He's healthy, but will he survive the plague? Possible constraints on mate choice for disease resistance

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Can females enhance their fitness by choosing a mate based on his disease resistance in addition to his current health and robustness (i.e. male condition)? The complex nature of disease resistance may constrain the evolution of female choice for this trait. Using a mathematical model, we showed that choice for immune function (an element of disease resistance) provided females with a fitness advantage. However, the fitness advantage was often small, much smaller than the fitness advantage females obtained from mating with males in good condition. Females choosing for a combination of male condition and male immune function sometimes showed no fitness advantage compared with females choosing for condition alone, even when condition and immune function were positively correlated. Our results suggest that when condition and immune function are correlated, selection for choice for male immune function may be driven by the fitness advantage that comes from mating with males in the best condition, even if a sexually selected trait correlates with male immune function. Moreover, females choosing for males with maximal immune function produced offspring with immune functions above the level needed for maximal fitness. In some species, females may gain little or no fitness advantage by choosing for male immune function *per se* in addition to male condition. This may explain why not all studies find evidence for female choice for male immune function.

Key words: ecological immunology, female choice, immunocompetence, mate choice, parasite, pathogen, sexual selection, immune

Most females mate selectively (Andersson 1994). Mate choice can be costly; therefore, it should provide a fitness advantage to choosy females, either directly (e.g. by improving female fecundity) or indirectly (e.g. by enhancing offspring fitness) to become established in a population (Andersson 1994). For example, by choosing healthy mates, females gain direct benefits by decreasing the risk of acquiring an infection during mating (Borgia & Collins 1989; Able, 1996). Choosing healthy mates could also provide females with a fitness advantage because healthy males are more likely to supply 'good genes' to their offspring (Hamilton & Zuk 1982). Some of these 'good genes' may enhance offspring disease resistance, thereby increasing female fitness (Hamilton & Zuk 1982).

An extension of this hypothesis proposes that females choose a mate based on his ability to resist future infections (i.e. disease resistance) in addition to his current health (see Wedekind 1994; Howard & Lively 2004; Piertney & Oliver 2006). In other words, females choose disease-resistant males not only by favoring the healthiest and most robust males, but, in addition, by assessing signals from males that advertise the quality of particular immune functions and then by mating with males with the best immune system (Møller & Petrie 2002). Males could signal their immune robustness (i.e. their ability to resist disease) if there is a genetic correlation between immune function and sexually selected traits (Lawniczak et al. 2007). Numerous studies have measured the phenotypic and/or genetic correlations between some aspects of male immune function and sexually selected traits (e.g. see Møller et al. 1999; Lawniczak et al. 2007). Not all studies find positive correlations (Møller et al. 1999; Lawniczak et al. 2007), and the data are mixed as to whether females choose males on the basis of particular immune

functions e.g. immune responsiveness (Lawniczak et al. 2007) and major histocompatibility complex (MHC) diversity (Piertney & Oliver 2006). In part this may be due to issues surrounding the assessment of disease resistance (Adamo 2004a; Corby-Harris et al. 2007). Disease resistance is not a monolithic entity that can be measured like length or weight. An animal's disease resistance is based on a large number of factors including the ability of its immune system to recognize and respond to invaders (Roitt et al. 2001; Gillespie et al. 1997). The complex nature of this trait may constrain the evolution of female choice for it. In this paper, we use a mathematical model to explore how the nature of disease resistance may affect whether females receive a fitness benefit by choosing for it. To increase the relevance of this model for animal behaviourists, the representation of disease resistance reflects the current empirical methods used to estimate it.

Using an earlier model, we showed that selection pressure for female choice for male immune function was weak when the number and type of pathogens varied across the generations (Adamo and Spiteri 2005). In this model we examine 3 further issues regarding selection for disease resistance.

First, it is unclear how much additional fitness advantage females accrue by assessing disease resistance directly (e.g. by assessing male immune function) as opposed to simply assessing a male for health and robustness (i.e. condition) (Milinski 2006). In other words, if there is an indicator trait that correlates with condition (e.g. train length in peacocks, Møller & Petrie 2002) and another that correlates with an immune response (e.g. the size of the ocelli in peacocks, Møller & Petrie 2002), does the female benefit by using information about an immune response? Females will not be selected to pay

attention to signals unless that information results in enhanced fitness for females. Kokko et al. (2003) argue against the view that males advertise specific components of viability such as immune function. By mating with males in the best condition (as reflected by condition-dependent sexually selected traits), females already choose males that are more disease resistant than average because of the decrease in condition caused by disease (Westneat & Birkhead 1998). Some sexually selected traits are very sensitive to immune activation, accurately reflecting the male's current health status (Faivre et al. 2003). Such studies demonstrate that male condition has a large impact on attractiveness to females. We test whether females gain any additional fitness benefits if they assess both male condition and some aspect of disease resistance (i.e. immune function) as opposed to choosing males based solely on their present condition.

