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Variation in immune defence as a question of evolutionary ecology

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The evolutionary-ecology approach to studying immune defences has generated a number of hypotheses that help to explain the observed variance in responses. Here, selected topics are reviewed in an attempt to identify the common problems, connections and generalities of the approach. In particular, the cost of immune defence, response specificity, sexual selection, neighbourhood effects and questions of optimal defence portfolios are discussed. While these questions still warrant further investigation, future challenges are the development of synthetic concepts for vertebrate and invertebrate systems and also of the theory that predicts immune responses based on *a priori* principles of evolutionary ecology.

Keywords: evolutionary ecology; immune defence; variation; review

1. INTRODUCTION

Until *ca*. 15 years ago, the study of immune defences was the almost exclusive domain of immunologists. Since then, the concepts of population biology, ecology and evolutionary biology have increasingly been applied and now shape one of the fastest growing areas of organismic biology (Sheldon & Verhulst 1996). On the one hand, this is done with the aim of understanding what the need to immune-defend means for the 'classical' study problems, such as sexual selection or life history. On the other hand, this approach holds promise of a deeper understanding of immunology itself, especially in terms of analysing adaptive defence strategies. So far, immune challenge by parasites has been the major interest although immune functions also include wound healing and the control of aberrant cells. Consequently, three main domains have dominated the research agenda: (i) immune defence, parasites and sexual selection; (ii) defence costs; and (iii) the problem of a general 'immunocompetence' versus an architecture of different specific response components.

Despite the obvious benefit of fending off disease and the pervasiveness of parasites, hosts remain susceptible, and observed immune responses vary widely across species and situations. The evolutionary-ecology approach attempts to explain why this variation exists and what the consequences are. Note, however, that defence against parasitism can be based on a variety of mechanisms, including behavioural defences (Moore 2002), herd immunity (Anderson & May 1985) and changes in life-history parameters (Minchella 1985). In this chain of events, the immune defence comes last (Schmid-Hempel & Ebert 2003).

2. THE PHYSIOLOGICAL BLACK BOX

Evolutionary ecology considers the fitness consequences of 'decisions', e.g. whether to maintain an immune system or to mount a response, and typically adopts a 'black box approach' largely ignorant of the actual molecular and physiological mechanisms of the immune system (Sheldon & Verhulst 1996). It is typically also unknown where the crucial step causing the observed variation in a response is located within the defence sequence, that is, whether it is in recognition, during the intermediate processes or in the effectors. Nevertheless, these mechanisms are potentially important, as they have a major impact on how studies are conducted and what questions can be asked.

The invertebrate immune response is based on both cellular and humoral components, on the actions of the pro-phenoloxidase (pro-PO) cascade (Ashida & Brey 1998) and on the inducible production of antimicrobial peptides (e.g. Boman 1995; Engstrom 1999). Because its activity is present before any parasitic challenge, the pro-PO cascade is partly constitutive ('innate immunity'). In addition, it can be activated by a wide array of antigenic challenges (Gillespie *et al.* 1997) and therefore also forms part of the 'induced' immune response (i.e. becomes active upon challenge). The activation eventually leads to the synthesis of melanin and to the production of humoral compounds (such as quinone) circulating in the haemolymph. These are toxic to micro-organisms (Söderhall 1998). In addition, building-up layers of melanized haemocytes can encapsulate and kill an invader.

The production of antimicrobial peptides in invertebrates is induced by cues that are conserved within broad classes of parasites (fungi, Gram-negative bacteria, Gram-positive bacteria, etc.). Signalling proteins and appropriate receptors (e.g. Toll and its allies; Imler & Hoffmann 2000) are involved but there are no genuine antibodies. The response is nevertheless specific because these broad classes each elicit a different array of peptides. These peptides are potent antibiotics capable of killing microbial parasites, for example, by changing the permeability of the parasite's cell membrane (Cociancich *et al.* 1993; Zasloff 2002). Even though invertebrates have no genuine antibodies, specific genotypic interactions between hosts and parasite strains are nevertheless found, for example, in water fleas infected by bacteria (Carius *et al.* 2001) and in bumble-bees infected by protozoan parasites (Schmid-Hempel *et al.* 1999).

