Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl

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Resistance to disease is frequently suggested to be important in mate choice, but information about how immune status can be conveyed is lacking. During the breeding season, male red jungle fowl with large combs, a sexually selected trait, have lower levels of lymphocytes, but greater cell-mediated immunity, indicated by a cutaneous hypersensitivity response. Before the breeding season, however, both cell-mediated immunity and proportion of lymphocytes are positively correlated with comb length. Cell-mediated immunity is particularly important to jungle fowl during the breeding season, because the likelihood of injury during sexual competition is high and cell-mediated immunity is essential for healing wounds and resisting infection. This seasonal change in one aspect of immunity but not another suggests that the birds adaptively maintain certain immune system abilities, and that it can be misleading to use a single aspect of immune response in evaluating immunocompetence.

Keywords: jungle fowl; immunocompetence; seasonality; ornaments; sexual selection

1. INTRODUCTION

Although many models of sexual selection predict that females choose mates of high quality to pass on superior fitness to their offspring (Andersson 1994), a practical definition of that quality remains elusive. Because parasites and pathogens are virtually universal threats to animals, resistance to disease, or immune system competence, has been invoked as an ideal criterion to be used by females in mate choice (Hamilton & Zuk 1982; Folstad & Karter 1992). Females in many animal species discriminate among males on the basis of costly secondary sexual characters such as bright plumage, and males with better-developed ornaments have also often been shown to have fewer parasites, which suggests that they have better immune systems (Zuk *et al.* 1990a; Zuk 1992).

Few studies have directly examined the link between ornamentation or mate choice and immune function, but these have sometimes found that highly ornamented individuals appear to pay a price by reducing some aspect of immunity. For example, male barn swallows (*Hirundo rustica*) with experimentally elongated tails had lower gamma globulin concentrations when their pre-manipulation tail length was shorter, suggesting that maintaining the tail imposes a cost to the immune system (Saino & Møller 1996), and song rate was also negatively correlated with gamma globulin and lymphocyte concentrations (Saino *et al.* 1997a). Lymphocytes were reduced in Arctic charr (*Salvelinus alpinus*) males with redder breeding coloration (Skarstein & Folstad 1996) and in red jungle fowl males with longer combs (Zuk *et al.* 1995). Male

house sparrows (*Passer domesticus*) with large black bibs had smaller bursae of Fabricius, an immune system organ, than did males with smaller ornaments (Møller *et al.* 1996). These measures of immune function are generally positively associated with fitness, however, as demonstrated in nestling and adult barn swallows and nestling European magpies (*Pica pica*), which showed higher survivorship or greater mass when immune response was highest (Saino *et al.* 1997b,c; Sorci *et al.* 1997).

Why should females prefer to mate with males that have apparently weaker immune responses? First, measuring lymphocytes or any other single component of immunity does not give a complete picture of immune response (Roitt *et al.* 1989; Westerman & Pabst 1990; Siva-Jothy 1995), and females may benefit if the males they choose are superior in other aspects of immunocompetence. The development of a meaningful assessment of immunity in the context of animals in the wild is still in its infancy, although of increasing interest to evolutionary biologists (Zuk 1994; Sheldon & Verhulst 1996). Second, the immunological cost of producing the ornament may be greater for low-quality males, creating an 'immunocompetence handicap' (Folstad & Karter 1992). In this case, ornaments such as antlers and fleshy ornaments like wattles are 'honest' signals of a male's ability to withstand the obligatory immunosuppressive effects of high testosterone titres. Cheating is unlikely because the cost of doing so, of presenting such characters when viability or fitness is low, is an automatic loss of immune function that makes the male too vulnerable to pathogenic effects of parasites for it to be maintained.

Third, the deficiency in immune function may only be temporary, associated with short-term stress such as that

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which occurs during a period of reproductive competition. Seasonal variation in immune response is common among a variety of animal species (Nelson & Demas 1996); white-crowned sparrows (*Zonotrichia leucophrys*), for instance, showed a decrease in the size of the spleen, another immune defence organ, before the breeding season began (Nelson & Demas 1996). If immunity varies seasonally, measurement at only one stage of the breeding cycle may give misleading results.

We examined the relationship between ornamentation and various measures of immune response in red jungle fowl (*Gallus gallus*). Red jungle fowl are the ancestors of domestic chickens, and are highly sexually dimorphic, with the male possessing a red fleshy comb and wattles as well as bright plumage, while the female is relatively drab and cryptic (Zuk *et al.* 1990*a,b*). In their native habitat of south-east Asia, the birds are polygynous, with a social unit consisting of a dominant male, one or more subordinate males, and several females and their chicks. Females often but by no means exclusively mate with the dominant rooster, and we and others have documented extensive female mate choice in this species (Zuk *et al.* 1990*a,b*).