Second, the relative disease resistance of different males is pathogen dependent (e.g. Gross 1980). Males that are resistant to one pathogen can be susceptible to others (Adamo 2004a). For example, among genetically distinct *Drosophila melanogaster* populations, the correlations between resistances to different bacteria are low (Lazzaro et al. 2006). Lazzaro et al. (2006) suggest that this lack of correlation reflects the complex and heterogeneous mechanisms underlying host-pathogen interactions. Therefore, it may be impossible to rank individuals in terms of disease resistance without knowing the identity of the pathogen (Milinski 2006). Females may be unable to find the most resistant male without knowing the identity of the pathogens that will be attacking their offspring. We explore how this pathogen-dependent nature of disease resistance may constrain the circumstances under which choice for resistance to specific pathogens will provide the female with a fitness advantage.

Finally, a third complication for mate choice for disease resistance is the growing appreciation that immunity carries substantial costs (e.g. Zuk & Stoehr 2002; Siva-Jothy et al. 2005). These costs may result in individuals with less disease resistance having the highest fitness (Viney et al. 2005). We examine how these costs may influence selection pressure for mate choice for maximal immune function in mates.

Methods

To examine female choice for male immune function, we developed a mathematical model similar to that of Kokko & Lindström (1996). The model is an extension of the one described in Adamo & Spiteri (2005). The model simulates real world conditions by exposing individuals to multiple pathogens. Individuals were exposed to a maximum of 7 pathogens (Table 1) that undergo independent cycles of increase and decrease. Therefore, in every generation, some pathogens were common whereas others were rare, and the identity of the common vs. rare pathogens varied over time. We used 7 pathogens in order to include all the common pathogen types attacking most animals (e.g. see Fuxa & Tanada, 1987). However, the model gives the same qualitative results with fewer pathogens (see Appendix B). We present the data for 7 pathogens to allow comparisons with our earlier paper (Adamo & Spiteri 2005).

Below is a general description of the model. Mathematical details are given in Appendices A and B.

In the model, infection reduced lifespan, leading to reduced fecundity and lowered fitness. Whether an individual survived an infection depended on the strength of

the individual's immune system and the individual's condition. However, a stronger immune system was more costly in terms of reduced fecundity. We created simulated populations, exposed them to pathogens, and examined the relative fitness advantage of females who chose males on the basis of immune function compared with non-choosers.

To ensure that we used biologically meaningful parameter estimates in our model, we used literature values for Orthopteran (e.g. grasshopper, cricket) species whenever possible (Table 1). We assumed our Orthopteran-based model had one generation per year, no parental care, and no overlap in generations. We modeled the immune system as having two types of immune responsiveness (constitutive immunity and inducible immunity; see Schmid-Hempel & Ebert 2003) and the ability to recognize pathogens. All three of these components of immune function are used to assess male immune ability by ecological immunologists (e.g. Milinski 2006; Lawniczak et al. 2007). Constitutive immunity (CI) is composed of the immune factors that an animal produces continuously, even without an immune challenge. Inducible immunity (IN) is composed of factors produced only during an immune challenge. Vertebrates and invertebrates have both constitutive and inducible immunity (Roitt et al. 2001; Gillespie et al. 1997). Our model reflects reality in that the two types of immune responsiveness differed in their impact on the organism's ability to survive attacks by different classes of pathogens (Table 1). We also ascribed different costs to each (see below), as suggested by the literature (see Adamo & Spiteri 2005). For both CI and IN, the stronger the immune response, the greater was the cost.

The ability to recognize pathogens was modeled either as one or two traits. When it was modeled as two traits, it was divided into non-specific recognition and specific

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recognition. Non-specific recognition (NSP) simulated the ability of immune systems to recognize broad classes of pathogens by their molecular signatures (e.g. lipopolysaccharide or peptidoglycan). We assumed that disease resistance increases as the number of non-specific recognition factors increases. Therefore, the cost of non-specific recognition was modeled as being proportional to its effectiveness. In other words, the cost of NSP, like the costs for CI and IN, was higher the greater the assigned value of NSP in the model.

Invertebrate immune systems are also capable of specific recognition and resistance (Little et al. 2005), although the mechanisms responsible for this ability are unknown. We hypothesize that individuals differ in their ability to recognize and/or resist specific pathogens because of the shape of particular recognition molecules or by the presence of fortuitous mutations (e.g. the lack of a docking protein for a virus). Therefore, increasing the effectiveness of specific recognition (SP) does not necessarily increase its cost. For this reason the cost of SP in our model did not vary depending on its effectiveness. The small assigned fixed cost for SP reflects the fact that recognition proteins may need to be synthesized. Unlike NSP, which we modeled as playing a role in recognizing all pathogens, each SP factor was modeled as increasing resistance to only 1 specific pathogen. Therefore, each individual had an SP score for each pathogen in the simulation. These SP values were chosen independently of each other. Individuals with high SP scores may be resistant to some pathogens, but susceptible to others, mimicking the natural situation (e.g. Gross 1980).