Recent studies indicate that the constitutive (innate) immunity of vertebrates is part of the same ancient defence mechanism as in invertebrates, which has been conserved in evolution (Medzhitov & Janeway 1998). As in invertebrates, broad classes of parasites are recognized. The immediate responses protect against viruses, mobilize killer cells or release cytokines that generate the inflammatory response (this is often used to characterize the immune response; Christe *et al.* 1998; Zuk & Johnsen 1998). In addition, the cytokines and other signalling molecules alert and instruct the induced (adaptive) response (Fearon & Locksley 1996; Brown 2001; Medzhitov & Janeway 2002). For this, presumably, appropriate cofactors are required (Matzinger 2002).

The highly specific induced (adaptive) immune response is based on the activation of B-cells and T-cells that produce specific antibodies capable of very specifically binding to antigens. Antibody production can be readily measured by taking titres and has therefore been used in various studies (e.g. Deerenberg *et al.* 1997; Nordling *et al.* 1998). The specificity of T-cells is linked to the major histocompatibility (MHC) gene complex present on all cells (MHC I) or on T-cell activating cells (MHC II). Consequently, the MHC has become one of the study subjects of evolutionary and ecological immunology (Von Schantz *et al.* 1996; Penn & Potts 1998; Reusch *et al.* 2001).

Immune defence is based on a variety of different cell types with specific functions. Because the vertebrate immune system is much better understood in this respect, specific cell types can be assayed. Examples include leucocyte counts (Saino *et al.* 1995; Zuk & Johnsen 1998), the ratio of heterophiles to lymphocytes (Ots *et al.* 2001), haematocrit estimates (Gustafsson *et al.* 1994) and various combinations thereof (Saino *et al.* 1998). By contrast, the role of different cell types in the invertebrate immune system is still not fully understood. Studies have thus often referred to the total haemocyte count, which is sometimes even considered to be a correlate of the overall capacity to respond, i.e. a general measure of immunocompetence (Kraaijeveld *et al.* 2001; Wilson 2001).

As with any fast-growing field, the terminology is muddled. For example, for immunologists, 'immunocompetence' is the ability of a (developing) cell or organ to respond, while for evolutionary ecologists, it typically means a (measurable) general capacity of the individual to mount an immune defence. Similarly, for an evolutionary ecologist an 'adaptive' response is the result of evolution by natural selection (understandable in terms of fitness costs and benefits), while for an immunologist it is the acquired, typically specific, response based on current or previous individual experience. The required individual immunological memory is characteristic of the advanced vertebrates and, therefore, immunologists sometimes restrict the use of all such concepts to vertebrates. To make matters worse, there is little interaction between the literatures on vertebrate (mostly birds) and invertebrate (mostly insects) evolutionary immunology. These terminological confusions and taxonomic splits unfortunately do not do justice to the common evolutionary history and the common principles guiding the strategies of all immune defences (Brown 2001). Here, I review selected findings from contemporary research in an attempt to merge ideas across taxa and backgrounds, with reference to variation in immune responses.

3. VARIATION RESULTING FROM THE COST OF IMMUNE DEFENCE

Life-history theory assumes that immune defence is a trait whose costs (in fitness units) are traded off against some other fitness components (Sheldon & Verhulst 1996). In fact, there are different kinds of costs that operate on different time-scales and have different implications.

(a) *Evolutionary cost*

Variation in the expression of a component of the immune system may simultaneously affect another fitness-relevant trait (e.g. growth, reproduction) of the organism (and vice versa). Over evolutionary time, this can become entrenched in negative genetic covariances, such that a 'hard-wired' trade-off between the immune trait and another fitness component is observed (Stearns 1992). This can appropriately be called the evolutionary cost of the immune system, since immunity evolves at the expense of another trait. An individual cannot change this genetic covariance and, hence, the evolutionary cost constrains the decisions made by the individual.

Is there evidence for evolutionary costs? Because the immune system is rather complex, there are many possible genetic covariance relationships. Only a very limited number of instances have been investigated to date, but the findings generally support this notion (table 1). Sometimes, the selective advantage of increased immune function leads to a correlated unavoidable loss in a fitness component that is expressed at a different age (antagonistic pleiotropy; Stearns 1992). In fact, several authors have implied that the (evolutionary) cost of the immune response may be the causal link that underlies the crucial life-history trade-off between current reproduction and future expected success (Richner *et al.* 1995; Deerenberg *et al.* 1997). Similar findings in organisms that have no genuine immune system support this general view. For example, selection for increased resistance to viruses in the bacterium *Escherichia coli* leads to lower competitive ability as a result of changes in coat proteins (Lenski 1988).

