PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2008) **363**, 321–339
doi:10.1098/rstb.2007.2142
Published online 18 July 2007

Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs

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Animals living in temporally dynamic environments experience variation in resource availability, climate and threat of infection over the course of the year. Thus, to survive and reproduce successfully, these organisms must allocate resources among competing physiological systems in such a way as to maximize fitness in changing environments. Here, we review evidence supporting the hypothesis that physiological trade-offs, particularly those between the reproductive and immune systems, mediate part of the seasonal changes detected in the immune defences of many vertebrates. Abundant recent work has detected significant energetic and nutritional costs of immune defence. Sometimes these physiological costs are sufficiently large to affect fitness (e.g. reproductive output, growth or survival), indicating that selection for appropriate allocation strategies probably occurred in the past. Because hormones often orchestrate allocations among physiological systems, the endocrine mediators of seasonal changes in immune activity are discussed. Many hormones, including melatonin, glucocorticoids and androgens have extensive and consistent effects on the immune system, and they change in systematic fashions over the year. Finally, a modified framework within which to conduct future studies in ecological immunology is proposed, viz. a heightened appreciation of the complex but intelligible nature of the vertebrate immune system. Although other factors besides trade-offs undoubtedly influence seasonal variation in immune defence in animals, a growing literature supports a role for physiological trade-offs and the fitness consequences they sometimes produce.

Keywords: immune; life history; passerine; rodent; seasonality; trade-offs

1. INTRODUCTION

Parasitism is one of the most common modes of life used to obtain resources. Consequently, one might expect that organisms would possess strong anti-parasite defences at all times of their lives to prevent or at least hinder potential infections. Counter-intuitively, a recent *zeitgeist* among ecology, physiology and life-history research has led to the observation that variability in these defences, particularly immune defences, is more often the rule than the exception (Nelson & Demas 1996; Ricklefs & Wikelski 2002). Variation in immune defence over the year may occur for several reasons including (i) changes in disease threat over time, (ii) dynamism in the relative benefits of immune defence at certain times of the year versus others, or (iii) changes in environmental signals which portend impending disease threats (Nelson *et al.* 2002). Another viable explanation that has only recently garnered attention involves the costs of immune defence and the subsequent trade-offs animals face between investing in immune defence versus other expensive processes (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000; Norris & Evans 2000). For years, it has been known that current reproductive investments often impinge upon future reproductive success (Stearns 1992). Until recently however, few mechanisms that could lead to these outcomes had been proposed (Nilsson & Svensson 1996; Wiersma *et al.* 2004; Monaghan & Haussmann 2006). Recent studies have suggested that direct antagonism between reproduction and immune defence could be responsible (Norris & Evans 2000; Ardia *et al.* 2003; Hanssen *et al.* 2005; Martin *et al.* 2006*a*). Apparently, the costs of immune activity themselves can be large (Lochmiller & Deerenberg 2000) and thus sometimes negatively affect reproduction (Ilmonen *et al.* 2000), growth (Prendergast *et al.* 2004) or other aspects of fitness.

In this paper, we propose an extension of this hypothesis to explain seasonal changes in immune defence in small mammals and birds (Sheldon & Verhulst 1996), emphasizing existing evidence identifying trade-offs between reproduction and immune function. Trade-offs are defined here as direct or indirect antagonistic interactions between two physiological processes, which can (but do not necessarily) have long-term fitness consequences for organisms. This paper focuses predominantly on work in small mammals and birds because these species provide some of the best examples that immune activity and reproduction are often in conflict (Sheldon & Verhulst 1996; Zuk & Stoehr 2002; Demas 2004). Further, the income-breeding strategy of these species (Drent & Daan 1980) makes them appropriate models for investigating counterbalances between current and future reproduction. Income breeders by definition do not possess the resource reserve capacity

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One contribution of 14 to a Theme Issue 'Adaptation to the annual cycle'.

Table 1. Most common assays used to measure immune activity in small mammals and birds.

arm measured	treatment	abbreviation	strong response	indicative of	reference
in vivo					
cell mediated	phytohaemagglutinin[a]	PHA	delayed-type hypersensitivity (DTH)	resistance to intracellular infection (e.g. viruses)	Lochmiller *et al.* (1993), Martin *et al.* (2006*c*)
	dinitrofluorobenzene	DNFB			Dhabhar & McEwen (1997)
	keyhole limpet haemocyanin	KLH			Martin *et al.* (2006*b*)
humoral	sheep red blood cells	SRBC	generation of antibodies against novel protein(s)	resistance to extracellular infection (e.g. bacteria)	Deerenberg *et al.* (1997)
	keyhole limpet haemocyanin	KLH			Demas & Nelson (1998)
	diphtheria–tetanus virus	DPT			Ilmonen *et al.* (2000)
innate	lipopolysaccharide	LPS	variable	resistance to multiple novel pathogens	Aubert *et al.* (1997), Owen-Ashley *et al.* (2004)
in vitro					
cell mediated	concanavalin A	Con A	extensive proliferation of responsive T-cells	cytokine production, T-cell activity	Fitzgerald *et al.* (1992)

[a] Unlike KLH and DNFB, PHA is a mitogen that activates a variety of T-cell types, which may lead to different degrees of swelling compared with other antigens (see Martin *et al.* 2006*a*,*c*)

(i.e. capital) to compensate for increased reproductive or immunological demands (Martin *et al.* 2006*b*), so in these species physiological trade-offs probably more often have consequences for fitness than would be the case in larger taxa.

In addition to reviewing the role of trade-offs in seasonal variation in the immune system, the hormonal mediators of these changes are discussed. Androgens, glucocorticoids, melatonin and other hormones mediate most changes in immune activity and are particularly important in seasonal contexts (Nelson *et al.* 2002). Finally, in an effort to refine future studies in ecological immunology, a recent hypothesis proposing a greater appreciation of the intricacies of the immune system is presented in the context of our review. Overall, our goal for this paper is to highlight how trade-offs might be important mediators of seasonal changes in immune defence; obviously, many other factors can also drive changes in immune activity across the year, but it is becoming apparent that trade-offs are indeed important.

2. THE VERTEBRATE IMMUNE SYSTEM

The vertebrate immune system is diffusely distributed throughout the body and consists of many interacting cells, tissues and soluble proteins (Janeway *et al.* 2004). Collectively, these substances protect the body from infection, as well as rid or control infections once they have taken hold. Broadly, the immune system comprises two arms that differ in function and evolutionary history: the adaptive arm and the innate arm. The adaptive immune system is unique to the jawed vertebrates and consists of two branches termed humoral (B-cell) and cell mediated (T-cell). Humoral immunity is predominantly responsible for extracellular pathogen control through generation of soluble proteins (antibodies) specific to particular components (antigens) of invading cells or organisms. Cell-mediated immunity is generally responsible for intracellular pathogen control (cytotoxic T-cells) and/or managing B-cell and other immune responses (T-helper cells). Unlike other immune cells, B- and T-cells are capable of targeted defence against non-self substances via a diverse set of membrane receptors generated early in ontogeny (see below). Although these defences can provide immunological memory of prior infections, they are developmentally expensive and slower to reach effectiveness in terms of pathogen control relative to the other main immune defence system, innate immunity.

The innate immune system consists of a diverse array of cell types, such as macrophages, granulocytes and natural killer cells, and a variety of substances secreted by these cells including antimicrobial peptides, destructive enzymes and complement, a protein complex responsible for rapid control of extracellular pathogens (Janeway *et al.* 2004). Innate immune defences are effective at controlling multiple parasite types, and they are much more quickly engaged than adaptive defences. However, they are often more expensive (especially if an acute phase response is induced; see below), and they can be more self-damaging than adaptive defences (Klasing 2004). Also, it is often innate defences that determine the nature and intensity of the adaptive immune response (e.g. a T-helper cell type 1 (Th1) versus T-helper cell type 2 (Th2) bias; Janeway *et al.* 2004). To date, many techniques have been used to measure both innate and adaptive immune responses in seasonally breeding birds and mammals. Table 1 summarizes the salient details of some of the most popular tests including the conventional interpretations of strong responses (the putative important parameter) for each test.