Resistance to disease in our model was also determined by an animal's condition, as it is in real animals (Westneat & Birkhead, 1998). Condition has a number of

definitions in the literature, e.g. phenotypic quality (see Birkhead et al. 2006). In empirical studies, condition is often estimated by assessing the animal's ability to assimilate resources (e.g. rate of growth or amount of energy stores). Such measures reflect the animal's ability to perform a number of physiological processes, such as digestion (Birkhead et al. 2006). In our model, condition was a composite score reflecting the animal's current health and its relative ability to perform all non-immunological physiological processes important for an animal's health and robustness. In essence, questions about mate choice for immune ability isolate immune function from the other physiological processes important for determining survival and reproduction and ask whether there is substantial selection pressure for females to choose males for this particular physiological function as opposed to, or in addition to, all other physiological functions. Condition influences female fecundity in our model (Equation 3) because traits such as enhanced digestive efficiency lead to increased energy available for reproduction. In the same way, condition also influences the ability to survive an infection. Animals in poor condition have reduced function in many organs systems (e.g. the liver). Liver function is critical for disease resistance, as are other physiological processes (Munford 2005). Such ancillary 'immune' systems are not assessed by the standard immune assays used by ecological immunologists when studying female choice for male immune function. Therefore, we model condition, a trait that summarizes the relative robustness of these physiological systems, as playing a role in whether animals survive an infection (see Appendix A, Equation 7).

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We assumed that females were able to perfectly assess condition, CI, IN, NSP, and all 7 SP values. The scores for condition, CI, IN, NSP, and the 7 SP scores were

chosen independently (i.e. scores were not required to be either positively or negatively correlated), although condition could be correlated with CI and IN. We allowed CI and IN to be correlated with condition in some simulations because they may be linked by similar physiological mechanisms (e.g. Smith et al. 2007). We examined the effect of the strength and sign of the correlation (i.e. negative or positive) between condition and CI and IN on the fitness advantage for choosy females. Immune recognition was not correlated with condition because immune recognition is not necessarily related to present condition (Dybdahl & Krist 2004).

Therefore, in our model, it was possible for an animal to have robust immune responses (CI and IN) and be in good condition, but still die of an infection, if the animal lacked the ability to recognize a particular pathogen.

The strength of the immune system was calculated as the immune function score (I). $I(i,j)$ of insect i with respect to pathogen j was determined according to the formula:

Equation 1. $$I(i,j)=recog(i,j)*(w1(j)*CI(i)+w2(j)*IN(i)),$$

where $recog(i,j)$, $CI(i)$, and $IN(i)$ were the recognition values, CI, and IN of insect i with respect to pathogen j and $w1(j)$, $w2(j)$ were the weights for pathogen j (see Table 1), where $w1(j)$ represented the importance of CI for resistance to pathogen j and $w2(j)$ represented the importance of IN for resistance to pathogen j. When decomposed into two traits, the recognition score of insect i for pathogen j was calculated as

Equation 2. $$recognition(i,j) = (SP(i,j)+NSP(i))/2,$$

where $SP(i,j)$ was the specific recognition that insect i had for pathogen j and $NSP(i)$ was the non-specific recognition of insect i.

The decrease in lifespan due to disease was calculated by estimating the individual's risk of death from each of the pathogens in a given year. Whether an individual survived an infection depended on the individual's immune function score, the animal's condition, pathogen prevalence, and pathogen virulence (Table 1) for each of the pathogens (see Appendix A, Equation 7). In our simulations pathogen prevalence can be set to a constant value for all generations or can fluctuate from generation to generation (see Adamo & Spiteri 2005). Each fluctuating pathogen population was assumed to have a cycle of 18 years. Pathogen cycle length was based on the cycle length of grasshopper pathogens studied by Smith (1965).

Fitness w(i) (see Equation 3) for insect i was modeled as being a product of fecundity and lifespan. Fecundity and lifespan were assigned ideal values of 1, which were then modified by condition (i.e. an individual's lifespan and fecundity were determined by their condition) and survivorship (the likelihood of surviving the pathogens prevalent during that generation). Low immune function reduced fitness by decreasing lifespan and hence fecundity. Furthermore, the cost of immunity also decreased fitness.

Equation 3.

w(i)= condition(i)*ideal fecundity* (1-cost of immunity(i))*ideal lifespan*survival (i)

where ideal fecundity = ideal lifespan = 1.

In each generation there were 500 females and 500 males. Each female was ranked by her fitness score to determine her mating precedence. "Dead" animals (i.e. those whose fitness score was 0) were excluded from mating. Starting with the top-ranking females, each female produced two male and two female offspring until the

original population was replaced. If there were insufficient numbers of surviving females to replace the original population within one mating cycle, the mating cycle was repeated (starting with the top-ranking females) until the population size was sufficient for the next generation. The values for CI, IN, recognition (SP and NSP), and condition were inherited from the father (for both male and female offspring). Therefore, this is a haploid model of inheritance (from the male), and female choice had an immediate effect on the fitness of the female's offspring. Thus, the fitness of the female's offspring was determined by her choice of mate. Female choosiness was inherited from the mother. Before inheriting values from the father, the values were mutated. Mutation maintained variability in immune parameters in the face of natural selection due to disease.