(b) *Use costs: maintenance and deployment*

The use of the immune system is bound to affect an organism's nutritional needs. This can give rise to a reallocation of resources that appears as traded off costs. Two different costs of use need to be distinguished: maintenance of the immune function and deployment of a response. The former is the cost associated with keeping the system at a given level of readiness; the latter is the cost associated with actually responding to a challenge. Maintenance is partly covered by the trade-offs inherent in how evolutionary costs are expressed. However, within the constraints set by the evolved physiology, maintaining the immune function is still a plastic trait, which can be influenced by individual decisions. For example, in rodents, immune functions are seasonally upregulated at the onset of winter (Nelson *et al.* 1998). Similarly, high-performance athletes are known to downregulate their level of immune functions (Kumae *et al.* 1994).

Table 1. Examples of experimental studies of the cost associated with the evolution of an immune defence component.

selective regime	organism	effect on other fitness components	references
earlier or later age at pupation (i.e. age at reproduction)	mosquito (*Aedes aegyptii*)	earlier reproduction correlates with lower encapsulation response, the opposite for later reproduction	Koella & Boete (2002)
increased resistance to nematode infections	mosquito (*Aedes aegyptii*)	reduced reproductive success	Ferdig *et al.* (1993)
increased encapsulation response to common larval parasitoids (*Asobara tabida*)	fruitfly (*Drosophila melanogaster*)	reduced competitive ability	Kraaijeveld & Godfray (1997)
increased encapsulation response to virulent larval parasitoids (*Leptopilina boulardi*)	fruitfly (*Drosophila melanogaster*)	lower survival rate of larvae	Fellowes *et al.* (1998)
increased resistance to bacterial disease	honeybee (*Apis mellifera*)	slower larval growth	Sutter *et al.* (1968)
increased resistance to bacterial disease	honeybee (*Apis mellifera*)	higher larval mortality	Rothenbuhler & Thompson (1956)
increased resistance to granulosis virus	Indian meal moth (*Plodia interpunctella*)	slower development, lower egg viability, but increased pupal mass	Boots & Begon (1993)
increased resistance or susceptibility to *Schistosoma* infections	snail (*Biomphalaria glabrata*)	susceptible lines produce more offspring, irrespective of infection status	Webster & Woolhouse (1998)
increased body mass	turkey (*Meleagris gallopavo*)	reduced immune function	Bayyari *et al.* (1997) and Nestor *et al.* (1996)

Unfortunately, the costs of maintenance are intrinsically difficult to measure (Lochmiller & Deerenberg 2000). For example, downregulation of an immune function as a result of physical exercise or dietary restriction is suggestive of a maintenance cost (table 2) but not proof, as the downregulation may be necessary to avoid self-damage rather than reflecting a cost. Råberg *et al.* (2002) compared the basal metabolic rates of normal and lymphocyte-deficient knockout mice, which have a constitutive but no induced immune function. They found, contrary to their initial expectation, that deficient mice had higher metabolic rates than mice with the immune system wholly intact, presumably because of pleiotropic effects on constitutive immunity, which was upregulated as a compensation. This indicates that an optimal combination of innate and adaptive immunity may save energy. Major steps in the evolution of the immune system might thus have required new biochemical pathways to upregulate or downregulate the associated costs as, for instance, with the regulatory function of leptin in the context of starvation (Lord *et al.* 1998, 2001). The constraints set by maintenance costs are therefore probably different for vertebrate and invertebrate systems. Iso-female lines available in *Drosophila* offer the opportunity to investigate such questions in invertebrate immunity (Fellowes & Godfray 2000).

In contrast to the cost of maintenance, the deployment cost occurring when an immune system responds is readily measured, and its general nature has been convincingly demonstrated. In fact, deployment seems to use up a tangible part of an organism's energy budget (Apanius *et al.* 1994; Demas *et al.* 1997). In mammals (humans, rats, mice and sheep), an increase in metabolic activity in the range of 10% to over 50% has been estimated (Lochmiller & Deerenberg 2000). A typical experimental protocol is to increase the demand on another fitness component (table 2). For example, an experimental enlargement of clutch size in birds increases the workload for the parents. Subsequent tests have typically revealed that parents then demonstrate a lower response in both the humoral and the cellular arms of immunity (table 2). Ots & Horak (1996) also reduced clutch size and found lower infection rates, suggesting that higher reproductive performance is traded off against deployment costs. Although the underlying mechanisms are different, increased workload also affects immune response in insects (table 2).