Table 2. Seasonal variation in *in vitro* and *in vivo* measures of immune activity in small mammals and birds.

species	measure	arm	in SD (winter)	reference
mammals				
Spermophilus beldingi	SRBC antibodies	humoral	decrease	Sidky *et al.* (1972)
Clethrionomys glareolus	SRBC antibodies	humoral	increase	Saino *et al.* (2000)
Sigmodon hispidus	spleen PFC to SRBC	cell mediated	increase	Lochmiller *et al.* (1994)
P. maniculatus	splenocyte proliferation	cell mediated	increase	Demas & Nelson (1998)
Mesocricetus auratus	splenocyte proliferation	cell mediated	increase	Brainard *et al.* (1987)
Phodopus sungorus	lymphocyte proliferation	cell mediated	decrease	Yellon *et al.* (1999)
	DTH to DNFB	cell mediated	increase	Bilbo *et al.* (2002)
	NK cell cytolytic activity	innate	increase	Yellon *et al.* (1999)
	phagocytosis	innate	decrease	Yellon *et al.* (1999)
	fever	innate	decrease	Bilbo & Nelson (2002)
	wound healing	integrative	decrease	Kinsey *et al.* (2003)
birds				
Sturnus vulgaris	splenocyte proliferation	cell mediated	increase	Bentley *et al.* (1998)
Philomachus pugnax	DTH to PHA	cell mediated	increase	Lozano & Lank (2003)
Passer domesticus	DTH to PHA	cell mediated	increase	Martin *et al.* (2004, 2005, 2006*a*)
			increase	Greenman *et al.* (2005)
			no change	Gonzalez *et al.* (1999)
Zonotrichia leucophrys	fever	innate	decrease	Owen-Ashley & Wingfield (2006)

3. SEASONAL VARIATION IN IMMUNE ACTIVITY

(a) *Intra-annual*

Environmental conditions are in temporal flux over much of the planet. At temperate and boreal latitudes, summer and spring represent conditions in which individuals of most species of small birds and mammals can thrive. Winter and its associated low temperatures and food availability, however, make breeding and sometimes even survival difficult (Nelson *et al.* 2002). For these reasons, parturition usually coincides with summer and spring in small mammals, whereas reductions in gonad size occur in response to shortening day lengths (Bronson 1988). In seasonally breeding passerine birds, most reproductive activities take place in spring, then males and females become refractory to long day lengths, spontaneously regressing their reproductive organs before winter begins (Gwinner 2003). These reductions in gonad size in males are typically accompanied by decreases in androgen secretion and sperm production (Korytko *et al.* 1997; Kuwahara *et al.* 2000).

In contrast to these decreases in reproductive activity, most small mammals and birds enhance thermogenic activity, cellular maintenance and other processes that promote survival in winter (Nelson *et al.* 2002). In particular, immune activity tends to be elevated at this time of year (Nelson & Demas 1996; Nelson 2004), presumably to improve the likelihood of survival to the next breeding season (Goldman 2001). Extensive work in birds, mammals and particularly reptiles has demonstrated that immunological tissues including thymus, spleen, bursa of Fabricius and gut-associated lymphoid tissues (GALT) change in size over the year (Nelson *et al.* 2002). Similarly, changes in the abundance and distribution of immune cells on a seasonal basis are common (Nelson *et al.* 2002), with circulating cell densities tending to be higher in the winter months. Although intriguing, such changes in immune organ size and numbers of immune cells in circulation are difficult to interpret in a functional sense; increased cellularity, for example, may represent greater infection or enhanced capacity for disease resistance (Norris & Evans 2000). Direct measurements of immune responses are therefore thought to better reflect an individual's capacity for immune defence.

Table 2 reports the extensive seasonal variation in immune responses found in both captive and wild vertebrates. To date, two conceptual models have proposed that trade-offs must be important for explaining seasonal variation in immune activity. One hypothesis posits that winter immunoenhancement reflects active upregulation of immune activity to counteract the immunosuppressive effects of stressors that occur in winter including low ambient temperatures and reduced food availability (Nelson & Demas 1996; Sinclair & Lochmiller 2000; Nelson 2004). Such stressors often increase circulating glucocorticoids in laboratory conditions. Thus, because elevated corticosteroids (over long periods) tend to decrease immune activity (McEwen *et al.* 1997), animals must *buffer* environmentally induced immunosuppression by increasing immune activity to higher levels than are maintained in less-demanding environmental conditions (e.g. summer; figure 1*a*). An alternative hypothesis proposes that the costs of immune activity underlie seasonal fluctuations (Martin *et al.* 2004, 2006*b*; Greenman *et al.* 2005). That is, immune activity is traded off during specific phases of animals' lives, such as breeding, owing to the incompatible costs of simultaneous demanding physiological activities (figure 1*b*).

Both hypotheses are supported by studies in multiple taxa and together they explain a significant proportion of the immunological variation seen in animals across the year. However, both hypotheses require refinement. For instance, it is unclear how animals could *buffer* themselves against winter stressors in the wild if (i) immune activity is costly, (ii) thermogenic requirements are high and also expensive at this time of year, and (iii) resource availability is low. If additional resources cannot be obtained then

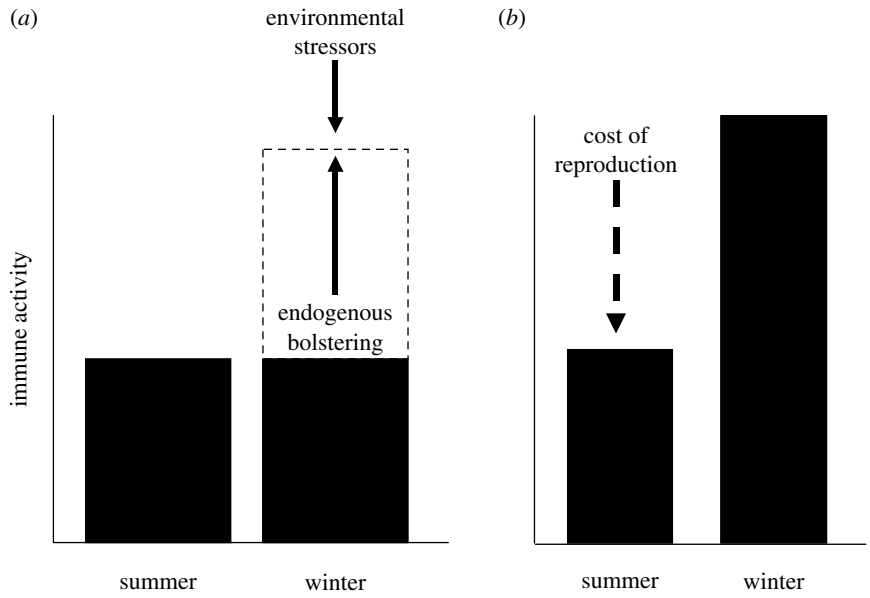

Figure 1. Comparisons of the (*a*) winter immunoenhancement versus the (*b*) trade-off models to explain seasonal variation in immune activity. According to the winter immunoenhancement model (*a*), immune activity is suppressed during winter (short days for most non-tropical seasonal breeders) due to the stressors of winter conditions (downward-pointing arrow), such as low ambient temperatures and food availability. This stress-induced immunosuppression is buffered by animals (upward-pointing arrow) in winter (short-day conditions in the laboratory) by increasing immune responses to levels greater than those that occur in long days. According to the trade-off model (*b*), immune activity is depressed when other costly physiological activities are concurrent, such as breeding (downward-pointing arrow). The schematic here depicts resource-driven immunosuppression during breeding, but this result would presumably hold for any other costly physiological process, such as tissue regeneration and growth, or perhaps even expensive behaviours (e.g. territory defence).

compensation must be made within some physiological system. Thus, if the immune system is to be bolstered and physiological trade-offs are unavoidable, other physiological systems must be weakened; just which system(s) are adjusted (other than the reproductive system) remain unspecified. Further, it is not apparent that *persistent* and *predictable* environmental conditions (e.g. low food availability and temperature) would necessarily induce stress responses each time they were encountered by wild animals. Arctic passerine birds only show elevated corticosteroid responses to large and prolonged storms, and baseline glucocorticoids are actually lower in most birds during winter when compared with summer (Romero 2002).