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Each population began with an equal number of choosy and non-choosy females. Choosy females mated only with males who were above average for the criterion of choice (e.g. fitness). In reality females are unable to assess male fitness directly, but we used choice for this trait both as a test of the model and as an example of the strongest fitness advantage we could expect from female choice in this system. Non-choosy females mated randomly.

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For choosy females, there was an additional cost of choice. The cost of choosing varies greatly among species, and in some animals it appears to be close to 0 (Gibson & Bachman 1992). However, there is evidence for a cost to female choice in Orthopterans (Gray 1999). We set the cost of female choice in our model at 1% of the fitness score. This value is used by other modelers (e.g. Kokko & Lindström 1996; Beck & Powell 2000).

We calculated the fitness advantage of choosers by subtracting their fitness scores from those of non-choosers for each generation of the simulation and taking the median. This method allowed us to assess the relative fitness advantage of choice for each immune attribute.

Simulations were run using Matlab version 2007a. Simulations were typically run for 100 different populations of 1,000 individuals each until the each population fixed at 0% or 100% choosers or to a maximum of 1800 generations.

Statistical analysis

Most of the data generated by the model were not normally distributed. Therefore non-parametric statistics were used throughout, following the procedures of Meddis (1984) and Sokal & Rohlf (1981). Ranking of data and most statistical analyses were done using Prizm (version 4) software. All statistical tests were two-tailed unless otherwise specified. When more than one statistical test was performed on the same data set, the alpha criterion was adjusted accordingly.

Results

The simulations were able to address all three issues raised in the introduction. First, choosing for a combination of male immune responsiveness (CI and/or IN) and male condition provided no significant fitness advantages to females compared with choosing for male condition alone under most circumstances (Fig. 1), unless CI and/or IN were positively correlated with condition (Fig. 2). Second, choice for a combination of male immune recognition ability and condition gave females no fitness advantage over females choosing for condition alone when pathogen prevalence changed every

generation (Fig. 4). Third, choice for immune responsiveness led to higher immune function values than those that gave females maximal fitness (Fig. 5). The details of these results are given below.

Female choice for male immune responsiveness (CI and IN) and male condition gave females little, if any, additional fitness advantage over females choosing for male condition alone.

Females that chose for fitness gained a fitness advantage over females that mated randomly (Fig. 1). Choice for fitness quickly fixed at 100% in all populations. Females who chose males in good condition also received a substantial fitness benefit over non-choosers (Fig. 1), and 100% of simulated populations (N=100) fixed at 100% choosers. The relative fitness advantage for choice for immune responsiveness was much lower than that for condition (Fig. 1; Kruskal-Wallis test: H=643, P<0.0001, Dunn's multiple comparisons, condition > CI, P<0.001, condition>IN, P<0.001, condition>CI+IN, P<0.001), with some populations having a negative fitness advantage score (i.e. females mating randomly in these populations had higher fitness). In these populations, choice for immune responsiveness was lost (13/100 for populations with females choosing for CI, 12/100 for females choosing for IN, and 1/100 for females choosing for CI+IN). Choice for condition + immune responsiveness gave an enhanced fitness advantage over choice for condition alone (Fig. 1), although the differences were not statistically significant at this sample size (Kruskal-Wallis test: H=643, P<0.0001, post hoc Dunn's multiple comparison, P=0.20). Rerunning the simulation with 1,000 populations, we found that

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choice for condition+CI+IN resulted in a significantly higher fitness score relative to non-choosers than when choosing for condition alone (Mann-Whitney *U* test: U=373,100, $N1$=$N2$=1,000, P<0.0001). Choice for condition, condition+CI, condition+IN, condition+CI+IN fixed at 100% in all populations.

Whether choice for both condition and immune responsiveness led to choosers having greater fitness than non-choosers compared to choice for condition alone depended on model parameters such as the value of condition. When the value of condition was reduced by 1/2 (i.e. when immune function was as important in determining fitness as condition), female choice for condition alone gave a greater fitness advantage relative to non-choosers than did female choice for condition and immune responsiveness (condition+CI, condition+IN, condition+CI+IN; Kruskal-Wallis: H=649, P<0.0001, Dunn's multiple comparison, P<0.01). Also, if females weighted condition at 80% in determining their choice, with immune responsiveness (CI) weighted at 20%, choosing condition and immune responsiveness resulted in enhanced fitness relative to non-choosers Kruskal-Wallis: H=75.5, P<0.0001, Dunn's multiple comparison, P<0.01). Conversely, if females weighted condition at 20% and CI at 80%, then choosing for condition alone resulted in a higher fitness score than choosing a combination of condition and CI (P<0.001, Dunn's multiple comparison).

