

Ecological immunology: life history trade-offs and immune defense in birds

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There has been considerable recent interest in the effects of life-history decisions on immunocompetence in birds. If immunocompetence is limited by available resources, then trade-offs between investment in life-history components and investment in immunocompetence could be important in determining optimal life-history traits. For this to be true: (1) immunocompetence must be limited by resources, (2) investment in life-history components must be negatively correlated with immunocompetence, and (3) immunocompetence must be positively correlated with fitness. To gather such empirical data, ecologists need to be able to measure immunocompetence. We review techniques used to measure immunocompetence and how they are applied by ecologists. We also consider the components of the immune system that constitute immunocompetence and evaluate the possible consequences of measuring immunocompetence in different ways. We then review the empirical evidence for life-history trade-offs involving immune defense. We conclude that there is some evidence suggesting that immunocompetence is limited by resources and that investment in certain life-history components reduces immunocompetence. However, the evidence that immunocompetence is related to fitness is circumstantial at present, although consistent with the hypothesis that immunocompetence and fitness are positively correlated. We argue that future work needs to examine the fitness effects of variation in immunocompetence and suggest that artificial selection experiments offer a potentially important tool for addressing this issue. *Key words:* fitness, immune system, immunocompetence, life-history trade-offs. [*Behav Ecol* 11:19–26 (2000)]

Since the publication of Hamilton and Zuk's seminal paper on the role of parasites in sexual selection in 1982, there has been considerable interest in ecological and evolutionary relationships between parasites and their hosts (reviewed by Clayton and Moore, 1997; Lochmiller, 1996; Loye and Zuk, 1991; Sheldon and Verhulst, 1996; Zuk, 1996). Several studies have shown a trade-off between investment in life-history components, such as reproductive effort or sexual ornaments, and infection with parasites; increasing investment in such life-history components often increases the prevalence or intensity of parasitic infections (e.g., Møller, 1994, 1997; Norris et al., 1994; Richner et al., 1995; Sheldon and Verhulst, 1996). More recently, interest has focused on the possibility that the relationship between life-history decisions and parasitism may be mediated by a trade-off between investment in life-history components and immune defense by hosts (i.e., the prevention or control of an infection by pathogens or parasites) (Deerenberg et al., 1997; Folstad and Karter, 1992; Saino and Møller, 1996; Saino et al., 1997a,b). If resources (e.g., energy, protein, nutrients) are limiting, it is reasonable to assume that investment of resources in, for example, a larger family or a more elaborate sexual ornament, might reduce the resources available to invest in immune defense (Sheldon and Verhulst, 1996). Because the immune system plays such a pivotal role in defending an animal against attack by pathogens and parasites (Roitt et al., 1998; Wakelin, 1996), such a reduction in immunocompetence (i.e., an animal's ability to mount an effective immune defense) is likely to reduce fitness, so trade-offs involving immune defense could be crucial in determining optimal life-history decisions. We define "immunocom-

petence" as the ability of a host to prevent or control infection by pathogens and parasites.

To demonstrate that a trade-off between life-history decisions and immunocompetence is evolutionarily significant, three pieces of empirical evidence are required: (1) immunocompetence must compete with life-history components for access to limiting resources, (2) increasing investment in a particular life-history component must reduce immunocompetence, and (3) a reduction in immunocompetence must cause a reduction in fitness. If these conditions are fulfilled, it is then important to describe how life-history decisions and immunocompetence relate to fitness in order to understand optimal levels of investment in life-history decisions and immunocompetence. To gather such empirical data, it is clearly important to be able to measure immunocompetence. Therefore, the main aims of this review are to critically examine the techniques employed by ecologists to measure immunocompetence and the limitations of the techniques and the evidence for trade-offs of evolutionary significance between investment in life-history components and immunocompetence.

Our discussion is taxonomically biased toward studies involving birds because birds have a relatively complex immune system and are ideal subjects for the experimental manipulation of life-history decisions. Birds provide a unique opportunity to manipulate life-history decisions and immunocompetence in wild populations, and, as a result, the majority of experimental studies on wild populations have involved birds. However, our arguments are general and relate to any taxon within which the question of immunocompetence trade-offs is being considered.