A shortcoming of the second (direct trade-off) hypothesis is that it cannot account for the known effects of environmental factors, particularly photoperiod, on immune activity in captive and wild non-tropical species (Nelson *et al.* 2002). That is, by using changing day length alone, many temperate and boreal species begin adjusting their phenotypes *before* changes in environmental conditions actually occur, and these changes occur even in laboratory conditions when resources are generally available *ad libitum*. Decreasing day lengths tend to enhance immune responses and induce regression of the reproductive system (Nelson & Demas 1996). Thus, seasonal changes in immune activity under laboratory conditions may represent manifestations of prior selection for phenotypes that use photoperiod to allocate resources appropriately among physiological systems (Ricklefs & Wikelski 2002).

Of course, these two hypotheses are not the only explanations for why immune defences may vary over the year. Another possibility that has been relatively unexplored involves changes in the abundance and distribution of pathogens over time. In temperate regions of the world, disease threats fluctuate depending on the time of year. In spring, vector-transmitted diseases, such as malaria, tend to peak; in autumn and winter, however, contact-transmitted diseases are more common (Nelson 2004). Thus, temporal changes in animals' immune defences may represent an effort to resist infection at those times of the year when certain diseases are most prevalent. Even though this hypothesis (and others) may be valid, it is apparent, based on a growing literature, that trade-offs must in part be responsible for seasonal variation in immune defence, the essence of the two hypotheses discussed earlier.

To establish the validity of these hypotheses, however, refinement and reinforcement are critical. Indeed, a historical emphasis on captive, temperate-dwelling seasonal breeders may have led to misconceptions regarding whether trade-offs alone can explain fluctuations in immune function in natural systems (Bronson 1988). For example, previous work by Lochmiller and colleagues on wild rodents highlights inconsistencies between patterns detected in captivity versus those found in the field. In one study, cotton rats (*Sigmodon hispidus*) from Oklahoma exhibited extensive intra-annual variation in both cell-mediated and humoral immune activity but not in any systematic (seasonal) fashion (Lochmiller *et al.* 1994). The authors suggested that the pattern they found reflected changes in the genotypic composition of the population more than phenotypic changes within individuals. In a second study, capacity of resistance to *Listeria monocytogenes* was compared over the year in both cotton rats and prairie voles (*Microtus ochrogaster*; Sinclair & Lochmiller 1999).

Neither species showed seasonal differences in mortality due to a large inoculation of bacteria (LD50) despite the extensive seasonal variation in multiple aspects of immune activity detected in both species. Such results could indicate that the seasonal decrements in immune function that ecologists have commonly detected might not be functionally important (Adamo 2004). Alternatively, studies in which potentially lethal doses of bacteria are directly administered to study subjects may be inappropriate for determining the relevance of changes in immune measures in natural contexts. Indeed, this method circumvents potentially important upstream immune defences. Moreover, longitudinal studies cannot always provide the resolution necessary to determine if trade-offs are important mediators of seasonal changes in immune activity. Variability in the physiological condition of individuals at different time points could obscure important relationships.

Altogether, the work of Lochmiller and colleagues indicates that an argument justifying trade-offs as mediators of seasonal variation in vertebrates immune defence has not been made. Although studies have indicated that immune activity is expensive in terms of resources/energy (Lochmiller & Deerenberg 2000; Demas 2004) or self-damage (Råberg *et al.* 1998), evidence has yet to be presented *en masse* to indicate that the costs of immune defence are important in explaining seasonal changes in immune activity. A first step towards testing this hypothesis would include examining evidence indicating that immune activity fluctuates on shorter time scales than seasons, particularly if immune activity was compromised when other expensive processes were ongoing. Such evidence is plentiful.

(b) *Intra-seasonal*

Pregnancy and lactation represent the most energetically demanding periods of the life cycle for small female mammals (Thompson & Nicoll 1986; Speakman 2000). Subsequently, multiple types of immune activity are compromised during these stages in rodents. For instance, lactating and pregnant Siberian hamsters (*Phodopus sungorus*) had lower antibody responses to a novel protein when compared with nulliparous animals (Drazen *et al.* 2003). Similarly, the degree of lipopolysaccharide-induced fevers of pregnant and lactating rats (*Rattus rattus*) was reduced when compared with virgin animals (Martin *et al.* 1995); lipopolysaccharide (LPS) is a component of Gram-negative bacterial cell walls that is commonly used to induce fever in vertebrates but is not a replicating pathogen. Moreover, in the latter study, if fever was induced within 24 h of parturition then no fever was detected, but almost all animals died 3–15 h after giving birth. Similar dosages of LPS did not cause mortality in either virgin or pregnant rats when given outside of this temporal window (Martin *et al.* 1995).

Changes in immune function during breeding are not limited to rodents. Insectivorous passerines exhibited weak cell-mediated immune activity when ambient temperature (and hence food availability) was low (Lifjeld *et al.* 2002). In nestling great tits (*Parus major*), cell-mediated responses decreased as the breeding season progressed, presumably in response to deteriorating environmental conditions (Dubiec & Cichon 2005). As with small mammals, increased parental responsibilities decreased both cell-mediated and humoral immune activity in avian species (Deerenberg *et al.* 1997; Nordling *et al.* 1998; Moreno *et al.* 1999). Even increased incubation efforts negatively impinged on antibody production in collared (*Ficedula albicollis*) and pied (*Ficedula hypoleuca*) flycatchers (Cichon 2000; Ilmonen *et al.* 2002).

Short-term trade-offs between immune activity and reproduction can be complex. For instance, pregnant greater mouse-eared bats (*Myotis myotis*) had weaker cell-mediated responses and harboured more ectoparasites than non-reproductive females from the same roost (Christe *et al.* 2000). Unexpectedly, T-cell immune activity progressively *increased* over the course of the pregnancy, reaching a maximum during lactation. This phenomenon may have represented a sampling bias whereby early breeding bats (lactating by the time the authors measured immune activity) had stronger T-cell responses than late-breeding bats (early pregnancy; *sensu* Lochmiller *et al.* 1994). Alternatively, this pattern could represent an effort by females to ensure that they reared their litters to independence in spite of the long-term consequences of concurrent, large reproductive and immune investments (Christe *et al.* 2000).

Similar shifts in allocation priority to current versus future reproduction (once a large reproductive investment has already been made) have been reported in passerines. Female house sparrows (*Passer domesticus*) injected with LPS were less likely to abandon their nests when their clutches were enlarged versus reduced (Bonneaud *et al.* 2003), which the authors suggested as evidence for terminal investment. Recently, we found evidence for terminal investment in rodents. Typically, seasonally breeding small mammals regress gonads in short days, presumably as an energy-saving mechanism to promote overwinter survival (Bronson 1988). Siberian hamsters injected with LPS, however, slowed gonad regression, indicating that when infected, hamsters may remain reproductively active late into the year to maximize current reproduction as future reproductive opportunities may never occur (Weil *et al.* 2006*c*).

Short-term changes in immune activity also occur during hibernation. Many small mammals and birds use hibernation or torpor to survive the winter when food resources are scarce. Immune function seems to operate in a unique manner during these energy-saving bouts. For instance, many species exhibit short, periodic bouts of normothermia during the hibernation period. Although it is unlikely that these arousals are solely for immune defence, a recent study in ground squirrels suggests that one benefit of these arousals may include reactivation of the immune system. Hibernating animals injected with LPS showed no fever until arousal, and LPS expedited arousals via increased prostaglandin secretion in the brain (Prendergast *et al.* 2002*a*).

4. ARE TRADE-OFFS RESPONSIBLE FOR SEASONAL VARIATION IN IMMUNE DEFENCE?

(a) *The costs of immune defence*

Presumably, all animals would benefit from strong immune defences at all times of their lives (Nelson *et al.* 2002; Nelson 2004). Some have

suggested that variability in defence is a consequence of selection against immunopathology, influencing organisms to decrease immune responses to avoid self-damage during sensitive periods of their lives (Råberg *et al.* 1998). Thus far, data to support this possibility are lacking. A more substantiated hypothesis proposes that immune activity varies because it is too expensive to use when other physiological activities are operating at a maximum (Sheldon & Verhulst 1996; Lochmiller & Deerenberg 2000). It is well documented that reproduction is expensive (Speakman 2008). In female rodents, food intake can double over the course of the gestational period and triple with the onset of lactation (Bronson 1988). In passerines, the costs of provisioning nestlings and even incubating, brooding and laying eggs can be substantial (Monaghan & Nager 1997). Recent work in ecological immunology shows that the costs of immune activity are often sufficiently large to influence fitness (Lochmiller & Deerenberg 2000; Martin *et al.* 2003).