Deployment costs of immunity have also been convincingly demonstrated with the reverse experimental protocol, that is, where the immune system is activated (typically without actual parasitic infections) and the consequences for host fitness are monitored. In many cases, the effects are condition dependent (Svensson *et al.* 1998; Moret & Schmid-Hempel 2000; Råberg *et al.* 2000). This illustrates a perhaps obvious but nevertheless important point: deployment of an immune defence is costly but these costs may often not be visible, because an organism can compensate for the extra demand with an extra intake of resources. Only if conditions deteriorate, will these costs become tangible and a trade-off with other fitness components be observed. Of course, it is always possible that no costs are observed because compensation within the immune system has taken place (*sensu* Råberg *et al.* 2002) and thus the wrong kind of response is measured.

An important and presumably much underrated cost of an immune response is the risk of self-reactivity. The underlying mechanisms have, again, been studied mostly in vertebrates. As a case in point, Råberg *et al.* (1998) and Westneat & Birkhead (1998) suggested that a lower

Table 2. Examples of studies of the cost associated with the use of immune defence components.

protocol	organism	effect of treatment	references
(*a*) nutrition and general stress			
restricted access to food	captive bumble-bee (*Bombus terrestris*)	reduces reproductive success but has no effect on encapsulation response	Schmid-Hempel & Schmid-Hempel (1998)
mechanical disturbance of 15 min duration	oyster (*Crassostrea gigas*)	various immune parameters down-regulated during stress, but stimulated for 30–40 min afterwards	Lacoste *et al.* (2002)
birds raised on supplemented diet or seeds only	captive zebra finch (*Taeniopygia guttata*)	seed-only diet reduces survivorship, and leads to reduced cell-mediated immune function in nestlings. No difference in adult birds, perhaps owing to compensation	Birkhead *et al.* (1999)
protein-rich or protein-poor diet	captive house sparrow (*Passer domesticus*)	protein-rich diet leads to higher cellular but lower humoral response	Gonzalez *et al.* (1999)
food deprivation or excess food	chicken (*Gallus domesticus*)	excess food decreases and deprivation increases various immune response parameters	Klasing (1988)
(*b*) manipulating workload			
clipping wings to prevent foraging and flying	free-flying bumble-bee (*Bombus terrestris*)	foraging bees show reduced encapsulation response	König & Schmid-Hempel (1995) and Doums & Schmid-Hempel (2000)
observation of activity	wild damselfly (*Matrona basilaris*)	after copulation or oviposition, encapsulation response decreased	Siva-Jothy *et al.* (1998)
comparing experimentally mated and unmated beetles	mealworm beetle (*Tenebrio molitor*)	mating reduces PO activity through juvenile hormone	Rolff & Siva-Jothy (2002)
experimental increase of parental effort by increasing brood size, and increasing daily work effort by different reward schedules	captive zebra finch (*Taeniopygia guttata*)	increased parental effort and workload reduce antibody titre against sheep red blood cells	Deerenberg *et al.* (1997)
clipping wing feathers to increase workload	wild tree swallow (*Tachycineta bicolor*)	clipping leads to lower humoral immune response, which correlates negatively with egg-laying date	Hasselquist *et al.* (2001)
experimental enlargement of clutch size	wild pied flycatcher (*Ficedula hypoleuca*)	no effects on offspring. Lower cell-mediated immune response in females with large broods. No effect on humoral response	Moreno *et al.* (1999) and Ilmonen *et al.* (2002)
experimental enlargement of clutch size	wild collared flycatcher (*Ficedula albicollis*)	reduced antibody production in response to antiviral vaccine. Higher infections by blood parasites. Condition has an effect in females with reduced broods	Nordling *et al.* (1998) and Cichon (2000)
experimental enlargement of clutch size	great tit (*Parus major*)	males have higher malaria infections	Richner *et al.* (1995)
(*c*) activating the immune system			
antigenic challenge by injection of LPS (lipopolysaccharides; the surface molecules of Gram-negative bacteria) and Sephadex beads	captive bumble-bee (*Bombus terrestris*)	reduced survival but only in bad conditions	Moret & Schmid-Hempel (2000)
experimental infection with microfilariae taken from mammalian host	mosquito (*Armigeres subalbatus*)	infection reduces egg development owing to common biochemical pathway	Ferdig *et al.* (1993)