When jungle fowl hens are presented with pairs of roosters, they typically prefer to mate with the male having a longer, redder comb (Ligon *et al.* 1990; Zuk *et al.* 1990*a,b*). Comb characteristics are condition dependent, and are adversely influenced by a variety of parasites as well as by nutritional state (Zuk *et al.* 1990*b*, 1995). Males with larger combs are also more successful in aggressive interactions with other males (Ligon *et al.* 1990), and the comb itself is a target during fights (M. Zuk, unpublished observations). Here we investigate the relationship between comb size in captive male red jungle fowl and two measures of immunocompetence, one non-specific and one cell-mediated, both during and outside of the breeding season. We wanted to determine if males with more attractive traits had higher or lower immunocompetence, and if the two measures of immunity showed similar relationships with comb length. We were particularly interested in how the relationship between ornaments and cell-mediated immunity changes from the time before the breeding season to the breeding season itself, when males are competing for access to females. Cell-mediated immunity is particularly important in wound healing, and because jungle fowl males risk injury during intrasexual competition, males with better cell-mediated responses at that time should be at an advantage. Females preferring such males will gain an advantage in sexual selection, and we therefore predicted that males with large combs would be better able to maintain their cell-mediated immune response.

2. MATERIALS AND METHODS

We evaluated one aspect of immunity by counting the relative numbers of circulating lymphocytes in the blood, a measure we and others have employed in other studies (Zuk *et al.* 1995; Saino *et al.* 1997*a*). Antibodies are produced by the B lymphocytes, and assuming that all males have had equal exposure to pathogens, males with a higher proportion of lymphocytes may have invested more in antibody-mediated immune response (Swails & Bell 1994; Zuk *et al.* 1995). Because our population is

captive and all males are kept under the same environmental conditions, this assumption seems warranted. For white blood cell counts, blood taken from the alar vein was smeared on a slide, air-dried, and fixed with methanol. The slides were stained with Giemsa and examined under $\times 1000$ oil immersion by two independent observers. Each observer identified 100 white blood cells as either lymphocytes, monocytes, eosinophils, basophils or heterophils; the mean count from the two observers was arcsin square root-transformed for analysis. Samples for blood cell counts were taken before the breeding season, both before and after protein injection, when the birds were eight months old, and again four months later, toward the end of the breeding season. Although we did not measure absolute numbers of white blood cells for these analyses, absolute lymphocyte and heterophil numbers were significantly positively correlated with the proportion of lymphocytes or heterophils in the smears in earlier data sets (Pearson correlation: lymphocytes, $r=0.53$, $p<0.001$, $n=44$; heterophils, $r=0.86$, $p<0.0001$, $n=44$). This correlation indicates that an increased proportion of lymphocytes reflects an overall increase in white blood cells.

We evaluated cell-mediated immunity, a generalized short-term response to grafts, allergens and wounds, using a delayed cutaneous hypersensitivity response (Roitt *et al.* 1989; Benjamini & Leskowitz 1991; Saino *et al.* 1997*b*; Sorci *et al.* 1997). This response is a measure of T-cell reactivity, and is assessed by subcutaneously injecting an inert protein and measuring the swelling that occurs within 24 h (Benjamini & Leskowitz 1991). Our procedure is adapted from Parmentier *et al.* (1993, 1994). The birds were given a sensitizing injection of 400 μg of phytohaemagglutinin, a plant protein often used to stimulate cell-mediated immunity, suspended in 0.4 ml of a phosphate buffer solution, administered subcutaneously into the abdominal region. The thickness of the wing web, a thin layer of skin between the radius and humerus, was measured to the nearest 0.01 mm using digital calipers after placing a metal disc 1 mm thick and 18 mm in diameter on both sides of the extended wing to standardize the measurements. For all measurements, the wing web was measured three times and the mean used in subsequent analyses; as in a study by Sorci *et al.* (1997), the measures were highly repeatable, with $r=0.94$ and $p<0.0001$. A week after the sensitizing injection, the wing web was measured and the birds were injected at the wing web with 0.1 ml of the same solution, and the wing web measurements repeated 6 and 24 h after this injection. Larger localized swelling indicates a more robust immune response. Different sets of individuals were used for tests before and during the breeding season to avoid confounding the results because of multiple treatments of the same birds with the protein.

Comb length was measured to the nearest 0.1 mm using digital callipers at the longest point of the comb on the same day males received their sensitizing injection. Measurements were taken both before and during the breeding season, which in our flock begins in late January and extends into May.

3. RESULTS

Before the breeding season, males with larger combs had significantly more pre-injection lymphocytes than did males with smaller combs (figure 1). During the breeding season, however, this relationship was reversed; in a previous study, comb size was significantly negatively correlated with the proportion of lymphocytes (Pearson correlation coefficient, $r=0.39$, $p=0.0017$; Zuk *et al.* 1995).