As expected, the stronger the correlation between immune function and condition, the greater the fitness advantage for females choosing the correlated immune function relative to non-choosers (for CI correlated with condition, non-parametric test for a specific trend, Meddis, 1984, Z=8.42, P<0.0001, for IN, Z=7.8, P<0.0001; for CI+IN, Z=9.2, P<0.0001). Even a relatively weak correlation (e.g. r=0.2) could increase the

fitness advantage for choice compared to trials in which immune responsiveness and condition were not correlated. For example, choice for CI fixed to 100% in more populations (100/100) under these conditions (Fisher's exact test, P=0.0002).

For some correlation values between condition and immune responsiveness, choice for condition alone resulted in a higher fitness score for females relative to non-choosers than choice for a combination of condition and immune responsiveness (Fig. 2a), e.g. r=-1, and 0.8 (Kruskal-Wallis: H=283, P<0.0001, Dunn's multiple comparisons) and no difference for others (r=1, P=0.99; r=0.5, P=0.32; r=-0.2, P=0.44; Dunn's multiple comparisons). When r= 0.2, females choosing immune responsiveness and condition had a higher fitness score relative to non-choosers than choosing for condition alone (Fig. 2b, Kruskal-Wallis: H=62.7, P<0.0001, Dunn's multiple comparison, condition less than condition+CI P<0.05, condition+IN, P<0.001, condition+CI+IN, P<0.001).

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We found that a negative correlation between condition and immune responsiveness resulted in a decrease in the fitness advantage of females that chose males based on their immune responsiveness compared to those that mated randomly. Not surprisingly, in these cases choosers were lost from the population. Choice for CI, condition + CI, condition+IN, or condition+CI+IN fixed to 0% when condition was negatively correlated with immune responsiveness.

Female choice for male immune recognition/resistance (NSP and SP) and male condition gave females no additional fitness advantage over females choosing for male condition alone

Females gained a fitness benefit from choosing males based on NSP. Choice for SP resulted in little fitness advantage for choosers under fluctuating pathogen conditions (Fig. 3). Choice for any SP was lost in at least 80% of all populations, although choice for the average recognition of all pathogens (i.e. choosing the male with the highest average SP) fixed to 100% in almost all populations (Table 2). The fitness advantage of choosers for SP was greatest for choice for an SP against the deadliest and most prevalent pathogens (Fitness advantage of choosers: Z=1.98, P<0.03; % Choosers: Z=1.93, P<0.05). Choosing the average resistance across many pathogens resulted in a higher fitness advantage to choosers over non-choosers than choice for resistance for any single pathogen (Kruskal-Wallis: H=1016, P<0.0001, Dunn's multiple comparisons, all P<0.001).

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Choice for a combination of condition and NSP (P=0.88) or a combination of condition and the average value of all 7 SP values (AgSp, P=0.65) did not increase the fitness advantage of choosers over non-choosers compared with choice for condition alone (Fig. 4; Kruskal-Wallis: H=456, P<0.005, Dunn's multiple comparison test). Choice for condition and specific resistance to the viral pathogen (SP #1) led to a significantly smaller fitness advantage over non-choosers than choice for condition alone (Dunn's multiple comparison test, P<0.05).

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Pathogen dynamics affected whether females choosing males on the basis of resistance to specific pathogens had a fitness advantage over non-choosers. The fitness advantage of choosy females increased when pathogen prevalence was constant (Kruskal-Wallis: H=47.6, P<0.0001; Dunn's multiple comparison, P<0.001). The percentage of

populations that fixed at 100% choosers for individual pathogen recognition increased as well (Table 2; Test for trends, Z=3.4, P<0.001).

Large increases in the virulence or prevalence of a pathogen increased the fitness advantage to females choosing resistance to that pathogen over non-choosers. For example, if the prevalence of parasitoids (pathogen #7, Table 1) increased from 0.17 to 0.7, the fitness advantage of females choosing males with higher SP #7 scores relative to non-choosers increased significantly (Mann-Whitney U=4055, P=0.02). The increased disease pressure also enhanced the fitness advantage of choosers for condition (Kruskal-Wallis: H=11.5, P=0.0003, Dunn's multiple comparison, P<0.05), and choice for immune responsiveness (CI+IN, Kruskal-Wallis: H=12.3, P=0.0003, Dunn's multiple comparisons, P<0.05). Choosers for NSP (Kruskal-Wallis: H=0.23, P=0.89) or for specific recognition for other pathogens (Kruskal-Wallis: H<0.013, P=0.99) had no significant increase in their fitness advantage over non-choosers. Choice for fitness led to higher values for specific resistance to parasitoids (SP #7) when prevalence increased (Mann-Whitney: U=1747, P<0.0001).