Although physiological costs may not always directly lead to fitness costs, fitness can be compromised by the activation of immune activity, especially if physiological costs are proportional to other fitness-related processes and compensation for increased resource use is not possible (Martin *et al.* 2003). Some of the first work demonstrating substantive costs of immune activity in wild vertebrates comes from work on the collared flycatcher (Gustafsson *et al.* 1994). Over the past several years, these results have been expanded to other species of birds and mammals. For instance, experimental increases of clutch size or brood size dampened immune activity (Nordling *et al.* 1998; Ardia *et al.* 2003). In some cases, these reductions in immune activity could be directly linked to compromised future reproductive success (Hanssen *et al.* 2004), but in others, individuals favouring strong immune responses over large reproductive efforts experienced greater future reproductive success by increasing lifespan (Ardia *et al.* 2003).

Although the above results indicate costs of immune activity, they do not demonstrate direct negative effects of induced immune activity on reproduction or other components of fitness (Sheldon & Verhulst 1996). Many other studies, however, have provided this evidence. Female house sparrows (*P. domesticus*) injected with LPS provisioned offspring at a lower rate than saline-injected birds, and hence had reduced reproductive success (Bonneaud *et al.* 2003). Studies in pied flycatchers (*F. hypoleuca*; Ilmonen *et al.* 2000) and blue tits (*Parus caeruleus*; Råberg *et al.* 2000) have detected similar outcomes. Fitness costs of immune activation are not limited to the effects on reproduction. Activation of the immune system reduced feather regrowth (moult) in house sparrows (Martin 2005). Similar suppressive effects of induced immune activity on the development of sexual ornaments in passerines have been reported (Kilpimaa *et al.* 2004). In white-footed mice (*Peromyscus leucopus*), immune challenge reduced testes and small intestine mass of adults (Derting & Compton 2003); likewise, immune challenge retarded both somatic and reproductive tissue growth in peripubertal Siberian hamsters (Prendergast *et al.* 2004) and Eastern bluebirds (*Sialia sialis*; Fair & Myers 2002) and attenuated reproductive behaviour (Aubert *et al.* 1997; Weil *et al.* 2006*a*). Even induction of one immune response can compromise another; female white-footed mice showed less inflammation at the site of wounds when T-cell-mediated activity was induced 1 day prior to wounding. Similarly, T-cell-mediated inflammation was compromised in mice wounded 1 day prior to T-cell challenge (Martin *et al.* 2006*c*). Some of the best evidence for the high costs of immune activity comes from work on domestic fowl. Turkeys (*Meleagris gallopavo*) selected for larger adult body size exhibited weaker cell-mediated responses, fewer lymphocytes in circulation and smaller spleens relative to birds selected for small body size (Bayyari *et al.* 1997). Conversely, chickens (*Gallus gallus*) selected for strong antibody responses to sheep red blood cells are smaller as adults than lines selected for weaker antibody responses (Parmentier *et al.* 1996).

To mount immune responses, animals require sufficient energy and nutrient resources. Oftentimes immune responses are condition dependent with animals in better health or possessing greater endogenous energy/resource reserves typically mounting larger immune responses than those in poor condition (Lifjeld *et al.* 2002). Adequate protein is critical to mounting many immune responses (Lochmiller *et al.* 1993; Klasing 1998). Likewise, adequate energy stores determine the strength and character of immune responses (Lochmiller & Deerenberg 2000; Demas 2004). Lipectomy in both prairie voles (*M. ochrogaster*) and Siberian hamsters reduced antibody production to a novel antigen (Demas *et al.* 2003). Similarly, immune challenge directly increases energy turnover in some, but not all, mammals and birds (table 3).

(b) *Parsing the costs of immune defence*

The lack of consistent increases in energy expenditure in response to immune challenges in the studies listed in table 3 and the lack of both physiological and fitness costs of immune activity in other studies (Williams *et al.* 1999; Lozano & Ydenberg 2002; Horak *et al.* 2003; Martinez *et al.* 2004; Verhulst *et al.* 2005) indicate that immune responses may not always be expensive. Indeed due to a publication bias against negative results, evidence supporting a lack of detectable immunity costs may be greater than that is apparent from the above studies. It is impossible to know, however, how large this bias may be, and more importantly the absence of costs in some studies cannot explain the presence of costs in others. We expect that some of the lack of detection of energetic costs may reflect the imprecise nature of using whole body energy turnover as an endpoint (Ksiazek *et al.* 2003; Klasing 2004). That is, energetic costs of using the immune system may be hard to identify owing to compensation among other physiological systems (Derting & Compton 2003). Furthermore, anorexia and inactivity are hallmarks of fever responses in many vertebrates (Hart 1988), so decreased energy turnover in some cases may be part of the defence strategy itself (Klasing 2004). For these reasons, most accounting of the costs of immune activity in domestic fowl has relied on critical amino acids as currencies. This work has provided strong evidence that the immune system is expensive but also

Table 3. Costs of immune responses in mammals and birds as a proportion of resting metabolic rate.

species	arm	percentage of increase	reference
mammals			
Cavia porcellus	humoral	—	Pilorz *et al.* (2005)
Mus musculus	humoral	27	Demas *et al.* (1997)
Peromyscus leucopus	humoral and cell mediated	—	Derting & Compton (2003)
birds			
Parus caeruleus	humoral	—	Svensson *et al.* (1998)
Parus major	humoral	8	Ots *et al.* (2001)
Passer domesticus	cell mediated	29	Martin *et al.* (2003)
		32[a]	Martin *et al.* (2006*a*)
Streptopelia risoria	humoral	9	Eraud *et al.* (2005)
Taeniopygia guttata	humoral	—[b]	Verhulst *et al.* (2005)

[a] Data from an equatorial population of house sparrows.
[b] Decrease in metabolic rate detected.

highlights that the (i) development, (ii) maintenance, and (iii) use of the immune system impart distinct costs (table 4).

Development of the innate immune system is not especially costly when compared with that of other physiological systems. Development of the adaptive immune system, however, is potentially the largest immunological investment vertebrates make. This high cost stems from the semi-random nature by which the T- and B-cell repertoires are generated. Early in ontogeny, T- and B-cells are produced that are either sensitive to self-antigens or not sensitive to self-antigens; self-sensitive cells are common whereas self-insensitive cells are rare (Cohn & Langman 1990). Those that are sensitive are immediately destroyed so as not to initiate autoimmune responses, whereas those that are not sensitive to self-antigens reach the circulation and are maintained in a semi-quiescent state until they encounter a pathogen that binds to the receptors they possess (Janeway *et al.* 2004). In chickens (*G. gallus*), approximately 90% of B-cells and 95% of T-cells produced are eliminated before they reach circulation (Reynaud & Weill 1996). At four weeks post-hatch, chicks export 5×10^8 B-cells per day from their bursae of Fabricius, constituting 0.04% of the weight of the growing bird (Paramithiotis & Ratcliffe 1994).

It has been suggested that the high costs of the adaptive immune system development are related to the extensive variation in the length of incubation/gestation periods among different vertebrate species. Duration of the developmental period varies from 18 to 660 days among placental mammals (Promislow & Harvey 1990); similar variation can be seen among avian species (Ricklefs 1992). Indeed, recent work has indicated that prolongation of the developmental period is correlated with greater cell-mediated immune responses in some cases (Tella *et al.* 2002), although these results require further validation (Palacios & Martin 2006*a*).

