargy and anorexia. On the other hand, when too much is invested in immune function then trade-offs with migration, reproduction, predator avoidance, and foraging might result in reduced fitness due to mortality on migration, reduced reproductive output, predation and starvation. Terms linked to fitness in the figure are in green and those linked to mortality are in red.

in Delaware Bay (Krauss et al. 2007; Hanson et al. 2008). Although the prevalence of this virus shed in red knot feces is <1%, the prevalence of antibodies against avian influenza have been found to be much higher (53.6% when assaying with enzyme-linked immunosorbent assays [ELISA]; Brown et al. [2010]), indicating that the birds are coming into contact with the virus and are mounting an immune response. The strains of avian influenza thus far found in Delaware Bay are not considered highly pathogenic; however, even low-pathogenic avian influenza has been found to have fitness effects in swans (van Gils et al. 2007). Influenza is an intracellular pathogen that requires mainly a Th1-cell-mediated response for effective viral clearance, (required response; Murphy et al. [2008]) although

specific antibodies are also produced. This response is relatively costly and has a long latency period if the bird has never encountered the virus before. However, our hypothetical knot is an adult that has encountered avian influenza in the past and already possesses protective memory cells. Therefore, this bird can mount a rapid and specific response at relatively low cost, and without the sickness that accompany the APR. Thus, in this example the birds' immune potential is greater than, or equal to, the response required by the pathogen and the bird can resist the disease. As stopover progresses, this bird can refuel and regain the capital needed to mount other more costly aspects of immune function, if needed. Research on Red Knots in Delaware Bay indicates that some indices of immune function

Table 2 A glossary of terms used in the text and in the model

"Acquired immune function" develops some time after initial infection, is specific to particular pathogens, and has memory.

"Actual defense" refers to the response used by an animal given its immune potential and the pathogen at hand. It is this response that will determine the animal's resistance to disease.

"Available ecological capital" refers to resources that can be used to pay the costs of immune function. The availability of these resources varies in time and space as do other aspects of the host's life, such as migration or reproduction, which are also expensive and compete for the same resources.

"Available evolutionary capital" refers to the potential to evolve towards the most adaptive combination of traits. It is comprised of "genetic potential" mainly in the form of genetic diversity (the amount of additive genetic diversity for a given trait). Without this diversity the trait can not change in response to selection over generations (Conner and Hartl 2004). This potential is constrained by "genetic constraint" including factors such as gene linkage, genetic drift and gene flow (Conner and Hartl 2004), and is depleted by genetic bottlenecks. It can also be limited in the sense that one trait may evolve at the expense of another.

"Bottlenecks" refer to periods when nutrients, energy, and time are limited due to temporal and spatial circumstances.

"Constitutive immune function" is constantly maintained, providing a system of surveillance and general repair.

"Current infection" refers to an existing infection by a given pathogen.

"Disease resistance" refers to an animal's ability to resist a particular pathogen's challenge. This is a subset of "disease risk," which refers to the risk of becoming ill and is affected by a myriad of factors, including exposure to pathogens, virulence of pathogens and the animals' condition at the time of challenge (susceptibility).

"Immune potential" refers to the immune strategies at an animal's disposal, given its circumstances at a particular time and place. Immune potential is constrained by bottlenecks that limit the amount of available capital to invest in immune function.

"Immunopathology" is defined as collateral damage caused when the immune system harms the host as well as the invaders.

"Induced immune function" is triggered only when a pathogen has established itself in the body.

"Innate immune function" is immediate and effective against a broad range of pathogens (not pathogen specific).

"Pathogen" refers to a disease-causing biological agent (including microorganisms and parasites). This may also include commensal organisms because they are kept in check by the immune system. For example, some *Escherichia coli* bacteria are beneficial in the gut but cause disease if allowed to establish in the bloodstream.

"Pathogen pressure" refers to the possible pathogens that an animal might encounter at a given time and place.

"Required response" refers to the best possible response against a given pathogen, assuming unlimited capital.

do increase as stopover progresses, although it remains unclear whether these increases are due to the rebuilding of a compromised immune system or to responses against infections acquired during stopover (Buehler et al. 2010).