Measuring immunocompetence

To examine trade-offs between life-history decisions and immune defense, ecologists need to be able to measure an individual's immunocompetence; that is, it is necessary to be able to quantify how immunocompetence changes as invest-

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Table 1
Techniques used to measure immune status and immunocompetence in studies of life-history trade-offs in wild bird populations

General type	Technique	Description	Examples
Monitoring	Leukocyte counts	Leukocytes, usually heterophils and lymphocytes, are counted in blood smears	Zuk and Johnsen (1998)
	H:L ratio	Ratio of heterophil to lymphocyte numbers in blood smears	Dufva and Allander (1995); Ots and Horak (1996)
	Buffy coat layer	Height of the buffy coat layer in a haematocrit capillary tube after centrifugation provides a measure of leukocyte abundance	Gustafsson et al. (1994)
	Plasma proteins	Concentration of plasma proteins assessed using standard spectrophotometry	Gustafsson et al. (1994)
	Serum proteins (e.g., immunoglobulins)	Protein levels assessed by standard agarose gel electrophoresis	Gustafsson et al. (1994)
Challenge	Assessment of humoral immune response	Humoral immune system is challenged by injection with a novel antigen (e.g., sheep erythrocytes) and subsequent antibody production quantified	Lochmiller et al. (1993); Deerenberg et al. (1997); Saino et al. (1997a); Nordling et al. (1998)
	Assessment of cell-mediated immune response	Cell-mediated immune system is challenged by injection with phytohemagglutinin and subsequent inflammatory response quantified	Lochmiller et al. (1993); Saino et al. (1997b); Christe et al. (1998); Zuk and Johnsen (1998)

ment in a life-history component changes. There are basically two different types of technique available for assessing immunocompetence that have been used by ecologists interested in life-history trade-offs: (1) monitoring techniques and (2) challenge techniques (Table 1). Monitoring techniques provide a measure of an individual's health and the status of its immune system at the time it was sampled. As a result, such techniques provide some information on an individual's immunocompetence but also reflect the immune response to current infections. This potentially makes certain techniques difficult to interpret if used as a measure of immunocompetence. For example, one monitoring technique used simply involves counting different types of leukocytes present in blood samples—heterophils (phagocytosing cells of the innate immune system) and lymphocytes (mainly T- and B-cells of the acquired immune system) (e.g., Dufva and Allander, 1989; Ots and Hórak, 1996; Zuk and Johnsen, 1998). Although it is possible that low lymphocyte numbers, for example, represent poor immunocompetence, such data could also reflect the lack of current infections requiring a specific immune response from the host. Conversely, high lymphocyte numbers could reflect high immunocompetence, the status of current infections, or a combination of both.

Such effects make it difficult to predict a priori the direction of correlations between investment in life-history components and immunocompetence measured using a monitoring technique. For example, reproductive effort in a hypothetical host is known to be positively correlated with the risk of parasitism. If we assume that this is caused by increased contact with vectors carrying the parasite rather than reduced immunocompetence, then we might expect the abundance of leukocytes in blood smears, for example, to be positively correlated with reproductive effort because birds experiencing high levels of effort also have a high current infection status. Conversely, assume that the relationship between parasitism and reproductive effort is caused by reduced immunocompetence. Under these circumstances we might expect a negative correlation between leukocyte abundance and reproductive effort. In reality, a combination of both possibilities is likely, so negative, positive, or nonsignificant correlations are possible depending on the mechanism linking parasitism and reproductive effort and the relative importance of immuno-

competence and current infection status in influencing the abundance of leukocytes in peripheral blood. In our opinion, therefore, the validity of certain monitoring methods as measures of immunocompetence *per se* is questionable.

For these reasons, ecologists have recently used challenge techniques, in which a component of the immune system, usually the humoral or cell-mediated acquired immune system, is exposed to a novel antigen and the subsequent immune response quantified (Table 1). The advantage of these techniques is that each individual in the study is exposed to a standardized challenge to their immune system, and the response of the immune system is quantified in a standardized way. Variation between individuals in the strength of the response to such a challenge is then taken as a measure of immunocompetence that can be related to life-history components or experimental manipulations of these components (e.g., Deerenberg et al., 1997; Saino et al., 1997a,b; Christe et al., 1998; Nordling et al., 1998). Although challenge techniques provide a potentially powerful way of examining trade-offs involving immune defense, the relatively simplistic approach to measuring immunocompetence used by ecologists contrasts with that advocated by immunologists interested in human health or the health of domestic birds (e.g., Dietert et al., 1994, 1996; Luster et al., 1992). Immunologists argue that, due to the numerous components in the immune system, several immune tests are required to measure immunocompetence effectively (Table 2). What are the possible consequences of an oversimplified view of the immune system, and what needs to be measured and why?

