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Westneat, D.F. and Birkhead, T.R. (1998) Alternative hypotheses linking the immune system and mate choice for good genes. Proceedings of the Royal Society Series B: Biological Sciences, 265 (1401). pp. 1065-1073. ISSN: 1471-2954

https://doi.org/10.1098/rspb.1998.0400

The Royal Society

Alternative hypotheses linking the immune system and mate choice for good genes

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Why do males often have extravagant morphological and behavioural traits, and why do females prefer to mate with such males? The answers have been the focus of considerable debate since Darwin's *The descent of man, and selection in relation to sex* appeared in 1871. Recently, the broadening of investigation to include fields outside evolutionary biology has shed new light on mate choice and sexual selection. Here, we focus on a specific set of hypotheses relating the biology of resisting disease-causing organisms with the production of condition-dependent sexual signals (advertisements). We present a framework that distinguishes three different hypotheses about trade-offs within the immune system that affect general condition. The original Hamilton & Zuk hypothesis suggests that hosts fight off disease via resistance to particular pathogens, which lowers resistance to other pathogens. Changes in pathogens over evolutionary time in turn favours changes in which genes confer the best resistance. Alternatively, the immunocompetence hypotheses suggest that the energetic costs of mounting a response to any pathogen compete for resources with other things, such as producing or maintaining advertisements. Finally, improving resistance to pathogens could increase the negative impacts of the immune system on the host, via immunopathologies such as allergies or autoimmune diseases. If both disease and immunopathology affect condition, then sexual advertisements could signal a balance between the two. Studies of hypothesized links between genes, condition, the immune system and advertisements will require careful consideration of which hypothesis is being considered, and may necessitate different measures of immune system responses and different experimental protocols.

Keywords: sexual selection; dimorphic traits; female preference; immunocompetence; condition-dependent handicap; disease resistance

1. INTRODUCTION

Females might choose males as sexual partners for a variety of reasons. Sometimes males provide resources useful to the female or her offspring (e.g. Alexander 1974; Kirkpatrick & Ryan 1991). Males always provide genes, and female preferences for particular males could evolve via genes passed on to their offspring. There are two broad (non-exclusive) classes of hypotheses for the evolution of female choice based only on genotypic (or indirect (Kirkpatrick & Ryan 1991)) benefits. One is Fisher's runaway model, in which female preference becomes correlated with the male trait and evolves owing to perturbations from equilibrium followed by correlated changes in both sexes (Fisher 1930). The other class of hypotheses have been termed 'good genes' models (Trivers 1972). A subset of these propose that a focal male trait is a signal of underlying quality, which can be inherited by offspring, thereby improving their survival (e.g. Zahavi 1975; Hamilton & Zuk 1982; Andersson 1994). One version of this idea suggests that male traits advertise the quality of their genotypes via general condition (condition-dependent handicap (Andersson 1994)). In this paper we focus explicitly on the hypothesis that quality is linked to the functioning of the immune system; males that can produce an extravagant advertisement have genetic make-ups that provide a better defence against pathogens.

We have approached this subject as relative outsiders. Both of us have long been interested in how female behaviour affects mating patterns (e.g. Westneat *et al.* 1990; Birkhead & Møller 1992), but we have only recently begun thinking about the immune system and mate choice. In doing so, we attempted to organize what we considered might be alternative routes by which the immune system, or some component of it, could have effects on condition. We have focused explicitly on condition-dependent signals and have ignored some systems (e.g. odour-mediated mate choice for major histocompatibility complex (MHC) compatibility (e.g. Potts *et al.* 1991; Yamazaki *et al.* 1994)) which do not involve extravagant advertisements. Not only did this help us to understand some of the complexity in immune responses, but we found that our approach also gave us some guidance about what might be appropriate tests of the alternatives. Here, we present our basic approach, the three routes by which the immune system might affect condition, and some comments on tests of these alternatives.

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Received 15 December 1997 *Accepted* 27 February 1998