Female choice for immune responsiveness led to immune function values higher than those that produced maximal fitness

The median values of CI and IN were lower when females chose for fitness than when they chose for CI, IN, or CI+IN (Fig. 5; Kruskal-Wallis: H=542, P<0.0001, Dunn's multiple comparisons, all comparisons P<0.001). When the costs of CI and IN were reduced to 0, the median values for CI and IN increased significantly when females were

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choosing for fitness (CI, Mann-Whitney: U=760, P<0.001; IN, Mann-Whitney: U=472, P<0.001).

Discussion.

Our central finding is that female choice for a combination of immune function and condition does not necessarily add fitness benefits compared to female choice for condition alone. For example, choice for both condition and immune recognition (NSP and SP) did NOT give choosers a greater fitness advantage than females choosing for condition alone for most model parameters (Fig. 4). Choosing for immune recognition provided a fitness benefit only when a pathogen produced severe and sustained mortality. Choosing for immune responsiveness, on the other hand, did give females a small fitness advantage over randomly mating females (Adamo & Spiteri 2005, Fig. 1). However, when females could choose for condition, choosing for condition produced a larger fitness advantage relative to non-choosers than choice for immune responsiveness (Fig. 1). Whether choosing males based on both condition and immune responsiveness (CI and IN) gave females a greater fitness advantage than choosing for condition alone depended on the model parameters. These results have implications for studies on mate choice for immunocompetence. Studies on mate choice for immunocompetence typically assume that a correlation between an individual's immune ability and its sexually selected traits is evidence for female choice for male immune function (e.g. Møller et al. 1999). Our study cautions that a correlation between an immune function and a sexually selected trait

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may exist solely because of an underlying correlation between condition and immune function. Condition, not immune function *per se*, may be the trait that females are seeking because specific immune functions can be poor predictors of fitness.

When immune responsiveness was positively correlated with condition, the fitness advantage of choosing for immune responsiveness increased dramatically. Superficially this appears to suggest that when immune responsiveness is positively correlated with condition, selection pressure for female choice for male immune responsiveness could be considerable. However, under the same circumstances the fitness benefit for choice for condition was larger. More critically, when condition was correlated with immune responsiveness, choosing for both condition and immune responsiveness did not significantly increase female fitness more than choosing for condition alone for most values of correlation. Therefore, when condition and immune responsiveness are correlated, choice may be driven by the fitness advantage that comes from mating with males in the best condition, even if the sexually selected trait correlates with male immune function (also see Kokko et al. 2003). In other words, a correlation between sexually selected traits and immune function does not necessarily imply that there is significant selection pressure driving female choice for male immune function. Females that are already choosing males on the basis of male condition may be under little selection pressure to use additional information about male immune function to find the fittest mate.

Studies typically find a complex relationship between immune function and sexually selected traits (Lawniczak et al. 2007). The lack of a consistent positive correlation between immune function and sexually selected traits in many species could

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exist for several reasons (see Møller & Petrie 2002; Viney et al. 2005; Lawniczak et al. 2007). One reason not usually considered is that there may be little selective pressure on females in most species to make mate choices based on immune function *per se*. The result of our model suggest that this possibility should be considered more seriously in future studies.

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Female choice for immune function could be substantial if females are using immune function to assess male condition. However, it is likely that immune responsiveness cannot be used to estimate condition in most species. Immune responses (e.g. lysozyme-like activity in insects) can be elevated in animals due to an acute infection (Adamo 2004b), previous exposure to pathogens (Jacot et al. 2005), or constitutively robust immune function (Adamo 2004b). Therefore elevated immune responsiveness could be a sign of males in both good and poor condition. Furthermore, there are more direct ways for females to assess male condition (see Birkhead et al. 2006).

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Pathogen Dynamics and the Fitness Advantage for Mate Choice for Disease Resistance

Selection pressure for female choice was stronger for immune mechanisms that increased resistance to a broad range of pathogens than it was for mechanisms that provided protection against a specific pathogen, unless the specific resistance was to a pathogen with sustained high prevalence and virulence. In our model, changes in pathogen prevalence led to changes in the fitness benefit of female choice for immune function. Empirical data have also shown that the fitness benefits of female mate choice may depend on environmental conditions (see O'Brien & Dawson 2007). Our model

suggests that female mate choice for immune function may be more likely to have a fluctuating pay off than has been previously appreciated. In that case, females that display mate choice only when it will increase their fitness would have a selective advantage (Qvarnström 2001). Therefore, mate choice for immune function may vary within a population. In fact, Howard & Lively (2004), using a different mathematical model from the one used here, found that choice for condition and choice for genetic resistance co-existed within the same population. Individual females may show different choice strategies, depending on factors such as early pathogen exposure, making it difficult to empirically demonstrate mate choice for immune function.

The Most Disease-Resistant Male May Not Be the Most Fit

Females that chose mates based on fitness chose males that had CI and IN values that were significantly less than females that chose for immune responsiveness (i.e. CI and/or IN). The cost of CI and IN lowered the values of CI and IN that produced the highest fitness. This result may explain why choice was sometimes lost for CI and IN; choice for these immune attributes resulted in females mating with males that had a higher level of immune responsiveness than that which led to maximal fitness. Given that real immune systems have costs (Zuk & Stoehr 2002; Siva-Jothy et al. 2005), it is possible that animals with less resistance are actually the fittest (Viney et al. 2005). The greater the cost of the immune function being chosen, the greater the risk that choice for maximal levels of the trait will actually reduce offspring fitness.