Most ecological studies that have used a challenge technique have employed a single technique that quantifies one component of the immune system, usually either the humoral or cell-mediated immune response (e.g., Deerenberg et al., 1997; Saino et al., 1997a,b; Christe et al., 1998; Nordling et al., 1998). For simplicity, consider the immune system as being composed of three primary components: innate immunity, humoral immunity, and cell-mediated acquired immunity. This classification approximates the components assessed using the immune assays proposed by Dietert et al. (1994, 1996) for domestic poultry (Table 2), the major components of immunocompetence identified by Luster et al. (1992) (see also Table 2 in Dietert et al., 1994), and those recognized by re-

Table 2**Example of a panel of assays used for assessing immune function recommended by poultry immunologists (adapted from Diertert et al., 1996)**

General immune system component	Immune measure	Assay	Examples of ecological studies using comparable measures
Acquired immune system	Integrated	ELISA ^a	Deerenberg et al. (1997); Saino et al. (1997a); Nordling et al. (1998)
	T- and B-cell status	CD4 and CD8 cell numbers and ratio	
	Cell-mediated immunity	Delayed hypersensitivity	Lochmiller et al. (1993); Saino et al. (1997b); Christe et al. (1998); Zuk and Johnsen (1998)
Innate immune system	Innate immunity	NO production by monocytes/macrophages	
Both	Innate immunity Immune stress response	Natural killer cell activity Heterophil:lymphocyte ratio in peripheral blood	Dufva and Allander (1995); Ots and Hōrak (1996); Zuk and Johnsen (1998)

^a Enzyme-linked immunosorbent assay, a measure of specific antibody production.

searchers examining immunocompetence in poultry (e.g., Cheng and Lamont, 1988). By using a single technique to assess only one of these components as a measure of overall immunocompetence, ecologists implicitly assume that there is a correlated response in the other components of the immune system not measured. That is, there is a reduction in investment in each component of the immune system as a result of resource limitation. If this assumption is correct, using a single challenge technique to measure immunocompetence would be adequate because it would provide an index of overall immunocompetence.

It is possible that resource limitation has a differential effect on the different components of the immune system (i.e., there is no correlated response). This could occur if individuals invested strategically in the different components of their immune systems. There could be sensible evolutionary reasons for doing this. There is evidence that limiting access to resources, such as energy or protein, compromises immune defense (e.g., Glick et al., 1981, 1983; Lochmiller et al., 1993; Saino et al., 1997b). As a result, it is possible that investment in one component of the immune system could compromise an animal's ability to invest in other components. Under these circumstances, animals could be forced to optimize their investment in each component, based on selection pressures generated by the important pathogens and parasites their immune systems have to combat. Investment of limited resources in immune system components should reflect the fitness effects on the host imposed by pathogens and parasites controlled by each component. How might such strategic investment influence the relationship between immunocompetence measures and resource limitation?

As resource limitation becomes more severe, investment in immune system components should be altered to maximize fitness for a given resource availability. This could conceivably mean that, as resource limitation increases, investment is initially reduced in one component (A) to maintain the effectiveness of another component (B), especially if component B is crucial in controlling potentially pathogenic infections. Clearly, if the immunocompetence of the host was measured by assessing a single component of the immune system under these circumstances, two different conclusions might be reached. If component A were assessed, it would be concluded that resource limitation reduced immunocompetence. However, if component B were assessed, it would be concluded

that resource limitation had no significant effect on immunocompetence.

There is evidence that selection can influence investment in one component of the immune system to the detriment of other components. Artificial selection can be used to produce domestic fowl lines that exhibit high (HA) or low (LA) antibody titers when immunized with sheep erythrocytes (Seigel and Gross, 1980). Gross et al. (1980) showed that HA birds were significantly less effective at controlling bacterial infections compared with LA birds. Bacterial infections are generally controlled by phagocytosing cells such as heterophils (i.e., innate immunity; Roitt et al., 1998), so these results suggest that selection for an improved immune response from a component of the acquired immune system could reduce investment in innate immunity. That is, there could be a trade-off between investment in different immune system components.

If investment in immune system components is shaped by selection in a similar way in wild bird populations, then ecological studies that examine the competence of only a single immune system component run an increased risk of type II statistical errors—failing to reject the null hypothesis that there is no relationship between immunocompetence and life-history decisions. This means that ecologists assessing only a single immune system component should be wary of nonsignificant results. However, significant results can be regarded as reliable evidence that life-history decisions do have consequences for immunocompetence.

Ecologists also implicitly assume that challenging a particular immune system component with a novel antigen produces a similar immune response, irrespective of the specific antigen used. Although this assumption has not been rigorously tested in wild bird populations, data from domestic fowl suggest that, indeed, immunocompetence measurements might not be antigen specific. For example, Gross et al. (1980) showed that chicken lines selected for a high-level antibody response to sheep erythrocytes also displayed higher immunocompetence when challenged with other antigens (e.g., Newcastle's disease virus vaccine).