Now consider two hypothetical shorebird species. The first approximates a Red Knot of the subspecies *C. c. canutus*, a long distance migrant that breeds in the high Arctic and winters in the tropics. The second approximates a non-migratory subantarctic Snipe *Coenocorypha aucklandica* (Miskelly 1990), resident in the South Temperate Zone. From the perspective of resource allocation, in both species, most of the available capital will be invested in reproduction, during breeding, and for the migrant resources will also be diverted into fuelling and flight during migration. However, outside of breeding and migration, the migrant species wintering in the tropics may have more capital to invest than will the resident species wintering in the South Temperate Zone, if we assume lower thermoregulatory costs and a more stable food supply in tropical climates. From the perspective of pressure from pathogens, during breeding the long distance migrant breeding in the

High Arctic may face fewer pathogen threats than the resident breeding in the Temperate Zone (hypothesized by Piersma [1997]). This idea remains to be tested for the vast majority of pathogens, but evidence that the likelihood of parasitic infection is reduced at high latitudes has been found for blood parasites (e.g., Greiner et al. [1975]; Bennet et al. [1992]). However, during migration, the migrant may encounter a higher proportion of novel pathogens (Møller and Erritzøe 1998) and while wintering in the tropics may face more extracellular parasites (i.e., mosquitoes, ticks) and the intracellular and extracellular bacteria and protozoa they carry (e.g., Mendes et al. [2005]). Conversely, the resident species may encounter more intracellular pathogens such as viruses during the South Temperate winter.

To illustrate how immune potential and required response combine to give actual defense, we now focus on the breeding season of the resident species. Working from the bottom up in Fig. 2 we assume that most capital in terms of nutrients, energy, and time will be invested in reproduction. There will be little surplus for costly defenses such as the APR or perhaps even Th1 or Th17 type immunity. Working

from the top downwards in Fig. 2 we assume that the bird has been bitten by a tick and infected by an intracellular protozoa that it has not previously encountered. The required response against this pathogen is first constitutive and non-specific immune function, followed, if the pathogen is aggressive (virulent), by induced and non-specific defenses such as inflammation and the APR. After a few days, a primarily Th1-cell-mediated response specific to the pathogen would be orchestrated that would eventually clear the infection. However, working to the middle of Fig. 2, given constraints on capital for immune investment, the bird's immune potential is less than the required response. The bird may rely on constitutive immunity alone to control the infection until a (perhaps a somewhat attenuated) Th1-cell-mediated response can be mounted; however, this will likely be insufficient, rendering the bird susceptible to the disease. In this case, either the infection will worsen and threaten the bird, or breeding will be aborted to free up capital for the APR (including fever, lethargy, and anorexia) to fight the infection until a full-fledged Th1-cell-mediated response, specific to the pathogen, can clear the infection.

These examples, and many aspects of this model, are, of course, simplifications. Animals experience a wider range of circumstances than we have portrayed and are faced with an incredible diversity of pathogens. Furthermore, the immune system and immune responses required to fight specific pathogens are more complex than depicted (Clark 2008; Murphy et al. 2008). However, this simplification provides a way to conceptualize how actual defense is shaped in individuals of differing condition, living in different situations, and faced with different threats from pathogens.

Towards testing the model: avenues for future research

The model presented above highlights the importance of detailed information on annual cycles, habitats, allocation of resources, immune costs, and an understanding of specific threats posed by pathogens. If this model is to be tested, we must develop ways to assay all aspects of the immune system over the course of the annual cycle in free-living environments (working from the bottom up in Fig. 2). We must also more precisely measure the costs of immunity in non-domesticated species and free-living individuals. Finally, we must learn more about the threats posed by pathogens and be better able to link measures of immune function to disease (working from the top down in Fig. 2).