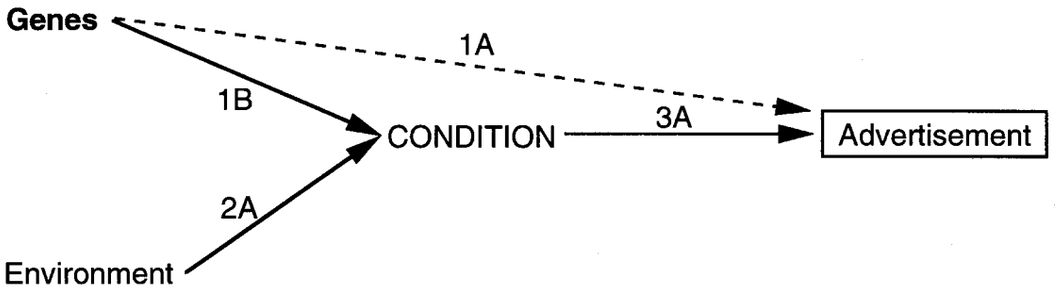

Figure 1. Path diagram illustrating relationships among key variables in a general model of condition-dependent advertisements in which the benefit of female choice is the genes (in bold) that the male can provide for the female's offspring. Path analysis seeks to explain causal sources of variation in important traits (Wright 1968) within populations. Empirically derived path coefficients at any one level of organization are measured as the partial correlation coefficients in a multiple regression. Hence, paths 1B and 2A represent the partial correlations of genes and environment, respectively, on condition, and paths 1A and 3A represent the independent effects of genes and condition on advertisements. Although path analysis is a statistical procedure, the arrows also represent underlying causal relationships. Thus, path analyses can be used either for data analysis or for organizing complex relationships among variables, and it is the latter use that we employ here. We recognize that any such diagram may leave out important variables, and, as in this case, might ignore covariances between variables at a given level (e.g. covariance between genes and environment). We deliberately omit some of these complications in order to clarify key processes (figure 2).

2. CONDITION-DEPENDENT ADVERTISEMENTS

The basic idea of condition dependence of male sexual signals (for reviews, see Grafen (1990), Kirkpatrick & Ryan (1991), Andersson (1994) and Ryan (1997)) is illustrated by the path diagram in figure 1. Variation in condition in a population of males will be the result of genetic variation (path 1B) and environmental variation (path 2A). Here, we broadly define condition as the ability of the organism to mobilize resources (energy or nutrients) for a particular task. Variation in advertisement is affected by variation in condition (path 3A), as well as a direct and independent effect of genetic variation (1A; in many models this path coefficient becomes small because selection drives genes to fixation (Andersson 1994)). In order for females to predict accurately which males have good genes, the magnitude of the paths leading from genes to condition (1B) and from condition to the advertisement (3A) must be high. As a consequence, the magnitude of the path leading from environment to condition (2A) must also be low (the latter may be less binding if there is a covariance between genes and environment, but this complexity is beyond our discussion here).

All condition-dependent advertisements depend on a positive value of the path from condition to advertisement. Many studies hint at this relationship, but few have directly tested it by manipulating some aspect of condition and measuring a change in advertisement (for a review, see Andersson (1994)). From here, we will assume that condition dependence of advertisements exists. We also assume that females choose partners based on these advertisements.

3. IMMUNE SYSTEM FUNCTION AND CONDITION-DEPENDENT ADVERTISEMENTS

Hamilton & Zuk (1982) developed the idea that because disease affects condition, a male's ability to fight off disease-causing organisms would affect his ability to put resources into sexual advertisements. They also suggested that because pathogens would be selected to avoid host defences, genetic resistance to pathogens would be part of an evolutionary arms race between host and pathogen. Their idea first stimulated a flurry of studies on potential pathogens, particularly parasites (for reviews, see Read (1988), Møller (1990*a*), Zuk (1992) and Andersson (1994)). More recently, attention has shifted to measuring the quality of the host's immune system (e.g. Folstad & Karter 1992; Gustaffson *et al.* 1994; Skarstein & Folstad 1996; Saino *et al.* 1997). Some of these latter studies have focused less on the coevolution between immune system and pathogen (and the cost of the disease itself), and more on the potential energetic costs of mounting an immune response (e.g. Wedekind 1992; Wedekind & Folstad 1994; Sheldon & Verhulst 1996) as the key factor affecting condition dependence. This shift in emphasis suggested to us that there might be multiple ways in which trade-offs involving the immune system would affect condition (see Zuk 1994). Clarifying the distinctions between alternatives seemed to us to be useful in testing the general idea of mate choice for immunity.

Any immune response includes two steps: (i) the host must first recognize a pathogen as an invader, and (ii) it must mobilize resources to destroy it. In the vertebrate immune system, once a pathogen enters the body, two different modes of defence act to destroy it (Campbell 1993). One mode, the cellular (also formerly called non-specific, natural or innate) response, is a system of macrophages, killer cells, blood proteins, interferons and inflammatory responses that destroy pathogens directly or resist their spread. Recognition of invaders in this system occurs via chemical gradients (e.g. histadines, prostaglandins or chemicals on the surface of the pathogen) or via interactions with the other mode, the humoral (also once called adaptive, learned or specific) response. The humoral response results in the increased production of proteins (antibodies) that bind specifically to portions of particular pathogen molecules, thereby neutralizing them or tagging them for destruction by phagocytes, T-cells or the complement pathway.