Given these complexities, how should ecologists measure immunocompetence? There are two obvious differences between the approach to measuring immunocompetence advocated by immunologists (e.g., Table 2) and that used by ecologists. First, to our knowledge no ecological study has assessed

more than one immune system component during a particular experiment on a wild population (see Dabbert et al., 1997; Lochmiller et al., 1993, for examples of a panel of assays applied to a captive population). Second, no direct assessment has been made of innate immunocompetence in any ecological study involving wild birds. In vivo challenge techniques are available for assessing innate immunity (e.g., Cheng and Lamont, 1988). However, such techniques have not been applied to wild birds, so methods need to be developed. Alternative monitoring methods are also possible. Heterophils in blood samples can be isolated and counted (see Table 1) and their phagocytosing activity assessed in vitro (e.g., Rodriguez and Lea, 1994; Rodriguez et al., 1997) as a potential measure of innate immunocompetence. However, this monitoring method also has the problem of assessing both immunocompetence and the immune response to current infections.

Humoral and cell-mediated acquired immunity are crucial to controlling a range of pathogens and parasites, including viruses, intra- and extracellular parasites, and ectoparasites (Roitt et al., 1998; Wakelin, 1996). Innate immunity is generally regarded as having an important role in the control of some of these infections, particularly during the initial stages, but innate immunity is the primary means of controlling bacterial infections (Roitt et al., 1998). Therefore, it seems prudent to suggest that these immune system components need to be assessed in future studies interested in immunocompetence trade-offs. This presents practical problems. If different immune system components are simultaneously challenged, does this influence the effectiveness of each component compared with a challenge to a single component? This seems likely if immunocompetence is limited by resources. As a result, it might be necessary to assess different immune system components in different individuals experiencing similar experimental treatments. This would have important implications for sample sizes required in immunocompetence studies and for the statistical power of feasible field experiments. Nonetheless, it is important that these methodological issues are addressed. A more comprehensive assessment of immunocompetence does not preclude a role for studies focusing on single components of the avian immune system. However, such an approach has clear limitations if used as a general measure of immunocompetence, especially if the data suggest no significant effect of life-history decisions on the competence of the immune system component being assessed.

Trade-offs and immune defense

A number of studies have examined aspects of the potential trade-off between life-history decisions and immunocompetence. Are such trade-offs significant?

Resource limitation and immune defense

The evidence that an immune response is energetically costly is equivocal (see Råberg et al., 1998, for a review). There is also evidence that a short-term increase in food intake can reduce immunocompetence (e.g., Klasing, 1988). Furthermore, some nutrients act as immunomodulators, influencing the specific type of immune response exhibited by a host (e.g., Lessard et al., 1997). Nevertheless, there is evidence that resources such as energy and protein can limit immunocompetence. Glick et al. (1981, 1983) showed that dietary levels of energy and/or amino acids influenced humoral and cell-mediated immunocompetence in domestic fowl. The relationship between nutrition and immunocompetence in domestic fowl is reviewed by Cook (1991). Lochmiller et al. (1993) showed that cell-mediated but not humoral immunocompetence was significantly suppressed in captive northern bobwhite chicks (*Colinus virginianus*) raised on a diet with a low

protein content. This study also highlights the importance of examining different immune system components within a single experiment. The majority of studies have been conducted on captive birds, but one study has shown that resource limitation can reduce immunocompetence in a wild bird population. Saino et al. (1997b) showed that nestling barn swallows (*Hirundo rustica*) provided with a protein-rich food supplement at regular intervals after hatching had significantly better cell-mediated immunocompetence than controls.

Life-history decisions and immune defense

Over the past 10 years, two life-history decisions have been studied in detail with respect to whether investment increases the risks of parasitism: reproductive effort and sexual ornamentation. There is now compelling evidence that there is a trade-off between the risk of parasitism and these life-history decisions (reviewed by Møller, 1997; Sheldon and Verhulst, 1996). However, this evidence, in isolation, is not sufficient to show that these life-history decisions have consequences for immune defense. This is because parasitism not only depends on the host's ability to control an infection using its immune system, but also on the chances of a host being infected with the parasite, which might be unrelated to immunocompetence (see also Norris et al., 1994). Therefore, it is important to show that life-history decisions have a direct effect on immunocompetence.

Fewer studies have explicitly examined the link between life-history decisions and immune defense in birds. However, there is evidence that life-history decisions can have a direct effect on immunocompetence. Increased reproductive effort reduces immunocompetence. Deerenberg et al. (1997) conducted a number of experiments that examined the relationship between reproductive effort and humoral immunocompetence in zebra finches (*Taeniopygia guttata*) by quantifying the production of antibodies toward sheep erythrocytes. They showed that all nonbreeding birds produced antibodies but that only 47% of breeding birds produced antibodies. There was also a reduction in antibody response with increasing brood size, suggesting that increasing reproductive effort progressively reduced humoral immunocompetence (Figure 1a). Furthermore, birds showed a reduced antibody response if they were forced to undertake energetically costly tasks, implying that the increased work required during breeding could directly result in reduced immunocompetence. Nordling et al. (1998) conducted a comparable brood manipulation experiment on a wild population of collared flycatchers (*Ficedula albicollis*) (Figure 1b). They quantified humoral immunocompetence by quantifying antibody production toward Newcastle disease virus (NDV) vaccine and showed that female birds raising larger broods (i.e., having greater reproductive effort) had reduced immunocompetence.