Maintenance of the immune system appears cheap relative to development. Most inactive immune cells possess little cytoplasm and thus have modest metabolic demands. Furthermore in chickens, the cost in terms of the amino acid lysine for maintaining the entire immune system has been estimated to be only 3% of the total body requirements (Klasing 2004). In small mammals, immunological maintenance appears comparably cheap. Administration of cyclophosphamide (a protein synthesis inhibitor with immunosuppressive effects) greatly decreased circulating lymphocytes in *P. leucopus*, but it had no effect on energy turnover (Derting & Compton 2003). Also, basal metabolic rate was higher in lymphocyte-deficient mice relative to normal mice (Råberg *et al.* 2002), which the authors suggested may have been related to increased reliance on innate defence in the lymphocyte-deficient groups. Further study of the maintenance costs of immune activity is critical, however, as maintenance costs may be important mediators of physiological trade-offs (Schmid-Hempel 2003; Verhulst *et al.* 2005; Martin *et al.* 2007)

In terms of use, the cost of the adaptive immune system is modest. Generating antibodies in domestic chickens (*G. gallus*) requires only 28 mg of lysine $kg^{-1}\,d^{-1}$ (Klasing 2004), a small amount relative to other physiological processes. Further, although activation of the adaptive immune system may elevate energy expenditure in some species (table 3), measurements in chickens indicate that the innate immune system is much more costly to mobilize, particularly processes associated with the acute phase response. Indeed, production of acute phase proteins during fever is more expensive (in terms of lysine) than any other metabolic process except somatic growth (Lochmiller & Deerenberg 2000; Klasing 2004). It has been suggested that usage costs of innate immune activity mediate many of the fitness consequences detected in ecological immunology studies. In pied flycatchers, for example, decreased reproductive success of females was originally attributed to the activation costs of the humoral immune system (Ilmonen *et al.* 2000). However, because the virus particles were emulsified in an adjuvant (aluminium phosphate) prior to injection, a technique specifically meant to induce a robust acute phase response and hence boost the antibody response (Janeway *et al.* 2004), it is probable that fitness costs in this study were due to induction of an acute phase response, not the generation of antibodies themselves (Klasing 2004). This issue does not invalidate the results of this and other studies; it simply highlights the need to appreciate the immunological details of the assays that are chosen. Indeed, even SRBC and PHA can induce mild acute phase responses in birds (Klasing & Peng 1987), which

Table 4. Parsing the costs of the vertebrate immune system. (Adapted from Klasing (2004).)

					effectiveness	
	development	cost of maintenance	use	pathological damage	novel pathogen	repeated exposure
arm						
innate	low	intermediate	high	high	good	good
adaptive	high	low	low	variable	poor	excellent

may explain why these challenges sometimes influence fitness (Garamszegi *et al.* 2004) or elevate energy expenditure (table 3).

5. GEOGRAPHICAL VARIATION IN IMMUNE DEFENCE

Although immune function varies temporally within species, little work has addressed whether investments in immune defence versus other physiological processes change depending on where animals live. If seasonal changes in immune activity are partly driven by resource availability over the year, then changes in environment across habitats (and especially latitudes and altitudes) should influence how resources are allocated among competing physiological systems. For instance, animals living in the humid tropics would presumably be exposed to greater numbers of and more diverse parasites than animals living in inland, boreal sites where most parasites (and their vectors) are active briefly each year (Piersma 1997). Some evidence from birds indicates that species living near the equator (Ricklefs 1992) or with broad geographical ranges show the highest prevalence of haematozoan infections (Tella *et al.* 1999). Variation in parasite threats across habitats can influence investments in immune defences in some cases. For example, small ground finches (*Geospiza fulginosa*) on large islands in the Galápagos carried more ectoparasites than conspecifics on small islands; investment in humoral (but not cell mediated) immune defence paralleled these patterns (Lindström *et al.* 2004).

Another apparently consistent geographical influence on immune activity is latitude. Temperate-dwelling house sparrows (40° N latitude), which are exposed to a more dramatic climatic variability, decreased immune investments during the breeding season but increased them later when reproductive activity ended; a near-equatorial population (9° N) exhibited static immune investments year-round (Martin *et al.* 2004). A more recent study on the same populations found that the immune systems of each population were distinct, and that the tropical birds generally invested more in defence than did the temperate ones (Martin *et al.* 2006*a*). Further, endocrine mediation of immune activities in these populations was distinct; corticosterone (see below) suppressed immune activity in the temperate but not the tropical population.

Such patterns are not limited to house sparrows. Tree swallows showed a similar latitudinal pattern in immune versus reproductive investments; more southerly populations (35° N) maintained immune responses irrespective of brood-size manipulations whereas northerly populations (65° N) decreased immune responses when brood sizes were enlarged (Ardia 2005). Some rodents also allocate to immune defence differently depending on latitude. Aztec mice (*Peromyscus aztecus*), which reside in southern Mexico, show no immunological responsiveness to the changes in day length (Demas & Nelson 2003), whereas more northerly distributed species (northern USA) show enhanced responses in short days coupled with reduced responses in long days when breeding typically occurs (Pyter *et al.* 2005*a*). Recent work in meadow voles (*Microtus pennsylvanicus*) indicates that the effects of latitude on immune activity may be complicated in some cases. Voles from the Northwest Territory of Canada enhanced cell-mediated responses in long days but reduced them in short days; voles from central Ohio (USA) showed the opposite pattern (Pyter *et al.* 2005*b*).

The character of trade-offs themselves may change with latitude. In high-latitude birds for example, breeding and postnuptial moult often overlap owing to the short window of time available to animals to breed and regrow feathers (Hemborg & Merila 1999). In the tropics, a larger temporal window allows birds to minimize or even eliminate this overlap. Subsequently, trade-offs between moult and immune function should be dramatic at temperate or boreal latitudes, but minimal near the equator. To date, only one study has addressed this possibility in passerines, but did not find an effect of latitude on trade-offs. Moult and immune activity did interact antagonistically in that study (Martin 2005), implying a trade-off between the two processes however. One possible explanation for this outcome may involve the relatively recent arrival of the study species (*P. domesticus*) to the tropics. Invasion status probably impinges on how the immune systems of birds and other vertebrates are organized (Lee & Klasing 2004; Lee *et al.* 2005), perhaps because parasites often perform better on local versus foreign hosts (Ebert 1994).

Conditions during birth can also influence both the immune and the reproductive systems. White-footed mice (*P. leucopus*) born at high latitudes are reproductively more sensitive to decreasing day lengths than low-latitude populations (Lynch *et al.* 1981). Similarly, high-latitude populations are slower to mature than low-latitude populations when exposed to decreasing day lengths (Dark *et al.* 1983), perhaps in an effort to delay the onset of puberty until environmental conditions permit rapid maturation. These reproductive strategies may ensure that immune defences are optimized to promote overwinter survival. Both male

and female Siberian hamsters born in short days and reared in short days (e.g. an autumn birth) exhibited stronger cell-mediated immune responses than hamsters short-day born, long-day reared (e.g. a spring birth) animals (Weil *et al.* 2006*d*).

6. PROXIMATE MECHANISMS MEDIATING TRADE-OFFS

Altogether, the above examples indicate that the costliness of immune activity probably drives some of the seasonal variation in this physiological system. Oftentimes, changes in immune activity are driven by short- and long-term fluctuations in hormones. In fact, the immune, endocrine and nervous systems are intricately connected and serve to communicate information about the internal and external environments to the control systems of the body. These relationships probably change seasonally, as hormone concentrations and receptor abundances and distributions vary as animals progress through different life stages (e.g. breeding, overwintering, moult, etc.). Below, we outline how several hormones influence the immune system, emphasizing those that probably mediate seasonal variation in immune function.

(a) *Melatonin*

Melatonin is thought to be a primary mediator of seasonality in the immune and reproductive systems (Guerrero & Reiter 2002; Hotchkiss & Nelson 2002). As a general principle, melatonin has negative effects on the reproductive axis, but its effects on the immune system are varied and depend on the specific arm of the immune system tested, the species studied and the timing of its delivery. One well-documented function of melatonin is to transduce day-length information into a physiological signal (Goldman & Nelson 1993). Pineal melatonin production occurs at night, but daylight suppresses its synthesis. It is the duration of the melatonin secretion rather than the amplitude that is the critical feature for the measurement of day length (Goldman 2001).

Melatonin, an indoleamine, is synthesized in a two-step enzymatic reaction from serotonin (Moore & Klein 1974). The rate-limiting enzyme (*N*-acetyltransferase) is produced via activation of sympathetic afferent nerves of the pineal gland (Moore 1996). Pineal-derived melatonin is critical in transducing day-length information to the mammalian reproductive system. Pinealectomy prevented photoperiod-induced regression of the gonads in Siberian hamsters, and both appropriately timed infusions and implantation of melatonin can induce the winter phenotype in long days (Bartness *et al.* 1993). This discovery led to the principle that melatonin is anti-gonadotrophic in mammals. Many avian species also use photoperiod to time reproduction. However, pineal melatonin does not appear to be the critical physiological cue to time reproduction for birds (Juss *et al.* 1993). The role of pineal melatonin in avian seasonality is difficult to generalize, in part, owing to the abundance of extra-pineal melatonin (Dawson *et al.* 2001). However, melatonin concentrations in birds show a similar circadian pattern of secretion that varies over the year. The persistence of this signal in birds suggests that it may be important for providing seasonal information for non-reproductive functions, including immune activity.