Here, we make an additional distinction about immune system components. Both the cellular and humoral

responses require recognition that an object is an invader. Both are also potentially involved in the invader's destruction. Some pathogens, however, are easier to recognize and destroy than others. Thus, the effectiveness of both cellular and humoral modes can be pathogen-specific (e.g. Golub & Green 1991; Playfair 1995). In addition, both cellular and humoral responses contain machinery that maintains and replicates the cells and biochemical agents involved in the response. Such machinery is common to all responses, and so is involved in the effectiveness of both modes of defence against any pathogen. Hence, a particular individual's resistance to a pathogen will be a result of both specific and general immune effectiveness (or resistance) (e.g. Wedekind 1994). We use these terms again below; we emphasize that they do not correspond to the standard distinction of specific and non-specific immune responses, which refers to whether or not the immune system can adapt its responses in ecological time to different pathogens (Playfair 1995). Obviously, the two dichotomies overlap, as the adaptive response of the humoral system improves its effectiveness against specific pathogens while simultaneously maintaining the ability to respond broadly to many different pathogens.

4. ALTERNATIVE HYPOTHESES ABOUT TRADE-OFFS WITH THE IMMUNE SYSTEM

Given that the immune system has components that act specifically against certain invaders and other components that are common to the responses to any invader, we identified three different trade-offs within the immune system that might affect general condition and hence influence sexually selected advertisements. We describe them separately below as alternative routes, linking the immune system with condition (figure 2*a*–*c*).

(a) *Route 1: specific resistance, disease and condition*

We interpret the first route (figure 2*a*) as presenting the idea originally suggested by Hamilton & Zuk (1982): high genetic resistance to specific pathogens (path 1C) will result in lower disease (path 5) and hence better condition (path 6) and more extravagant advertisements (path 3A). In this route, there is no direct link between the immune system and condition; only the disease caused by the pathogen affects condition. The key cost of immunity is that modifications to either cellular or humoral responses, which more effectively combat a particular pathogen, reduce the immune system's effectiveness against other pathogens (e.g. Frank 1994; Wedekind 1994). For example, particular MHC proteins are more effective in recognizing or presenting some antigens (e.g. Briles *et al.* 1983; Lamont *et al.* 1987; Hill *et al.* 1991). Similarly, particular forms of lytic enzymes might be necessary to overcome the protective capsules of some bacteria, such as *Mycobacterium tuberculosis* and *Brucella* (Playfair 1995). Hamilton & Zuk (1982) noted that specific resistance creates strong selection on the pathogen to escape host defences. Hence, an evolutionary change in the pathogen (or an increase in the abundance of another species) will in turn change the underlying basis for resistance, resulting in an unending coevolutionary war between host and pathogen species. Because of the effect of disease on condition, the advertisements always signal the level of resistance.

A number of reviews have presented the key assumptions and tests of this idea (e.g. Read 1988; Møller 1990*a*; Zuk 1992). Our conceptual framework echoes many of these and we discuss them only briefly for comparison with the other two routes described below. For females to gain genes for resistance via this route alone, the paths 1C, 3A, 5 and 6 must all be substantially different from zero (paths 5 and 6 must be negative). However, in order for the genetic benefits of female choice to persist, the pathogen species must evolve to cause a reduction in the effects of particular resistant genotypes on the disease caused by the pathogen (i.e. the magnitude of path 5 must change over evolutionary time for a given host genotype). We can broaden this idea by noting that the magnitude of path 5 could be different as well for different pathogen species that all coevolve (Eshel & Hamilton 1984) or fluctuate ecologically.

Because tests of Hamilton & Zuk's (1982) hypothesis have been recently reviewed (e.g. Read 1988; Møller 1990*a*; Zuk 1992; Andersson 1994), we will not explore them in great detail. Many studies have measured correlations between parasites, condition and advertisements (paths 6 and 3A; reviewed by Møller (1990*b*), Zuk (1992) and Andersson (1994)); fewer have experimentally manipulated parasites or condition and measured the causes of such correlations (e.g. Milinski & Bakker 1990; Møller 1990*b*; Zuk *et al.* 1990). Relatively few studies of sexual selection have measured path 1C, a genetic effect on variation in immunity. A study of particular interest concerns the role of the MHC in spur length in the pheasant *Phasianus colchicus* (von Schantz *et al.* 1996). As noted above, MHC fits the idea of specific immune effectiveness embodied in Hamilton & Zuk's model. von Schantz *et al.* (1996) found that spur length is a condition-dependent sexual advertisement and appears to be a target of female preferences. Spur length was correlated with particular MHC genotypes, and the males with those genotypes also had higher survival. Interestingly, the positive relationship between spur length and MHC genotype switched among different genotypes between years (von Schantz *et al.* 1997), suggesting that the effectiveness of particular combinations of MHC against pathogens changed with time. We do not know if (i) spur length was longer in these particular males because they were protected from a particular pathogen, or (ii) the important pathogen changed with time, either by evolving or being replaced by another one. Both (i) and (ii) are critical for the Hamilton & Zuk idea.