The reproductive effort of parents also appears to have consequences for immune defense of offspring. Saino et al. (1997b) showed that there was a negative correlation between cell-mediated immunocompetence in nestling barn swallows and the size of the brood. A brood manipulation experiment showed that this relationship was causal: chicks in broods enlarged by one nestling had significantly lower cell-mediated immunocompetence compared with broods reduced by one nestling (Figure 1c). Saino et al. (1997b) argued that this resulted from a lower per capita feeding rate in larger broods, so chicks in such broods were more likely to be resource limited. This interpretation was supported by an experiment in which chicks in a number of different broods were provided with a protein-rich food supplement. Food-supplemented chicks had significantly higher immunocompetence compared with controls.

Investment in sexual ornamentation also appears to affect

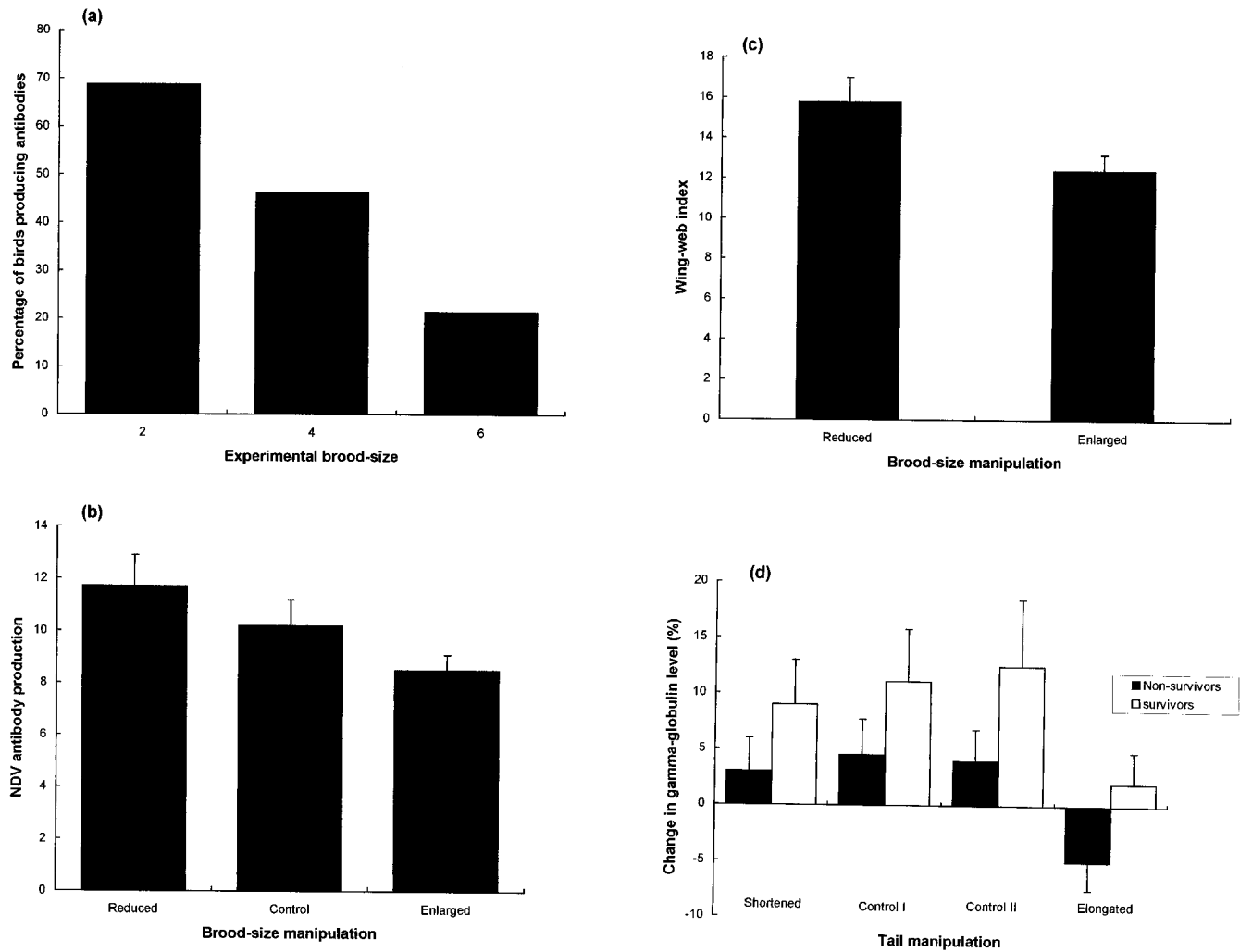


Figure 1

Life-history decisions and immune defense. (a) Humoral immunocompetence in relation to brood size manipulation (i.e., numbers of chicks in brood) in zebra finches. Immunocompetence was measured as the percentage of adult birds producing antibodies against sheep erythrocytes. Redrawn from Deerenberg et al. (1997). (b) Humoral immunocompetence in relation to brood size manipulation in female collared flycatchers. Immunocompetence was assessed by quantifying antibody production to Newcastle disease virus (NDV). Reduced broods had 2 nestlings removed, enlarged broods 2 nestlings added. Redrawn from Nordling et al. (1998). (c) Cell-mediated immunocompetence (assessed using phytohemagglutinin injected into wing web) in nestling barn swallows in relation to brood size manipulation. Reduced broods had one nestling removed, enlarged broods one nestling added. Redrawn from Saino et al. (1997b). (d) Humoral immunocompetence in relation to tail length manipulation in adult male barn swallows. Immunocompetence was assessed by quantifying the change in γ -globulin in plasma over a 3-week period following injection with sheep erythrocytes and expressing it as a percentage of the initial level. Males surviving and not surviving to a subsequent breeding season are shown separately. Redrawn from Saino et al. (1997a).

humoral immunocompetence. Male barn swallows have elongated tail ornaments that are important in mate choice by females; males with long tails are more likely to obtain a mate, mate earlier in the season and are more successful at mating with extrapair females (Møller, 1994). There is evidence that investment in this sexual ornament by males can reduce immunocompetence. Saino et al. (1997a) experimentally manipulated the tail length of male swallows and examined the impact of this manipulation on antibody production toward sheep erythrocytes. They showed that males with elongated tails had reduced γ -globulin levels, compared with control males (i.e., no change in tail length) or males with shortened tails (Figure 1d). This implies that humoral immunocompetence is compromised by increasing investment in tail ornaments.