(*Continued.*)

Table 2. (*Continued.*)

protocol	organism	effect of treatment	references
antigenic challenge by injection of tetanus vaccine	wild pied flycatcher (*Ficedula hypoleuca*)	reduced foraging effort and fewer offspring	Ilmonen *et al.* (2000)
antigenic challenge by injection of diphtheria–tetanus vaccine	blue tit (*Parus caeruleus*)	birds exposed to cold temperatures have lower antibody response. Basal metabolic rate under normal conditions increased	Svensson *et al.* (1998)
antigenic challenge by injection of diphtheria–tetanus vaccine	blue tit (*Parus caeruleus*)	females reduce contribution to nestling feeding, which may have condition-dependent reproductive costs	Råberg *et al.* (2000)
antigenic challenge by injection of sheep red blood cells	great tit (*Parus major*)	during week following injection: increase in basal metabolic rate and leucocyte stress index, but loss of body mass	Ots *et al.* (2001)
antigenic challenge by injection of sheep red blood cells after completion of first clutch	European starling (*Sturnus vulgaris*)	no effect on second clutch or other parameters of reproductive success	Williams *et al.* (1999)

immune response may be the result of an active suppression of the immune system. Damaged tissues and organs, and the stress-related expression of heat shock proteins, are suspected to promote antigens that are a favourite target of autoimmune reactions. However, 'autoimmunity' also occurs in invertebrates, although it naturally assumes other forms. During the insect cellular immune reaction (e.g. phagocytosis and encapsulation), severely cytotoxic molecules are produced as a by-product, in particular quinones and reactive forms of oxygen (Nappi & Vass 1993; Nappi *et al.* 1995). These molecules may help to kill an invader, and excess production is normally detoxified. But such toxic compounds pose a serious autoreactive threat to the organism, especially if it has an open circulatory system as found in insects.

(c) *Evolutionary cost versus use costs*

There are interesting parallels between immunity and the antibiotic resistance observed in bacteria. In both cases, we have to assume that these traits are costly yet vital for the organism. Studies in bacteria show that selection for increased resistance reduces the cost of resistance by simultaneously favouring genes that compensate for the cost of antibiotic resistance. As a result, resistant bacteria may be even fitter than non-resistant bacteria in the absence of the antibiotic (Schrag *et al.* 1997). The immune system is a well-regulated network, which may similarly have evolved to compensate costs incurred by one component with correlated changes in another. Indeed, the experimental demonstration of a use cost for the immune response often fails, as the examples in table 2 show. For example, experimentally increased parental effort in birds may have no, small or very diffuse effects on their immune response, or only affects the condition of dependent young. By contrast, many studies typically find a strong effect of condition or individual 'quality' on immune response (Horak *et al.* 1999; Ilmonen *et al.* 1999, 2002; Merila & Andersson 1999; Moret & Schmid-Hempel 2000). This hints at the possibility that immune systems may have evolved such that use costs are minimized under 'normal' circumstances. Thus, the use cost may be partly compensated by the evolutionary cost of the trait. Whatever the precise situation, variation in these cost structures is an important determinant of variation in immune function.

4. VARIATION RESULTING FROM SPECIFICITY

Specific host responses to different variants of the same parasite species are arguably a second important source of variation. Terminology again gives rise to some confusion, as for vertebrate immunologists 'specific' immunity means the specific binding properties of antibodies. Evolutionary ecologists, however, typically refer to the outcome of the host–parasite interaction regardless of the underlying mechanisms. Such specificity readily explains variation in observed immune responses, and is a factor that is currently underrated (Schmid-Hempel & Ebert 2003). If based on genotypic differences, specific immune defence also plays a potentially important part in generating rapidly fluctuating antagonistic coevolution, a process that has been invoked in the maintenance of sexual reproduction and recombination (i.e. the Red Queen dynamics; Peters & Lively 1999). Remarkably few attempts have been made to integrate these two major sources of variation in immune response, i.e. costly trade-offs and specificity, into a common framework (Frank 2000; Jokela *et al.* 2000).