Recently, many researchers have begun measuring the magnitude of the humoral immune system response using novel but non-replicating antigens (e.g. Saino *et al.* 1995; Zuk *et al.* 1995; Ros *et al.* 1997). These are useful techniques (see below), but on the basis of how we have defined route 1, they do not provide a measure of the immune system that is relevant to route 1. The key idea embodied in route 1 is that there is a trade-off between becoming more resistant to one pathogen at the expense of being resistant to other pathogens. When such specific resistance is involved, responses to a novel antigen will tell us little about how the organism can respond to the real pathogens it currently faces.

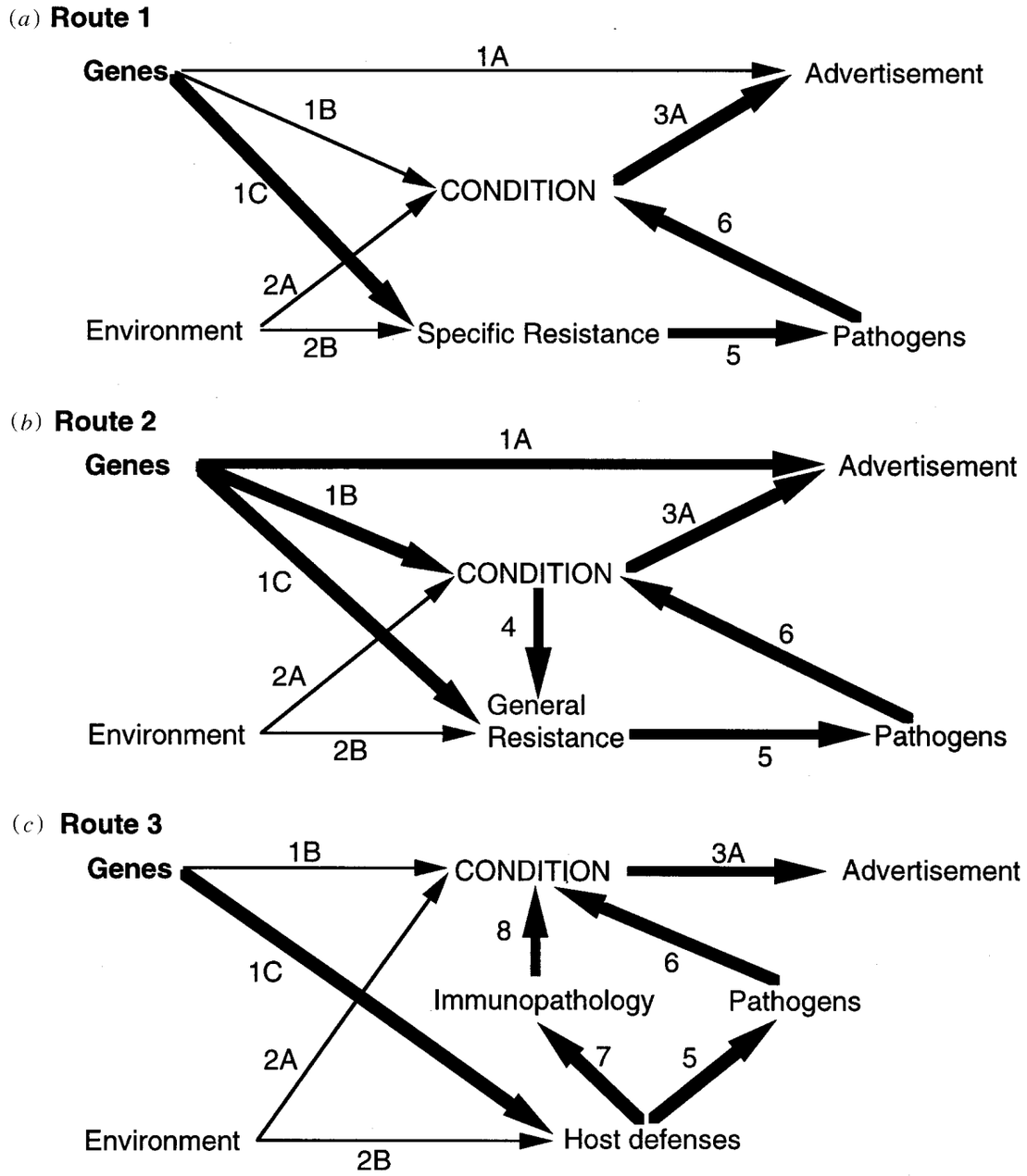

Figure 2. Path diagrams illustrating three different routes linking genes, immune responses, condition and sexual advertisements. (*a*) Route 1, depicting Hamilton & Zuk's (1982) idea that genes affecting specific immune effectiveness (specific resistance; path 1C) would reduce pathogen prevalence (path 5), which would improve condition (path 6), thereby resulting in a more extravagant advertisement (path 3A). (*b*) Route 2, depicting condition-dependent general immune effectiveness (general resistance). Genes affect condition directly (1B), the level of advertisement given a particular condition (path 1A), or the level of immune response given a particular condition (path 1C). General resistance trades-off with the advertisement over condition (paths 3A and 4), with the benefit of higher immunity being lower disease (path 5) and the benefit of higher advertisement being increased mating success. (*c*) Route 3, depicting dual effects on condition of the immune system via pathogen resistance on the one hand and the risk of immunopathologies on the other. Genes affect the aggressiveness of the immune system (path 1C), which affects disease (path 5) and immunopathology (path 7) in opposite directions. Both disease and immunopathologies have negative effects on condition; the optimal balance of those two will maximize condition and produce the most extravagant advertisement (via path 3A). In all three routes, some effects of environment are included; path 2A is the effect of environmental variation on condition, path 2B is the influence of environment on the immune system (e.g. via prior exposure to pathogen and 'memory' of the immune system).

(b) *Route 2: condition and the energetics of general resistance*