Direct evidence of a trade-off between sexual ornamentation in male birds and immunocompetence comes from a re-

cent study on selected lines of domestic fowl. Verhulst et al. (1999) produced lines of domestic fowl selected for different levels of antibody response to sheep erythrocytes (low or high response) and control lines. They showed that lines selected for a high antibody response had significantly smaller sexual ornaments (i.e., comb size) than the lines selected for a low antibody response, with control lines intermediate. This is consistent with the idea that investment in immunocompetence reduces a male's ability to invest in his sexual ornaments, but the study also elegantly demonstrates a negative genetic correlation between immunocompetence and this life-history component.

Immune defense and fitness

Although there is experimental evidence that immunocompetence is influenced by resource availability and that investment in life-history components, such as reproductive effort

and sexual ornaments, has consequences for immunocompetence, reduced immunocompetence must reduce fitness for these trade-offs to be evolutionarily significant. That is, changes in immunocompetence of the magnitude recorded in experimental studies in relation to changes in investment in life-history components must affect fitness. This distinction is important because it is not sufficient simply to argue that a reduction in immunocompetence must have fitness consequences because the immune system is so important in pathogen and parasite prevention and control. How fitness changes in relation to changes in immunocompetence needs to be quantified to investigate optimal life-history decisions empirically.

Because the immune system is designed to prevent and control infection by pathogens and parasites, it is reasonable to expect that a reduction in immunocompetence might lead to an increased risk of infection. There is evidence consistent with this idea. For example, studies on reproductive effort have shown that increased effort is associated with reduced immunocompetence (e.g., Deerenberg et al., 1997; Nordling et al., 1998) and increased parasitism (Møller, 1997; Norris et al., 1994; Oppliger et al., 1996; Ots and Hörak, 1996; Richner et al., 1995). However, it is possible that correlations between reproductive effort and parasitism represent the risk of exposure to parasites rather than the influence of immunocompetence. To our knowledge, only a single study has reported the effects of reproductive effort on both immunocompetence and parasitism within a single experiment. Nordling et al. (1998) showed that female collared flycatchers raising enlarged broods had reduced humoral immunocompetence and an increased intensity of infection by *Haemaphysalis* parasites. This is consistent with the hypothesis that reproductive effort influences immunocompetence, which in turn influences parasitism, although these data are, in isolation, insufficient to demonstrate a causal link (see below for discussion).