The effects of melatonin on the immune system are well established (Guerrero & Reiter 2002; Hotchkiss & Nelson 2002). Receptors for melatonin are found on mammalian and avian splenocytes, thymocytes and lymphocytes (Nelson & Demas 1996). In general, melatonin is associated with enhanced immune function in most laboratory animals. In domestic mice, pinealectomy reduced antibody-dependent cellular toxicity, antibody production and mitogen-stimulated splenocyte proliferation (Nelson & Demas 1996). However, house mice are not reproductively photoperiodic, and many strains have a genetic defect that prevents synthesis of melatonin (Reppert & Weaver 1995). Therefore, it is difficult to draw definitive conclusions regarding seasonality of immune activity from studies of domesticated mice.

Strong evidence exists that melatonin mediates many of the effects of photoperiod on the immune system in non-domesticated species. Generally, exogenous melatonin reproduces the immunological effects of short day lengths. Short-day deer mice, for example, enhanced *in vitro* cell-mediated immunity; exogenous melatonin produced the same effect in long days (Demas *et al.* 1996). Similarly, short photoperiods or exogenous melatonin prevented deer mice (*Peromyscus maniculatus*) from developing chemically induced tumours (Nelson & Blom 1994). However, melatonin can also inhibit certain aspects of the immune system. For example, Siberian hamsters in contrast to other photoperiodic rodents reduced *in vitro* cell-mediated immune activity and attenuated antibody production in short days when compared with long days (Prendergast *et al.* 2001*b*). Addition of physiological concentrations of melatonin to cultured lymphocytes reduced proliferative responses in long but not in short days. Pinealectomy also blocked the short-day reduction in antibody responses to sheep red blood cells (Yellon *et al.* 1999). Further, pineal melatonin can affect some components of the innate immune system in hamsters (Yellon *et al.* 2005), but this part of the immune system has been understudied when compared with the adaptive arm in this context.

The effects of photoperiod and melatonin on the immune system are temporally similar to the effects on the reproductive system. Photoperiodic rodents become refractory to short day lengths after prolonged exposure (approx. 20–24 weeks) and then 'spontaneously' regrow their testes. Melatonin does not affect immune activity *in vivo* or *in vitro* in refractory rodents (Prendergast & Nelson 2001; Prendergast *et al.* 2002*b*). Latitude of origin is related to responsiveness to photoperiod as deer mice from a high-latitude population, but not mice from a low-latitude population, responded reproductively to photoperiod and exogenous melatonin (Bronson 1985). The same pattern emerged for immune responses; animals from the high-latitude population, but not the low-latitude population, increased *in vitro* cell-mediated immune function in response to both melatonin and short day lengths (Demas *et al.* 1996).

It is important to note that manipulation of melatonin in the whole animal is associated with alterations in several neuroendocrine and physiological systems. Thus, it is difficult to determine whether immunological effects of melatonin are direct or indirect (Hotchkiss & Nelson 2002). Some evidence suggests that melatonin can directly affect immune cells and tissues. For instance, addition of melatonin to splenocyte cultures enhanced mitogen-stimulated proliferation in house mice and prairie voles; treatment with luzindole, a melatonin receptor antagonist, attenuated this effect (Drazen *et al.* 2000*a*; Drazen & Nelson 2001). Studies of animals that fail to respond reproductively to melatonin have illuminated the direct effects of this hormone on immune activity. European starlings (*Sturnus vulgaris*) are seasonal breeders that become refractory to long day lengths and require exposure to short days before they can again respond to long days. Exogenous melatonin prevented the decrease in splenocyte proliferation associated with photostimulation in castrated versus intact birds (Bentley *et al.* 1998).

(b) *Glucocorticoids*

Glucocorticoids are the end product, primary effectors and principal negative regulators of an important neuroendocrine axis (hypothalamus–pituitary–adrenal (HPA) axis). Originally described for their role in energy mobilization, glucocorticoids are now recognized as powerful mediators of many physiological processes including reproduction and immune activity. Unlike other hormones, glucocorticoids tend to suppress both reproduction and immune function. This observation has led to the principles that elevated glucocorticoids promote physiological and behavioural responses that (i) favour immediate survival at the expense of other processes (i.e. the emergency life-history stage hypothesis; Wingfield *et al.* 1998) and (ii) maintain homeostasis in the face of environmental changes (i.e. allostasis; McEwen & Wingfield 2003).

Interaction between glucocorticoids and the immune system is complex and bidirectional. Stressor-induced elevated glucocorticoid concentrations can modulate immune activity; however, activation of the immune system can also drive the production of glucocorticoids (Turnbull & Rivier 1996; McEwen *et al.* 1997). Because glucocorticoids tend to suppress inflammation but be induced by proinflammatory stimuli, they have been conceptualized as 'brakes' on the immune system, having evolved to prevent runaway inflammation and promote fine-tuning of the immune response (Sapolsky *et al.* 2000). A wealth of information demonstrates how glucocorticoids suppress immune function (McEwen *et al.* 1997), which led to the conjecture that glucocorticoids are largely responsible for decrements in immune activity in free-living animals in winter (Nelson *et al.* 2002).

Now there is compelling evidence that in certain contexts glucocorticoids can enhance aspects of immune function. In many cases, immunosuppression may be immunoredistribution in disguise (Braude *et al.* 1999). From an adaptationist perspective, one might predict that animals would enhance immune function in parts of the body at times when injury is probable, such as during a territorial dispute or failed predation event. Recent data in mice and rats support part of this prediction; in response to acute stressors, circulating leucocytes do not die, but instead they leave the bloodstream and move into peripheral tissues (skin, gut and lymph nodes). In the absence of injury or infection, these cells return to the general circulation quickly (Dhabhar *et al.* 1995). One of the best examples of these stress-induced immunoredistributions comes from work on delayed-type hypersensitivity (DTH). The DTH response is characterized by T-cell-mediated trafficking of immune cells into the skin (Dhabhar & McEwen 1997). Thus, if acute stress truly enhances front line immune defences, then DTH responses should be enhanced in response to short-term stressors. Laboratory rats (*R. rattus*) restrained daily for three weeks prior to antigenic challenge displayed the expected suppression of DTH responses. In contrast, when animals were stressed briefly just before immune challenge, they enhanced responses relative to non-stressed control animals (Dhabhar *et al.* 1996). These stress-induced alterations in the DTH response were directly mediated by corticosterone.

Photoperiod affects the character of these stress-induced immunological redistributions, suggesting that these hormones probably influence seasonal changes in immune activity in wild animals. Siberian hamsters acutely stressed prior to immune challenge had elevated DTH responses, and this response was significantly augmented in short versus long day animals (Bilbo *et al.* 2002). This enhancement in skin immune function was associated with an enhanced glucocorticoid response and expedited movement of leucocytes out of the blood during restraint stress in short versus long day-housed hamsters (Bilbo *et al.* 2002). As with photoperiod, latitude of origin can also influence corticosteroid effects on the immune system. Tropical-dwelling, but not temperate, house sparrows failed to show immunosuppression in response to chronically elevated corticosterone (Martin *et al.* 2005).

(c) *Androgens*

Androgens, primarily testosterone (T), are necessary for the organization and expression of many male sexual behaviours and morphological traits. In contrast, androgens have long been considered immunosuppressive, particularly because females and gonadectomized males tend to have stronger immune responses than intact males (Klein 2000). Antagonistic effects of androgens on the immune system were suspected as early as 1898 when it was reported that rabbits castrated pre-pubertally had larger thymuses than intact animals (Calzolari 1898). In recent years, similar observations led to the immunocompetence handicap hypothesis (ICHH), a mechanistic version of the Hamilton–Zuk handicap hypothesis of sexual selection (Hamilton & Zuk 1982; Folstad & Karter 1992). This model, which provided one of the first attempts to link immune activity and reproduction in an ecological context, has recently been challenged because the effects of androgens on the immune system are apparently less consistent than once believed (Owen-Ashley *et al.* 2004; Roberts *et al.* 2004). In the laboratory, males of seasonally breeding rodent species

housed in long days tend to maintain higher androgen concentrations in circulation and have weaker immune responses than conspecifics maintained in short days (Nelson *et al.* 2002; Nelson 2005). Hence, an early explanation of short day increases in immune activity involved the reduction in circulating androgens detected in these conditions (Nelson & Demas 1996).