Immune systems have costs beyond the energy needed for their maintenance and activation. High levels of immune responsiveness can lead to immunopathology (Sadd &

Siva-Jothy 2006) resulting in decreased condition. These costs decrease the selection pressure for choice for high values of immune responsiveness and/or other immune functions. Moreover, some immune factors are multifunctional and play a role in other physiological systems, such as lipid metabolism (Adamo et al. 2008). Determining the value that leads to maximal fitness could be complicated because it would likely represent a compromise between the needs of two physiological systems. Regardless of the underlying mechanism, if the maximal immune function value does not lead to maximal fitness, it is unlikely that there will be much selection pressure for females to choose for it.

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Limitations of the Model

Similar to our earlier model (Adamo & Spiteri 2005), the model used was strongly biased in favor of finding selection pressure for female choice for male immune function. Complexities that have been ignored by our model are all likely to reduce, not enhance, selection pressure for female choice. For example, in our model, immune responsiveness and recognition led directly to disease resistance; however, the relationship between traits such as CI, IN, NSP, and SP and disease resistance is not straightforward (Adamo 2004a; Avecedo-Whitehouse & Cunningham 2006; Lazzaro et al. 2006; Miniski 2006). We assumed that all traits, including condition were heritable, although there is some debate about how heritable these traits are (Gleeson et al. 2005). As heritability declines, so would female choice for that trait. We also assumed that females could accurately assess male immune function; we did not consider how such sexually selected indicators would evolve and be maintained as honest signals. In our

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model female choice was restricted to condition or to some aspect of immune function, whereas real females have a much wider array of traits they may need to balance during mate choice (Andersson 1994). Such balancing is likely to reduce choice for any one attribute such as immune function.

We ignore host-parasite co-evolution (e.g. our pathogens do not mutate) but evolving pathogens should decrease the ability of the female to predict which male will be the most resistant in the next generation. This will reduce the pressure for female choice for male immune function, especially for immune functions specific for a single pathogen.

In our model, female choice for multiple traits was no more costly than choice based on a single attribute. However, the cost of choice may increase as choice becomes more complex. For example, averaging the values of multiple traits probably requires more cognitive processing power than choice for a single attribute. Increasing cognitive ability reduces fitness (Dukas 2008). If the cost of choice does increase with choice complexity, then the fitness benefits of choice for multiple attributes will be reduced.

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Female Choice for Male Disease Resistance May Be Species-Specific

One reason why choice for condition is thought to provide females with a large fitness advantage is that it allows them to choose males based on their overall genetic quality because condition depends on a large number of genes (Tomkins et al. 2004). Choice for immune function *per se* is likely to provide females with a smaller fitness advantage than condition because immune function is only one of many physiological

systems that determine an animal's fitness. In some species, specific traits may have a large enough impact on fitness for the female to benefit from choosing for it. To determine whether immune function might be such a trait, it would be important to know the identity of the major pathogens for a specific species, how variable the prevalence of these pathogens are, and to what extent these pathogens reduce an individual's fitness. Knowing the pathogen identity would also help determine which immune functions should be examined. However, for many species, focusing on the male's immune system (i.e. the relationship between immune function and sexually selected traits) is probably misleading in terms of understanding to what extent different factors are driving selection for female choice (also see Lailvaux & Irschick 2006). For example, the liver is a large, metabolically expensive organ (Desmet 2001). One of the main functions of the liver is to detoxify food (Desmet 2001). Its ability to detoxify substances varies considerably among individuals within a species (Dorne et al. 2004). Investing in liver function can increase the range of foods available to a herbivore and increase the chance that it will survive the accidental ingestion of a poisonous plant, a common occurrence for herbivores (Karban & Agrawal, 2002). Females choosing males with better liver function could enhance their offspring's fitness, just as they can by choosing a disease-resistant mate. The relative importance of different physiological pathways in determining female fitness will vary depending on the species.

Acknowledgements

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Figure Legends

Figure 1. Fitness advantage of choosers for immune responsiveness. The y-axis denotes the relative fitness advantage of choosers vs. non-choosers for each group. When values are positive, choosers have greater fitness. Each bar denotes choice for a different trait: Fit – fitness, CI – constitutive immunity, IN – inducible immunity, CI+IN – the average of constitutive and inducible immunity scores, Con – condition, Con+CI – the average of condition and constitutive immunity scores, Con+IN – the average of condition and constitutive immunity scores, Con+CI+IN – the average of condition, constitutive immunity, and inducible immunity scores. Box-and-whisker plot. The central line represents the median, the bars represent the 1st and 3rd quartiles and the error bars denote the sample range.