Even if individuals with low immunocompetence were more likely to be infected with pathogens and parasites, it would still be necessary to show that such infections reduce fitness. Although it is generally accepted that parasites can reduce the fitness of their hosts (Clayton and Moore, 1997; Loye and Zuk, 1991), links among life-history decisions, immunocompetence, parasitism, and fitness must be established to show that immunocompetence trade-offs are likely to be of evolutionary significance. It is possible that life-history decisions influence immunocompetence, which in turn influences parasitism, but that increased parasitism has negligible fitness consequences in specific cases. For example, Norris et al. (1994) showed that increased reproductive effort by male great tits increased the prevalence of their hematozoan parasites. If we assume this was due to reduced immunocompetence, is this sufficient evidence of a significant trade-off? Circumstantial evidence would suggest not, as a number of studies have failed to show that increased reproductive effort in great tits causes a reduction in adult survival or future fecundity (e.g., Pettifor et al., 1988; Tinbergen and Daan, 1990), which would be expected if reduced immunocompetence and increased parasitism reduced the fitness of males.

There is experimental evidence from work on domestic fowl of direct links among immunocompetence, pathogens and parasites, and fitness. Gross et al. (1980) investigated the ability of lines of domestic fowl, selected for aspects of humoral immunocompetence, to control experimental infections with a range of pathogens and parasites. Two specific types of selection were included in the study with respect to humoral immunocompetence: (1) lines selected for high (HA) and low (LA) antibody response toward sheep erythrocytes, and (2) lines selected for persistent (PA) and nonpersistent (NPA) antibody responses to sheep erythrocytes. With the exception of

bacterial infections, the HA and PA selected lines had higher fitness than the LA and NPA lines when exposed to a range of viral and parasitic infections. This implies a link between immunocompetence and fitness. However, it could be argued that differences in fitness between strains (i.e., HA and LA or PA and NPA) could have arisen for reasons other than immunocompetence. This explanation seems unlikely because, for certain infections that could be treated, the administration of drugs removed the fitness difference between strains (Gross et al., 1980).

If a reduction in immunocompetence has fitness consequences because it renders hosts more susceptible to infection with pathogens or parasites, then immunocompetence should be positively correlated with survival. There is some evidence to support this idea. Saino et al. (1997a) showed that male barn swallows surviving to a subsequent breeding season showed a significantly stronger antibody response toward sheep erythrocytes than non-surviving males. Christe et al. (1998) showed that nestling house martins (*Delichon urbica*) of low rank within the brood hierarchy, which also had poor cell-mediated immunocompetence, were less likely to survive the pre-fledging period than higher ranking, more immunocompetent siblings. Nordling et al. (1998) showed that female collared flycatchers raising enlarged broods had lower humoral immunocompetence, increased parasitism, and that parasitism was associated with a reduction in survival probability to a subsequent breeding season.

Although these data are consistent with the hypothesis that immunocompetence influences fitness, they are also consistent with an alternative hypothesis (see Sheldon and Verhulst, 1996). It is possible that both survival and immunocompetence are condition-dependent traits. That is, an individual in good condition might have a high survival probability because it is, for example, better able to escape from predators or less likely to starve. Such an individual might also be able to invest resources in mounting an effective immune defense. The converse situation could be true for an individual in poor condition. Under these circumstances, there would be a correlation between survival and immunocompetence even if immunocompetence had no direct impact on survival. If life-history decisions influence condition, then one could erroneously conclude that there was a significant trade-off between such decisions and immunocompetence, even though there was no causal link between immunocompetence and fitness. Although consistent with the hypothesis that immunocompetence influences fitness, therefore, correlations between fitness components (e.g., survival and immunocompetence) might not reflect a causal link.

Future prospects

There is clearly a need for more studies on life-history decisions and immunocompetence in wild bird populations, both from a methodological perspective and to provide further empirical information on life-history trade-offs involving immune defense. There are few studies on wild birds that examine the impact of resource limitation on immune defense; further field experiments are required. This is especially important given the current debate concerning the extent to which immune defense is resource limited (see Råberg et al., 1998; Sheldon and Verhulst, 1996). Furthermore, only a small number of life-history decisions (reproductive effort and sexual ornaments) have been examined experimentally. It would be valuable to investigate immune defense trade-offs in other decisions to see how general immune defense trade-offs might be in life-history evolution. However, in our opinion, the most pressing issue is to understand the relationship between immunocompetence and fitness in bird populations. It is appar-

ent that it is difficult to establish a causal link using circumstantial evidence due to the possibility of condition dependence in fitness and immunocompetence. The only way to overcome this problem directly is to manipulate immunocompetence (Norris et al., 1994; Sheldon and Verhulst, 1996). There appear to be two possible ways of doing this: (1) administration of immunosuppressive or immuno-enhancing drugs, or (2) use of lines artificially selected for high and low immunocompetence.