It was soon recognized, however, that this explanation could not account for short-day increases in immune function seen in females, as T concentrations are generally low in females at all times of year. Further, because oestrogens tend to enhance immune activity, if photoperiodic changes in immune function are mainly due to fluctuations in sex steroid hormones, then females in short days that have low oestrogen concentrations should show reduced immune function when compared with long-day animals (or at least not exhibit male-like increases). Because these predictions were not substantiated in male and female deer mice (Demas & Nelson 1998) and gonadectomy and exogenous testosterone administration did not alter short-day enhancement of the DTH response in Siberian hamsters (Prendergast *et al.* 2005), this hypothesis has fallen out of favour.

Still, it is apparent that the immune and reproductive systems are intimately interconnected and that androgens are important components of these interactions. Indeed, the immune system can be modulated by androgens in some cases; conversely, activation of the immune system, particularly the innate arm, is associated with suppression of the reproductive neuroendocrine axis (Turnbull & Rivier 1997). Further, in domestic fowl, birds selected for strong antibody responses have smaller combs, sexually selected traits that are testosterone dependent and lower testosterone titres (Verhulst *et al.* 1999). Conversely, chickens infected with an intestinal parasite exhibited similar concentrations of testosterone and similar-sized combs as uninfected birds (Johnsen & Zuk 1998). As evidenced by these studies, trade-offs between reproduction and immune function may be mediated directly via communication between the reproductive and immune systems in certain contexts (Avitsur & Yirmiya 1999; Weil *et al.* 2006*b*), although these interactions tend to be complex.

The apparent immunosuppressive effects of androgens may sometimes be due to glucocorticoids (Evans *et al.* 2000; Berger *et al.* 2005), at least in certain experimental contexts. For example, captive dark-eyed juncos (*Junco hyemalis*) implanted with testosterone had reduced PHA responses when compared with those without implants. This effect could not be attributed directly to testosterone, as birds with implants also had higher circulating corticosterone (Casto *et al.* 2001). Similarly, house sparrows implanted with testosterone had decreased antibody response but increased corticosterone concentrations post-implantation (Evans *et al.* 2000). In this study, after controlling for corticosterone concentrations statistically, testosterone was positively associated with antibody production. In a related study, house sparrows implanted with testosterone and held in short days showed no suppression of cell-mediated immune activity, indicating that testosterone is not obligatorily immunosuppressive year-round in this species (Greenman *et al.* 2005).

(d) *Prolactin*

Prolactin is a peptide hormone released from the anterior pituitary (Goffin *et al.* 1999) that influences reproduction, growth and development, as well as water and electrolyte balance, maintenance of integumentary structures and components of the immune system (Yu-Lee 2002; Nelson 2005). In mammals, prolactin is regulated positively by hypothalamic thyrotrophin releasing hormone (TRH) and negatively by dopamine (Freeman *et al.* 2000). In birds, prolactin release is controlled by hypothalamic vasoactive intestinal peptide (el Halawani *et al.* 1996). Despite these regulatory differences, the short-day reduction in circulating prolactin concentrations is a nearly ubiquitous phenomenon that occurs even in short-day breeding species (Goldman & Nelson 1993).

The relationship between pituitary prolactin and immune function was suggested as early as 1930 when hypophysectomized (anterior pituitary removed) rats were observed to undergo thymic involution (Smith 1930). More recent support comes from studies showing deficits in cell-mediated and humoral immunity after hypophysectomy that are reversible via exogenous prolactin (Reber 1993). Prolactin receptors are expressed in primary lymphoid organs such as the thymus, lymph nodes, bone marrow and spleen and on peripheral T-cells, B-cells and macrophages (Leite De Moraes *et al.* 1995). Most studies of the effects of prolactin on the immune system have indicated enhancive effects (Yu-Lee 2002). Prolactin stimulated *in vitro* proliferative responses of natural killer cells, as well as T- and B-cells, to multiple mitogens (Matera *et al.* 1992). However, high concentrations of prolactin can be detrimental as evidenced by reduced proliferative responses to mitogens, reduced tumoricidal activity of natural killer cells and increased susceptibility to autoimmune diseases (Gerli *et al.* 1987; Matera *et al.* 1992; Vera-Lastra *et al.* 2002). Interestingly, prolactin opposes glucocorticoid-induced cell death *in vitro* (Fletcher-Chiappini *et al.* 1993) and improves macrophage function after trauma-haemorrhage or infection (Zellweger *et al.* 1996). These results and work in Snell dwarf mice, which are deficient in anterior pituitary hormones including prolactin, led to the hypothesis that prolactin may be part of a physiological system that opposes glucocorticoids and some inflammatory mediators by maintaining immunological homeostasis during stress (Dorshkind & Horseman 2000, 2001).

Few studies have directly tested the effects of prolactin on the immune system in a seasonal context in small mammals; some work on this subject has been conducted on larger mammals however (Auchtung & Dahl 2004). Furthermore in laboratory studies, prolactin treatment tends to be negatively associated with immune function in seasonal breeders. For instance, nearly 90% of female deer mice housed in long days and injected with the chemical carcinogen 9, 10-dimethyl-1, 2-benzanthracene developed squamous cell carcinomas; no mice housed in short days developed tumours. When long-day mice were treated with the prolactin release inhibitor, bromocriptine,

the incidence of tumours declined by nearly 50% (Nelson & Blom 1994). This study suggests that the elevated prolactin during long days may decrease immune function to favour reproduction and/or somatic growth. In sum, characteristics of prolactin secretion indicate that it may be an important mediator of seasonal changes in immune activity, but insufficient evidence exists as yet to support this possibility.

(e) *Leptin*

The adipocyte-derived hormone leptin was originally discovered as the product of the *ob* gene (Zhang *et al.* 1994). Mice deficient in leptin are obese due to both overeating and decreased energy expenditure (Coleman 1978). Thus, early research on leptin focused on its role in body weight regulation and metabolism. However, leptin may also be an important regulator of the reproductive and immune systems and their interactions, as it communicates adiposity and satiety. Although there is no universal relationship between day length and leptin, many seasonal animals undergo annual variation in food intake and adiposity. Not surprisingly, seasonal variation in leptin has been reported in a variety of species including woodchucks (*Marmota monax*) and Siberian hamsters (Klingenspor *et al.* 1996; Concannon *et al.* 2001; Marie *et al.* 2001).

Leptin interacts with the immune system both directly via receptors on immune cells and indirectly by increasing metabolic fuel availability. Obese *ob*-/- mice have fewer T-cells, reduced macrophage responsiveness and impaired wound healing (Lord *et al.* 1998). Similar outcomes were induced in wild-type mice following severe food restriction (leading to decreased leptin production); these effects could be reversed by exogenous leptin treatment (Lord *et al.* 1998). In genetically unmodified mice, leptin enhanced phagocytosis and T-cell proliferation (Baumann *et al.* 1996). Inflammatory stimuli such as LPS or recombinant proinflammatory cytokines, including IL-1β and tumour necrosis factor alpha (TNFα), acutely increased leptin concentrations and may be involved in the anorexia associated with inflammation (Grunfeld *et al.* 1996).

Siberian hamsters have stronger humoral immune responses in long versus short days. Treatment with exogenous leptin eliminated the short-day reduction in humoral immunity, body mass and food intake, but it did not affect these measures in long-day animals. In addition, leptin did not enhance immune function in short days when food intake was limited to levels consumed by short-day hamsters not supplemented with leptin (Drazen *et al.* 2001). Previous studies have indicated a photoperiod-induced differential sensitivity to leptin in this species (Klingenspor *et al.* 2000). A certain proportion of Siberian hamsters, as other photoperiodic animals, do not respond to short day lengths with reproductive regression (Prendergast *et al.* 2001*a*). Such animals produced anti-KLH IgG concentrations reflective of their photoperiodic exposure, not their reproductive state; that is, immune function of the reproductive non-responders was not different from reproductive responders when leptin was provided. However, leptin concentrations of the short day non-responders resembled that of long-day hamsters (Drazen *et al.* 2000*b*). A recent study demonstrated that leptin directly regulates humoral immunity in response to energy stores in Siberian hamsters. Hamsters undergoing lipectomy and treated with leptin did not display the decreased antibody responsiveness to a novel antigen that lipectomized, vehicle-treated hamsters did (Demas & Sakaria 2005). Taken together, these data indicate that leptin may be an important signal driving seasonal counterbalances between immune function and reproduction, but as with prolactin further study is necessary.