To really examine trade-offs within the immune system as hypothesized by Lee (2006), Buehler et al. (2008b), Buehler et al. (2008c), Buehler et al. (2009c), and to test the concept of immune potential, it will be necessary to concurrently assay constitutive immunity, induced innate immunity, and the different types of helper-T-cell mediated acquired immunity (i.e., Th1, Th2, Th17, and T_{reg}) in free-living animals. However, from a practical standpoint, this remains difficult because it is still impossible to measure induced innate or acquired immunity from a single blood sample. One approach to this problem could involve measuring cytokine proteins in serum or the expression of the genes coding for cytokine proteins and their receptors in cells (Graham et al. 2007; Adelman and Martin 2009). This is a promising avenue for future research because the character of the acquired immune response, in particular whether the response is dominated by Th1-, Th2-, Th17- or T_{reg}-helper-T-cells, is shaped by the cytokines released by antigen presenting cells. Another promising possibility might be to delve into the genetic basis of immune function. A full discussion of this topic remains outside the scope of this article, but briefly, we could begin to study the effect of genes at the population level by relating genetic polymorphisms in immune genes with selection pressures by pathogens (this would fall under evolutionary capital in Fig. 2). We could also begin to study the effect of genes and gene regulation on flexibility in immune function, at the individual level, by measuring levels of gene expression under different circumstances and against different pathogens. Many candidate genes for study are possible, and both structural genes important for the recognition of pathogens (i.e., genes of the MHC, toll-like receptors [TLR]) as well as regulatory genes coding for the intensity of different immune responses (i.e., interleukin-10 [IL-10]) should be considered (Maizels 2009). These assays are not yet developed for non-model organisms; however, they represent promising possibilities for the future.

To test ideas presented in the model about resource allocation in terms of capital available for immune investment, we need to measure the costs of immunity more precisely, particularity in non-domesticated species and free-living individuals. This is not a trivial exercise due to the compensatory nature of energy allocation strategies (Piersma et al. 2004; Mendes et al. 2006). Energy costs have been approximated for inflammatory/cell-mediated responses (i.e., Martin et al. [2003]) and humoral responses (i.e., Mendes et al. [2006]), but are still needed for constitutive and induced innate

immunity. Estimates for a wider variety of species would allow us to see if costs change with life-history traits [cf. Mendes et al. (2006)]. The field could also benefit from measuring the nutrient costs of different aspects of immune function (Klasing 2004) in a variety of non-domesticated species. Finally, we need to develop ways of measuring costs of immunopathology under different circumstances, i.e., research in humans (summarized by Clark 2008; Murphy et al. 2008).

With regards to the pathogen pressure that selects for immune function (working from the top down in Fig. 2), we need to learn more about threats by pathogens and be better able to link measures of immune function to disease. We could begin by addressing the question: 'What are the diseases that threaten species of interest, and how is pathogen pressure distributed over time and space?' In many species, a good starting point would be tapping into large-scale screening for wildlife diseases such as avian influenza or malaria. Furthermore, the advent of rapid DNA sequencing may allow ecologists to begin to understand the gastro-intestinal flora and fauna of a wide range of free-living animals by applying metagenomics techniques (Ley et al. 2008).

Next we could address the question: 'What is the effect of relevant diseases on immune indices?' Comparing the immune profiles of healthy and sick individuals will help to establish a link between changes in immune indices and current infection, and will help us to understand how the immune system responds to disease. Finally, we could address the question: 'Do high scores on immune indices signify better resistance to disease?' This question must be answered by performing experiments that link individual scores on immune indices with subsequent resistance to disease. If scores prior to inoculation with the pathogen are predictive of high resistance, then that index is a definitive proxy for resistance against that disease. If no relationship is found, then the index can tell us nothing about that disease, but may still be linked to other diseases. If a negative relationship is found then there may be trade-offs within the immune system with resistance to one type of disease causing susceptibility to another type of disease.

Once we have established that immune index scores correlate with resistance to disease, we could study whether increased resistance improves survival and reproduction. Doing this would bring the field a step closer to linking immune function to fitness, and to understanding how patterns of immune function and susceptibility to disease affect the viability of populations in the wild. This is an important goal in a world where both human overpopulation and climatic change threaten to alter the resources available for immune investment, as well as the distributions of pathogens against which defenses need to be mounted. Finally, we would be in a much better position to study how aspects of immune function and disease affect topics of interest in ecology and evolution (e.g., the maintenance of migratory routes or the evolution of life histories).

Supplementary Data

Supplementary data are available at *ICB* online

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