Multiple infections by different parasites abound in free-living animals. Interactions between co-infecting parasites are known to occur, some of which are mediated by the specificities of the immune system itself (Beegle & Oatman 1974; Richie 1988; Fellowes & Kraajveld 1998). In vertebrates, acquired immunity against one parasite can also provide protection against another set of parasites (Cohen 1973). Such cross-immunity or concomitant immunity can even affect the evolution of parasite diversity itself (Haraguchi & Sasaki 1997; Lythgoe 2002). These aspects of specificity urgently require the further

Table 3. Examples of studies of immune defence and sexual selection.

study question	organism	finding	references
Does testes size variation fit the expectations of the immuno-handicap hypothesis?	greenfinch (*Carduelis chloris*)	males with larger testes have higher parasite loads and brighter plumage (as expected from hypothesis)	Merila & Sheldon (1999)
Does testosterone reduce immune response?	house sparrow (*Passer domesticus*)	testosterone implants lead to dominance, higher ectoparasite loads and a larger status badge. Testosterone reduces (immuno-suppression) but also increases (status badge) success	Poiani *et al.* (2000)
Does testosterone reduce immune response?	wild and captive house finch males (*Carpodacus mexicanus*)	in captive males it increases infection by coccidia, but opposite relationship is observed in free-living males, perhaps owing to condition-dependence	Duckworth *et al.* (2001)
Does testosterone reduce immune response? (Over prolonged times?)	wild and captive dark-eyed junco males (*Junco hyemalis*)	long-lasting testosterone implant reduces antibody production in captive males, but cell-mediated immunity in free-living males	Casto *et al.* (2001)
Does sexual activity reduce immune response?	damselfly (*Matrona basilaris*)	encapsulation response is lower shortly after courtship and copulation activities	Siva-Jothy *et al.* (1998)
Does sexual activity reduce immune response?	fruitfly (*Drosophila melanogaster*)	males exposed to many females have lower antibacterial activity in haemolymph	McKean & Nunney (2001)
Does testosterone reduce immune response? (Over prolonged times?)	sand lizard (*Psammodromus*)	long-lasting testosterone implant lowers immune haematological parameters and leads to higher tick loads and lower survival	Salvador *et al.* (1996)

development of models of immunity under multiple infections (Brown & Grenfell 2001).

Selection experiments indicate that increased resistance to a given parasite can be caused by specific responses (in snails; Webster & Woolhouse 1998) or by generalized responses (in *Drosophila*; Fellowes *et al.* 1999). Similarly, increased defence against one parasite may not be correlated with defence against another (Ferrari *et al.* 2001). As these invertebrate examples show, the interplay of general and specific responses to different parasites complicates or even precludes the search for a general measure of immunocompetence. Vertebrate studies have therefore reverted to the assay a number of immune measures simultaneously (tables 1–3).

5. VARIATION DUE TO SEX

Whether and how sexual selection targets variation in immune response has been one of the most active research fields over the past years, especially using birds as study systems (Hamilton & Zuk 1982). Collectively, the evidence suggests that immunocompetent males generally have higher success in mating and offspring production (table 3; review in Møller *et al.* 1999). The underlying reasons, however, are not completely understood (Westneat & Birkhead 1998). Female mate choice has been linked to immune response specificity associated with MHC variation (Jordan & Bruford 1998; Reusch *et al.* 2001).

In vertebrates, immune response and resistance to infection appear to be consistently lower in males than in females (Møller *et al.* 1998, 1999). The 'immuno-handicap' hypothesis explains this by suggesting that an increased level of testosterone in males increases mating success but suppresses the immune response (Folstad & Karter 1992). Although the empirical evidence suggests that there is a relationship between testosterone and reduced immune function (c.f. table 3), the precise role of testosterone is still debated (e.g. Casto *et al.* 2001). Indeed, vertebrate studies have overshadowed the fact that the difference between the sexes is actually taxonomically widespread and is, in particular, also found in insects and other invertebrates that lack testosterone (Zuk & McKean 1996; Gray 1998; Wedekind & Jakobsen 1998; Kurtz *et al.* 2000; Sheridan *et al.* 2000; Rolff 2001). Generally, sexual selection is part of the life history, and correlated effects of sexual selection affect the sexes differentially (Chippindale *et al.* 2001). Thus, immune function may vary generally as a result of different life histories for males and females (an evolutionary cost) (Adamo *et al.* 2001; Rolff 2001).