Figure 1. Relationship between comb length and the percentage of lymphocytes in white blood cells from unmanipulated male red jungle fowl sampled just before the breeding season began in January ($n=36$). The line is the least-squares regression ($r=0.47$, $p=0.004$). Eliminating the male with the extremely small comb does not alter the results of the analysis.

In contrast, tests for a cutaneous hypersensitivity response both before and during the breeding season showed a positive relationship between comb size and the amount of swelling (figure 2), suggesting that males with larger ornaments also have more robust cell-mediated immunity. This finding is mirrored by changes in the relative numbers of heterophils, white blood cells equivalent to the neutrophils of mammals, which are essential elements in the cell-mediated immune response, particularly at wound sites (Abramson & Wheeler 1993). Heterophil numbers increased significantly after the injection was administered (paired t -test, $t=2.10$, $p=0.04$). In addition, males with larger combs showed a significantly larger increase in heterophil number (figure 3).

4. DISCUSSION

Male red jungle fowl with larger ornaments have lower proportions of lymphocytes during the breeding season than at other times, but appear to continuously maintain their ability to heal injuries. This seasonal change is consistent with other studies of immunity over the course of the year in animals living in temperate climates, although the focus in most of those studies was on the ability of animals to compensate for the stresses of winter (Nelson & Demas 1996). Red jungle fowl, although tolerant of a wide temperature range, do not experience a temperate winter in their native habitat, but they do show marked seasonality in breeding physiology and behaviour. The breeding season may be stressful regardless of temperature changes simply because of the demands of courtship, mate competition, and in some species, parental care. In both collared flycatchers (*Ficedula albicollis*) and great tits (*Parus major*), it has been suggested that immune function may trade off against aspects of life history, such as clutch size or parental effort (Gustafsson *et al.* 1994, 1995; Richner *et al.* 1995; Oppliger *et al.* 1996). In these studies, individuals pay a price for reproductive effort by

Figure 2. Relationship between comb length and cutaneous hypersensitivity response in male red jungle fowl (a) before breeding began (January) ($r=0.38$, $p<0.05$), and (b) in a different set of birds ($n=51$) during the breeding season (May) ($r=0.33$, $p=0.02$). The thickness of the swelling in response to injection of phytohaemagglutinin into the wing web is a gauge of cell-mediated immunity.

Figure 3. Relationship between comb length and the change in the percentage of heterophils (arcsin square root-transformed) after phytohaemagglutinin injection into the wing web of male red jungle fowl during the breeding season ($n=31$). White blood cells were counted from smears made immediately before a sensitizing injection and 24 h after a second injection given one week later. The line is the least squares regression ($r=0.44$, $p=0.01$). Eliminating the male with the extremely small comb does not alter the results of the analysis.

decreasing investment in immune response. In contrast, our study suggests that the ability of males to maintain cell-mediated immunity is also greater in more ornamented individuals, so that high-quality males may indeed be better able to pay the price of maintaining elaborate secondary sexual characters. Whether this ability is due to genetic quality or some other source remains to be seen.

Sexual competition among male jungle fowl is extreme; males routinely engage in exhausting battles with other males, and their maximum aerobic capacity is higher than that of females even after accounting for differences in body weight (Chappell *et al.* 1997). Although we prevented injury to males during our own experiments, observations of free-ranging birds as well as the practice of cockfighting in many cultures suggests that the risk of wounding is extremely high. The ability to resist infection and promote healing should therefore be advantageous to breeding males. The immune system trade-off may be mediated through corticosterone; this hormone is elevated in sexually competing, stressed males, and causes heterophils to increase and lymphocytes to decrease (Dhabhar *et al.* 1995). Stress alone appears to enhance delayed-type hypersensitivity in rats (Dhabhar & McEwen 1996). Because comb length is positively correlated with both immune responses at times other than the breeding season, however, females should still benefit by choosing males with well-developed combs, even if those males temporarily pay a price in reduced immunocompetence. If dominant males, which also tend to have large combs (Ligon *et al.* 1990), also allocate more resources to cell-mediated immunity, they should benefit because of their greater predilection for engaging in fights.

Along with other researchers, we caution behavioural ecologists against adopting a unitary definition of immunocompetence, and against measuring one or even a few aspects of immunity and using those as universal gauges (Lochmiller 1995; Sheldon & Verhulst 1996). Likewise, it may be too simplistic to expect that more ornamented individuals will have uniformly 'better' or 'worse' immune responses; different parts of the immune system may be important under different circumstances. Like behaviour, the actions of the immune system are context dependent, making generalizations about the function of particular components difficult unless the current and past exposure of animals to disease, the time of year, and other variables are known (Zuk 1994).

We are grateful to the many people who help maintain the jungle fowl colony and assist with measurements. Mats Grahn, George Lozano, Frode Skarstein and four anonymous reviewers made useful comments on previous versions of the manuscript. This research is supported by grants from the UCR Academic Senate and the National Science Foundation to M.Z.

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