In route 1, the costs of making and maintaining the components of the immune response were considered negligible. Wedekind (1992) and Wedekind & Folstad (1994) suggest that the energetic costs of making and maintaining immune system components is the key effect on condition, which might create a link between the immune system and sexually selected advertisements. This cost seems likely to be found in the machinery

common to the response to any pathogen. Thus, in this route (figure 2*b*) we focus on general resistance. Host resources are used up by the advertisement, the immune system and by the disease (paths 3A, 4, and 5), and so only those individuals in good condition can mount a strong immune defence (or handle the disease (e.g. Skarstein & Folstad 1996)) and produce an extravagant advertisement.

We suggest that genetic variation could have four different effects on advertisements in this route. First, there could be genetic variation for the rules by which condition is allocated to the immune system or the advertisements. Such variation might persist because the optimal balance between immune system or advertisement changes owing to shifts in the presence of pathogens in the environment or in their effects on the host (a shift in the magnitude of path 6). In figure 2*b* we assume that this allocation rule is fixed; three other paths then describe possible independent effects of genetic variation. One (path 1B) represents the effect of genetic variation on variation in condition. One possible mechanism could be via genetic differences in foraging behaviour. Individuals that forage appropriately would have high condition, and could produce a more elaborate advertisement, mount a larger immune response, handle a higher invasion of pathogens, or some combination of all three. Alternatively, genetic variation could affect the cost of the advertisement (path 1A), perhaps via more optimal growth or better timing of displays. Quality individuals would have more resources to be allocated resulting in a more extravagant advertisement. Finally, genetic variation could affect the cost of the immune system, perhaps in two ways. First, a given level of response could be less costly to produce, perhaps via conformational changes in key enzymes that make production of antibodies or growth of cells more efficient (path 4 would be less in these genotypes). Alternatively, improved specific resistance might reduce the quantitative response needed to fight a pathogen (see below). Both of these would result in a shift in use of resources to other tasks, including a more elaborate advertisement.