7. FUTURE PROSPECTS

A recent effort has been made to delineate the best way to study the immune systems of animals in ecologically relevant contexts (Norris & Evans 2000). A recent comparative study in birds indicated that single measures of immune activity do not adequately characterize inter- and intraspecific variation in immune defence (Matson *et al.* 2006). If these data hold for other taxa, then single measures of immune activity are probably insufficient to characterize immunocompetence. To resolve problems associated with measuring something as complicated and dynamic as immunocompetence, some have suggested a shift in focus to measuring disease resistance because only such measures reliably indicate whether changes in immune defence are directly relevant to fitness (Adamo 2004). A related proposal suggests that immune responses be ignored altogether and the fitness consequences of immune challenges or disease resistance endpoints be favoured (Viney *et al.* 2005). Although these approaches may be appropriate in certain contexts, a third approach may be more realistic given the difficulties associated with characterizing resistance to multiple pathogens and/or measuring lifetime reproductive success, be more amenable to using the techniques currently available and be more relevant in studies in which the immune system itself is the subject of interest. This approach, the immune defence component model (IDCM; Schmid-Hempel & Ebert 2003), suggests that consideration of the general characteristics of the immune system can achieve these ends.

The IDCM model (Schmid-Hempel & Ebert 2003) separates the immune system into four quadrants composed of two intersecting axes: one a continuum of specific to non-specific defence, the other a continuum of constitutive to inducible defences (figure 2). These categorizations do not necessarily match the conventional 'adaptive' versus 'innate' categorizations of immune activity (Janeway *et al.* 2004); rather, they account for more basic features of immune defences that the conventional classifications lack. For instance, the IDCM recognizes that some animals may favour constitutive over inducible defence if they live in habitats where parasite threats are high. Organisms in disease-depauperate environments might allocate resources solely to inducible defence mechanisms and invest more in physiological systems promoting reproduction. Likewise, the high costs of generating lymphocyte diversity may be prohibitively costly for short-lived species (Ricklefs 1992; Martin *et al.* 2006*a*). The short-lived species may favour

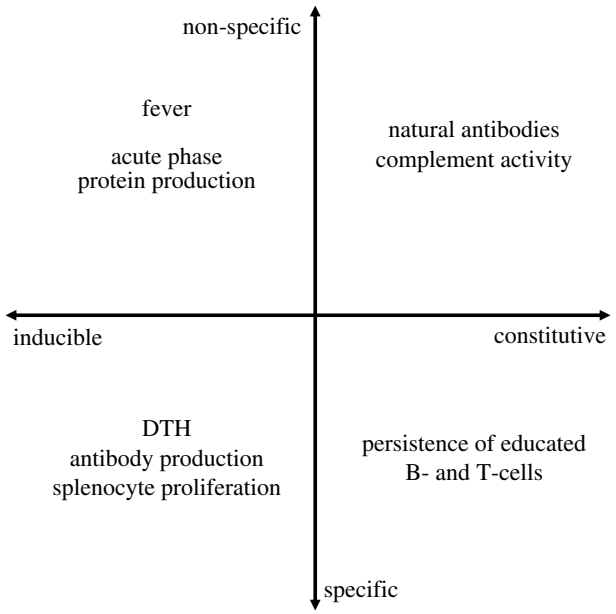

Figure 2. The immune defence component model (IDCM). This conceptualization of the immune system, originally proposed by Schmid-Hempel & Ebert (2003) separates immune defences into four quadrants representing the four types of defences available to animals. One axis represents a continuum from non-specific to specific defences, whereas the other axis represents static (constitutive) versus dynamic (induced) defences. In the figure, we categorize several of the assays currently favoured in ecological immunology (see table 1 for details of each assay). Adapted from Schmid-Hempel & Ebert (2003).

cheaper non-specific immune defences, especially if they are vagile and subsequently rarely participate in host–parasite arms races (Klasing 2004). Altogether, the IDCM leads to a new perspective for ecological immunology; instead of looking for changes in 'immunocompetence' over an animal's lifetime, attention should be focused on potential redistribution among different physiological components, including aspects of the immune system. In other words, trade-offs between reproduction and immune defence may be just that, or they may represent reallocations within the immune system itself (perhaps in a cost-effective manner) that would not be detected unless multiple, particular aspects of immune activity were measured (Martin *et al.* 2006*c*).

An additional benefit of this model is that unlike the conventional 'innate–adaptive' dichotomy, the IDCM is as applicable to other animal species as it is to mammals and birds. Additionally, the IDCM is amenable to the immunological techniques currently available to ecologists, although it emphasizes incorporation of potentially beneficial prophylactic defences (e.g. behavioural avoidance, grooming). Behavioural defences may be important as these defences may often alleviate the need for strong downstream investments in fever and antibody production, but they are rarely studied in this context.

A further critical requisite of the IDCM is that the immune assays that ecological immunologists currently favour should be understood as more than simple measures of immunocompetence. For example, PHA-induced wing-web swelling cannot be coarsely characterized as a cell-mediated immune index, as the immune activity underlying the swelling response involves more than just T-cells (Martin *et al.* 2006*a*). Moreover, care must be taken when initially categorizing immune measures ecologists commonly use. Figure 2 depicts our assessments of some favoured immunological assays, but these categorizations are open to debate. According to the IDCM, antibody production should be classified as a specific inducible defence. However, B-cells and constitutively expressed cells of the innate immune system can both influence antibody production. Likewise, DTH responses are typified as cell-mediated assays because T-cells orchestrate local inflammation even though macrophages and granulocytes are responsible for much of the swelling. For these reasons, the development of new immune assays and more in-depth studies of currently favoured assays is imperative. Years of successful research in other fields indicate that ecologists need not abandon favoured techniques just yet (Adamo 2004; Viney *et al.* 2005); they need only to appreciate that immune function is no more a monolithic entity than nervous system function (Matson *et al.* 2006).

8. SUMMARY

Research in the past decade has revealed that changes in the way organisms invest in immune defence over their lifetimes may help explain long-standing unresolved phenomena in evolutionary ecology. Refinements of current approaches in ecological immunology, particularly more attention to the nature of immune defence itself, may expand our appreciation of linkages among disease resistance, immune function and fitness. Studies in domestic chickens have shown that selection for antibody responsiveness can impinge on growth and reproductive output (Norris & Evans 2000). Conversely, lines of chickens selected for rapid growth versus prolific egg production use very distinct innate immune defences when given bacterial challenges (Leshchinsky & Klasing 2001). The natural extension of these studies in terms of seasonality in reproduction and immune activity in small birds and mammals might involve selective breeding. Such research could identify how immune investments impinge on life-history characters in an evolutionary sense, and they could intimate the endocrine mechanisms that drive these relationships.

In addition, emphasis on pre-emptive (e.g. constitutive) defences may help us better understand resistance or control of infection. Sickness behaviour, for example, is a strategy common to most vertebrates, but only recently have the adaptive benefits of these behaviours been proposed (Hart 1988). Finally, the physiological mechanisms by which immune challenges affect fitness remain unresolved. Are trade-offs between reproduction and immunity predominantly mediated through activation of acute phase responses (Klasing 2004)? If so, then proinflammatory cytokines (in the periphery and the brain) should modulate many important outcomes, and research on this level would lead to identification of the molecular mechanisms, which to this point in ecological immunology are generally unknown. Altogether, it is becoming apparent that

variation in immune defence in vertebrates is the rule more than the exception. It is also apparent, however, that we are only at the threshold of a new and enormously interactive realm of biology.

All studies cited from our laboratory comply with US NIH guidelines for animal research and were approved by the appropriate institutional animal care and use committees.

The authors thank Michaela Hau, Kristen Navara, Leah Pyter and Brian Trainor for their comments on previous drafts. Preparation of this manuscript and studies cited from our laboratory were supported by NIH grants MH57535 and MH66144 and NSF grant IBN 04-16897.

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