6. VARIATION RESULTING FROM NEIGHBOURHOOD-MODULATED IMMUNITY

Neighbouring individuals affect the defence of a host. For example, in social insects, colony organization (Schmid-Hempel 1998; Hart & Ratnieks 2001) and cooperative hygienic behaviour, such as removing fungal spores from nest mates (Rosengaus *et al.* 1998), reduce the risk

of infection. Under certain circumstances, resistance by only a fraction of individuals within a social group may prevent the spread of a disease ('herd immunity') (Anderson & May 1985). A socially organized neighbourhood typically also entails a hierarchy of dominance among individuals, which in turn affects individual immune responses according to rank (Barnard *et al.* 1996).

A relevant neighbourhood is also present when hosts live in dense aggregations, a situation that is likely to facilitate the transmission of a pathogen. In several insect species, it has been found that the standing activity of the pro-PO cascade is increased under crowded conditions (Wilson & Reeson 1998; Barnes & Siva-Jothy 2000; Wilson *et al.* 2002). Since the pro-PO cascade is part of a constitutive defence, this is seen to provide prophylactic immunity in a risky environment ('density-dependent prophylaxis').

7. THE DEFENCE PORTFOLIO AND OPTIMAL STRATEGIES

Immune responses can be mapped onto three major axes: strength, timing (immediate or delayed) and specificity. A response therefore reflects an entire portfolio, such as a mixture of immediate constitutive and delayed induced responses, whose evolution and use is subject to costs and benefits (Harvell 1990). If this adaptive hypothesis holds, the defence portfolio is of course expected to vary across populations and species in relation to the prevailing selection pressures exerted by parasites. In view of the extant variation in immune defences, a theoretical understanding of adaptive immune responses will be one of the major challenges for the future, but can currently rely on only a small body of work.

For example, Perelson *et al.* (1976), using control theory, modelled the optimal time when B-cells should switch from proliferation into becoming effector cells. Segel & Bar-Or (1999) analysed autoimmunity risks and were able to predict the optimal strength of the immune reaction that minimizes both damage to the host and pathogen replication. Modelling a more complicated portfolio, Shudo & Ywasa (2001) considered the problem of how to organize a swift constitutive and a delayed induced response that operate at different costs. As one would intuitively expect, if parasites grow fast within the host or the delayed response comes late, only the swift response should be used. De Boer & Perelson (1993) used a model that includes the constraints of self-recognition and autoimmunity to ask what diversity of parasites an immune system should recognize. This capacity is possibly limited by the available developmental time for screening and eliminating self-reactive antibodies (Müller & Bonhoeffer 2003). In all, optimal defence theory has more often been applied to plants (and especially to herbivory), perhaps because the trade-offs and defence mechanisms (e.g. toxins) are easier to understand and to model (Iwasa *et al.* 1996; Tuomi *et al.* 1999; Pavia *et al.* 2002).

The evolutionary-ecology approach requires that variation in the level of immunity maps onto a corresponding variation in fitness in the natural habitat of the organism, a problem that has been little studied. For example, Hasselquist *et al.* (2001) found that the level of the experimentally elicited immune response in tree swallows was correlated with the egg-laying date, which is an important fitness parameter in birds. Similarly, in bumble-bees, a positive correlation between the level of encapsulation response in the first brood and the eventual fitness of the colony was discovered (Baer & Schmid-Hempel 2003). Von Schantz *et al.* (1996) discuss the correlation between different MHC types and male viability. More such studies are needed and could be based on correlative evidence, field tests with selected lines or common garden experiments with lines differing in the desired characteristics.

8. CONCLUSIONS

Contemporary research on the evolutionary ecology of immune defences has uncovered a range of factors, such as costs, sex differences and specific and context-dependent responses, that help to explain why immune responses vary across species and situations. The multitude of factors also suggests that a simple general measure of immunocompetence will be hard to define. The simpler system of invertebrates may perhaps provide better prospects. However, this also requires the existing split between vertebrate and invertebrate literature to be overcome. After this first phase of testing and data collection, the next challenge is to develop concepts and models capable of predicting variation in immune responses based on *a priori* principles of evolutionary ecology.

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