An important difference between routes 1 and 2 is that in route 2, there is a general aspect of the host's defence system that is costly. Others have reviewed whether or not generating an immune response entails resource-based costs (Sheldon & Verhulst 1996; Deerenberg *et al.* 1997; Råberg *et al.* 1998). We point out here that, whereas studies showing a reduction in weight gain during production of antibodies (e.g. Deerenberg *et al.* 1997; Klasing *et al.* 1987) are suggestive, measures of the impact of antibody on basal metabolic rate reveal negligible costs (Svensson *et al.* 1998). Indeed, some internal processes, such as destruction of leukocytes that are designed to suppress the immune system, do not appear to be as efficient in conserving energy as one would expect if the energetic cost of immunity were important (Sapolsky 1992). A few studies have experimentally manipulated condition and measured general components of the immune system. They have shown that an improvement or reduction in condition can enhance or reduce immune function, respectively (e.g. Cooke *et al.* 1993; Lochmiller *et al.* 1993; Praharaj *et al.* 1995). Few studies have focused on the resource-based costs of mounting an immune response, especially in free-living populations. Although it seems likely that maintenance of cell-mediated responses as well as production and maintenance of humoral responses will all entail some cost, the evidence that this is an important cost for models of sexual selection is equivocal.

An important prediction of route 2 is that manipulations of condition (via diet or workload) should result in parallel effects on the advertisement and the immune system response. In this case, using novel (to the host) but non-replicating antigens (e.g. sheep red blood cells or SRBCs) is a conceptually relevant way to measure immune system response. However, interpretations of novel antigen studies should be made cautiously. Responses to just one type of antigen (e.g. SRBCs) may provide misleading information. For example, in lines of mice selected for their antibody production in response to immunization with SRBCs (Weiner & Bandieri 1974), the line selected for low antibody production significantly deviated from controls. However, this line turned out to have a very strong cell-mediated immune response (via phagocytosis), which possibly reduced the invasion of SRBCs so quickly that antibody production became less important. In studies of chicken lines selected for high antibody titres to SRBCs, responses to other antigens were sometimes high (e.g. Parmentier *et al.* 1994; Scott *et al.* 1994), suggesting a common component to antibody production, whereas in others it was low (e.g. Kreukniet *et al.* 1992), indicating unique components. Some studies also found selected lines were more resistant to some pathogens but not others (e.g. Gross *et al.* 1980; Gross 1986). Hence, a low (or high) response to one antigen presentation (e.g. SRBCs) alone does not mean that the individual has poor (or good) general resistance. One method of confirming that a general component of the immune system is being measured might be to use multiple measures of immune system response (e.g. multiple antigens). Covariance between the responses obtained from separate novel antigens would suggest a common component to them all; the results of the few such studies to date are mixed (e.g. Cheng *et al.* 1991; Bacon 1992; Luster *et al.* 1992; Kean *et al.* 1994; Dietert *et al.* 1996). These studies have not used free-living individuals with natural variation in condition or genetics, and so much could still be gained from attempting to measure general resistance in such situations by employing an array of assays.

We also note that, although the use of novel antigens removes the potential cost of the disease (path 6) from the experiment (an important benefit), it may be potentially less demanding on the immune system than a real pathogen, which replicates inside the organism. Thus, only the initial components of host defences might be necessary to combat this experimental 'infection'. We would predict that a higher cost might be evident if novel antigens were used in such a way (e.g. rapidly repeated immunizations) that might more closely approximate the load on the immune system from an actively replicating organism.

Separating the three genetic pathways (paths 1A, 1B and 1C) has not been attempted, and seems likely to be difficult. Measures of heritability of resistance to experimental inoculations of parasites (e.g. Hillgarth 1990; Møller 1990*b*; Zuk *et al.* 1990) do not necessarily test paths 1A–C in this route because they do not distinguish

between general (route 2) and specific resistance (route 1). Divergence of lines selected on immune responses (e.g. Weiner & Bandieri 1974; Kreukniet *et al.* 1992; Kean *et al.* 1994) strongly suggests a genetic basis for such responses, although which paths (1A–C) are involved is not clear. From the point of view of whether or not females receive indirect genetic benefits by choosing upon advertisements, separating the three paths does not matter. Nevertheless, a complete understanding of this route would need to examine each path. A possible, but difficult, experiment that could measure all three separately would be to cross-foster individuals and have independent measures of the effect of genotype on condition, immune system response and advertisement.

(c) *Route 3: resistance and immunopathology*

The immune system could be viewed as a biochemical signal detector, because it must distinguish between self and non-self, and between benign and harmful invaders. Thus, the immune system faces the same problem any signal detector does: errors of rejecting or accepting an object when it should not are inevitable, and the system cannot simultaneously reduce both (Wiley 1983). A possible trade-off faced by the immune system, therefore, might be between the risk of a pathogen passing through undetected (acceptance error) versus the risk of immunopathology (i.e. rejecting parts of the host or completely benign invaders (rejection errors) and attacking them (e.g. de Boer & Perelson 1994; Sherman *et al.* 1997)). In this route (figure 2*c*), a change in the immune system would have simultaneous and opposite effects on paths 5 and 7. Acceptance and rejection errors both have negative effects on condition (paths 6 and 8), thereby affecting the advertisement. The genetic effect (path 1C) might be thought of as influencing immune system 'aggressiveness', and the genes most beneficial to females would be those that optimally balance the benefits of aggressively attacking real pathogens with the risks of immunopathology. Changes in the biotic environment could lead to shifts in the optimal balance point, thereby maintaining the genetic variation being signalled by the advertisement (figure 3). Route 3 is distinctly different from route 1; the cost here is the effect of the immune system on the host, whereas in route 1 the cost was in terms of limits to immunity to one pathogen versus another.