A range of biotic and abiotic factors are known to influence the immunocompetence of domestic fowl (see Dietert et al., 1996) and could potentially be used to experimentally alter immunocompetence. Furthermore, specific drugs have been used to suppress particular immune system components in domestic fowl. For example, Arnold and Holt (1995) used cyclophosphamide and testosterone propionate to suppress humoral immunity in chickens. These drugs prevent development of the bursa of Fabricius and so selectively reduce humoral immunocompetence without influencing macrophages or cell-mediated immunity. Drugs such as cyclosporine A act on T-cells and can therefore be used to reduce cell-mediated immunocompetence (e.g., Arnold and Holt, 1995). It is unclear at present whether similar treatments would be suitable for experimental manipulation of immunocompetence in wild bird populations to examine subsequent fitness effects. This is because a suitable drug must (1) only influence immunocompetence and no other physiological or behavioral mechanism likely to affect fitness and (2) act over a sufficient time to render the host more susceptible to natural pathogens and parasites. Although drugs such as cyclophosphamide are likely to have a long-term effect on humoral immunocompetence, drug treatment using particular protocols can reduce host survival (Arnold and Holt, 1995). Thus there are significant methodological and ethical considerations in developing and applying comparable techniques in wild bird populations.

Selected lines provide an alternative way to establish a link between fitness and immunocompetence, as well as providing an elegant technique for examining trade-offs between life-history decisions and immunocompetence (e.g., Verhulst et al., 1999). The experiments on domestic fowl reported by Gross et al. (1980) and Seigel and Gross (1980) provide an example, as well as a range of experiments cited by Verhulst et al. (1999). It is at least feasible to produce selected lines among bird species that readily breed in captivity, which could then be released into the wild. It is also feasible to use artificial selection regimes to generate a range of immunocompetence among a host population to describe the relationship between immunocompetence and fitness. However, it would also be necessary to demonstrate that any fitness differences between strains (i.e., high and low immunocompetence) within selected lines in the wild was due to immunocompetence differences rather than other differences that affected fitness caused by the artificial selection regime. One way to do this would be to study a host population in which pathogens and parasites were well known and could either be controlled directly using drugs or varied in occurrence between host populations. This would allow the fitness of different strains in the presence and absence of pathogens and parasites to be examined.

Given the obvious difficulties of performing such experiments on wild bird populations, we are not arguing that all studies examining trade-offs between life-history decisions and immunocompetence need to undertake them. Rather, it would be valuable to perform such experiments on a few model systems. Gallinaceous birds seem to be ideal model hosts for this kind of experimental study because wild-caught birds can be bred and raised in captivity and released successfully into wild populations. Furthermore, for certain commercially

important species (e.g., red grouse *Lagopus lagopus*), pathogens and parasites are well known and vary consistently between host populations.

There are also specific methodological issues that require attention. First, it would be valuable to develop methods for assessing the innate immune response, in vivo, in wild bird populations. Using a standardized challenge would provide a comparable technique to those used by ecologists to assess humoral and cell-mediated immunity. However, counts of heterophils in peripheral blood and assessment of phagocytic activity in vitro may be a feasible alternative. Second, studies are required to examine how selection influences investment in immune system components; studies on the evolutionary ecology of the avian immune system are needed. Selection experiments on domestic fowl clearly show that the immune system could potentially be influenced by selection in wild populations mediated by the fitness effects of the range of pathogens and parasites experienced by particular hosts. It would be interesting to experimentally expose hosts to particular parasites over a number of generations against which the immune response is well documented to see whether such exposure alters the competence of the particular immune system components involved in defense to the detriment of other immune system components. Selected lines could also be used to examine the consequences of selection for high and low immunocompetence in one immune system component on the competence of other immune system components. It would also be interesting to see how the competence of different immune system components (e.g., innate, humoral, and cell-mediated) change along a gradient in resource availability within a population of hosts. Finally, it would be valuable to investigate experimentally whether there are interactions between immune responses to different challenges. If different immune system components are simultaneously challenged, does this influence the effectiveness of each component when compared with a challenge to a single component?

Conclusions

There is evidence to show that immunocompetence in birds can be limited by resources and that life-history decisions can have consequences for immune defense. However, field experiments on wild populations are still limited. Only one study has examined the relationship between resource limitation and immunocompetence in the wild, and experimental studies on the relationship between life-history decisions and immunocompetence have only focused on reproductive effort and sexual ornamentation. Furthermore, the evidence that immunocompetence correlates with fitness is, at present, only circumstantial. It is a substantial future challenge for ecologists to devise experiments that directly manipulate immunocompetence, thereby allowing them to examine fitness effects. Until such experiments are undertaken, it will remain uncertain whether trade-offs involving immune defense are significant in terms of determining optimal life-history strategies, even though evidence consistent with this view is steadily growing.

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