Immunopathology is common and is clearly associated with mistakes of recognition or an overzealous immune response (or both). Autoimmune disease, in which the immune system attacks host tissues (e.g. rheumatoid arthritis or haemolytic anaemia (Golub & Green 1991)), is clearly a mistake in recognition. A number of other debilitating consequences of immune function appear to be mistakes in level of response. Allergies, for example, are the result of a strong response to foreign substances that are not harmful on their own. Similarly, dangerously high fever, excess granuloma formation or excess deposits of antibody–antigen complexes (which can lead to problems such as renal failure (Playfair 1995)) all have negative effects on the host.

Some evidence exists for the idea of a trade-off between fighting disease and damaging self. Some studies indicate that the prevalence of immunopathology and the strength of immune responses are negatively correlated (e.g. Kinlen

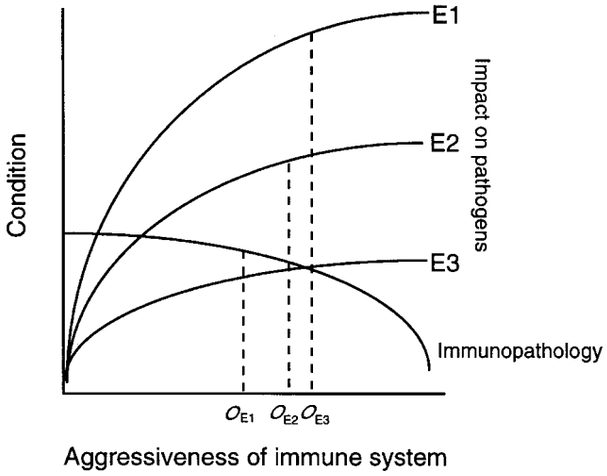

Figure 3. Optimal immune system responses (O_{E1}, O_{E2} and O_{E3}) as a function of trade-off between the impact of immunity on pathogens and the effect of immune system function on immunopathologies. The optimal points occur when the slopes of the curves relating the effect on condition of the immune system fighting off the pathogen and the effect on condition due to immunopathology sum to zero (indicated by vertical dotted lines). Differences in prevalence or type of pathogen among environments (either in space or time) result in shifts in the benefit curve (E1, E2 or E3) and hence produce different optimal levels of aggressiveness that might be indicated by a condition-dependent male advertisement.

1992; Gridley *et al.* 1993). Females generally have higher immune responses than males (Grossman 1985) and, in humans, also have higher rates of autoimmune disorders (Nelson & Steinberg 1987). Hormone studies have shown that progesterone inhibits the immune system and when women are either pregnant (when progesterone levels are high) or using oral contraceptives that contain progesterone, some autoimmune conditions are reduced. Similarly, disruption of the normal feedback regulation of the immune system via the secretion of glucocorticoids (Besedovsky & del Rey 1996) results in a prolonged immune response and increased autoimmune disease (e.g. Wilder 1995). Indeed, immunosuppression during stress has recently been suggested to be an adaptive response to reduce the impact of immunopathology at sensitive times (Råberg *et al.* 1998).

An important issue is whether or not both disease and immunopathologies affect condition in ways that would influence the development of sexual advertisements. Some immunopathologies are clearly very costly (e.g. death due to anaphylaxis), and other consequences of immune responses (e.g. cell lysis) could affect resources available to other parts of the body (e.g. haemolytic anaemia) (Playfair 1995). Nevertheless, little attention has been given to how immunopathologies might affect sexual advertisements.

A prediction of the resistance versus immunopathology hypothesis is that the optimal immune response will be intermediate: that is, it will be a balance between the ability to resist pathogens versus the dangerous effects on self. Furthermore, immunopathology could result from either specific or general resistance. Improved immune

effectiveness against particular pathogens could result in more immunopathology if the pathogen is evolving to escape immune defences by mimicking the host (e.g. group A streptococcus, which mimics mammalian heart tissue (Playfair 1995)). A larger or longer response (general resistance) to a pathogen can lead to more cell lysis, more antibody–antigen deposits, more granulomas, or an increased risk of autoimmunity (as happens with injections of the bacterial protein lipopolysaccharide, which can stimulate self-reactive B cells directly (Playfair 1995)). It is therefore not clear whether use of novel antigens as a measure of the immune response is appropriate for testing route 3. If the focus is to look at the effects of general resistance on immunopathologies, then novel antigens would be a useful way of measuring such responses, although because the optimal response is intermediate, we would expect a nonlinear relationship with condition (individuals with both high and low responses should have low condition, although for different reasons). However, if specific resistance is thought to be important, then use of novel antigens would be inappropriate, and it seems likely that investigators would have to uncover the specific host–pathogen relationship to test route 3 adequately.

As with route 1, a complete test of this route will need to document shifts in biotic environments that cause shifts in the optimal balance between aggressive immunity from pathogens and the risk of pathology (figure 3). It seems to us that we are a long way from testing this idea, as we will need to know a great deal more about the pathogens involved, the portions of the immune system of relevance and the costs associated with increased immune aggressiveness.

5. THE REAL WORLD

Our discussion of the three alternative trade-offs and the predictions of each assumed that each was the only route of importance. It is obvious that in the real world, all three could be involved simultaneously. For example, fever is a potentially effective weapon against some diseases (e.g. Playfair 1995; route 1), involves metabolic costs (e.g. Baracos *et al.* 1987; route 2), and can have debilitating effects on the functions of host tissues (e.g. neural damage) if it is too extreme (e.g. Playfair 1995; route 3). Furthermore, there are possible interactions between the routes. For example, better recognition of antigen by antibody, or more effective presentation of antigen by MHC (specific resistance), can produce a stronger production of antibody (general resistance) (Golub & Green 1991). In some cases, improved recognition of pathogen (specific resistance) might make the risks of cross-reactivity with self go down (e.g. Steward 1981). Changes in one route could also affect selection on the others. For example, evolution of more effective destruction of a particular pathogen (e.g. better lytic enzymes; route 1) might cause evolution of a reduced numerical response (lowered antibody production) and a reduced energetic (route 2) or immunopathogenic cost (route 3).

Environmental influences (paths 2A and 2B), such as fluctuations in food availability, seem likely to have large effects in all these models, and will reduce the reliability of advertisement as a signal of genetic benefits. Relatively little study of environmental influences on sexually selected traits has been attempted, yet besides the potential dampening effect such variation will have on the reliability of advertisements, environmental variation could be the key driving factor maintaining genetic variation in all three routes discussed above. Changing environments could change the array of pathogens of most danger to hosts, thereby shifting the value of specific resistance in route 1. Similarly, changes in environment could alter which path and which genes within that path have the most influence on condition in route 2. Finally, fluctuating pathogens alter the optimal balance between disease resistance and risk of immunopathology in route 3 (figure 3). Thus, one exciting area of study might be the ways in which environmental variation influences sexually selected advertisements and the underlying benefits females acquire by choosing adorned males.

Given these complications, how should one proceed in testing these ideas? The real world also undermines glib assertions of what constitutes a rigorous test of particular hypotheses, as many of the issues we have discussed (e.g. measures of paths in figure 2*a–c*) are likely to be very difficult to accomplish. Consideration of the key differences between the three routes we have described suggests some general approaches. These include searching for measures of specific versus general resistance (which are likely to emerge from combining study of the immune system and the key pathogens affecting the host), the energetic cost of the immune system, and the consequences of immunopathologies on general condition. Although behavioural ecologists and immunologists are still a long way from fully understanding each other (e.g. Svensson & Skarstein 1997), some of the tools to achieve these measures may be already available. We hope that the framework we have presented will allow integration of immunology and behavioural ecology such that specific measures of the key variables can be found, and the ways in which mate choice is influenced by the immune system can be tested.

Ideas come from many sources, and those in this paper were greatly influenced by conversations with many people, especially D. Hasselquist, B. J. Hatchwell and M. Siva-Jothy. We also thank D. Hasselquist, L. Råberg and Eric Svensson for providing us with unpublished data; they independently developed a graphical model of optimal immune response similar to our figure 3. D.F.W. was supported by the National Science Foundation and the University of Kentucky, and T.R.B. by NERC and the University of Sheffield during the writing of this paper. Fred Davison, Dennis Hasselquist, Joe Poston, Lars Råberg, Andrew Read, Paul Sherman, Marlene Zuk and three anonymous reviewers kindly provided many useful comments on the manuscript.

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