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Figure 2a. The fitness advantage of choice when condition (Con) and immune responsiveness (CI+IN) are strongly negatively correlated (r=-1). The y-axis denotes the relative fitness advantage of choosers vs. non-choosers for each group. When values are positive, choosers have greater fitness. All traits show a significantly smaller fitness advantage than condition.

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2b. The fitness advantage of choice when condition (Con) and immune responsiveness (CI+IN) are weakly positively correlated (r=0.2). All traits except IN show a significantly larger fitness advantage than condition. Each bar denotes choice for a different trait. Con – condition, Con+CI – the average of condition and constitutive immunity scores, Con+IN – the average of condition and constitutive immunity scores,

Con+CI+IN – the average of condition, constitutive immunity, and inducible immunity scores, CI – constitutive immunity, IN – inducible immunity, CI+IN – the average of constitutive and inducible immunity scores. Box-and-whisker plot. The central line represents the median, the bars represent the 1st and 3rd quartiles and the error bars denote the sample range.

Figure 3. The fitness advantage of choosers for specific recognition. The y-axis denotes the relative fitness advantage of choosers vs. non-choosers for each group. When values are positive, choosers have greater fitness. Each bar denotes choice for a different trait. Fit – fitness, S – survivorship, Con – condition, CI – constitutive immunity, IN – inducible immunity, CI+IN – average of constitutive and inducible immunity scores, NSP – non-specific recognition, AgSP – average of the 7 specific recognition scores, SP1 – specific recognition for pathogen 1, SP2 – specific recognition for pathogen 2, SP3 – specific recognition for pathogen 3, SP4 – specific recognition for pathogen 4, SP5 – specific recognition for pathogen 5, SP6 – specific recognition for pathogen 6, SP7 – specific recognition for pathogen 7. Box-and-whisker plot. The central line represents the median, the bars represent the 1st and 3rd quartiles, and the error bars denote the sample range.

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Figure 4. The fitness advantage of choosing for both immune recognition and condition compared with choice for condition alone. The y-axis denotes the relative fitness advantage of choosers vs. non-choosers for each group. When values are positive, choosers have greater fitness. Each bar denotes choice for a different trait. Con+NSP –

average of condition and non-specific recognition, Con+AgSP – average of condition and average of the 7 specific recognition scores, Con+SP1 – average of condition and specific recognition for pathogen 1, NSP – non-specific recognition, AgSP – average of the 7 specific recognition scores, SP1 – specific recognition for pathogen 1, Con – condition. Box-and-whisker plot. The central line represents the median, the bars represent the 1st and 3rd quartiles, and the error bars denote the sample range. Asterisk denotes values significantly different from condition.

Figure 5. Values of CI and IN depend on the trait chosen by females. a) Values of CI b) Values of IN. Each bar denotes choice for a different trait: Fit – fitness, Con – condition, CI – constitutive immunity, Con+CI – the average of condition and constitutive immunity scores, IN – inducible immunity, Con+IN – the average of condition and constitutive immunity scores, CI+IN – the average of constitutive and inducible immunity scores, Con+CI+IN – the average of condition, constitutive immunity, and inducible immunity scores. Box-and-whisker plot. The central line represents the median, the bars represent the 1st and 3rd quartiles, and the error bars denote the sample range.

Table 1. Values used to model the effect of different pathogens (taken from Adamo & Spiteri 2005)

Pathogen	Virulence*	Maximum Prevalence**	w1[13,14]	w2[13,15]
Virus				
1. Cricket Paralytic Virus[1]	0.80	0.55	0.95	0.05
Bacteria				
2. *Serratia marcescens*[2,3,4]	0.90	0.02	0.05	0.95
3. *Ricketsiella grylli*[4, 5]	0.80	0.15	0.05	0.95
Fungi				
4. *Entomophaga grylli*[6,7]	0.98	0.40	0.05	0.95
Protozoan				
5. *Nosema locustae*[8,9,12]	0.90	0.38	0.95	0.05
Metazoan				
6. *Mermithidae*[10,12]	0.98	0.21	0.95	0.05
7. *Parasitoid*[11,12]	0.98	0.17	0.95	0.05

*Virulence denotes the probability of mortality once the pathogen has entered the host.

**Maximum prevalence sets the maximum likelihood an individual will become infected with a given pathogen. The values were set to prevent populations from going extinct. In the field, populations rarely go to 0, even during epizootics (e.g. Smith 1965; Anderson & May 1981 (Table 6); Carruthers et al. 1997).

[1] Evans & Entwhistle 1987; [2]Zelazny et al. 1997; [3]Benz 1987; [4]Kreig 1987; [5]Adamo 1998; [6]Carruthers and Soper 1987; [7]Carruthers et al. 1997; [8]Maddox 1987; [9]Johnson & Dolinski

1997; [10]Kaya 1987; [11]Adamo et al. 1995; [12]Smith 1965; [13]Gillespie et al. 1997 and Hoffman et al. 1996.

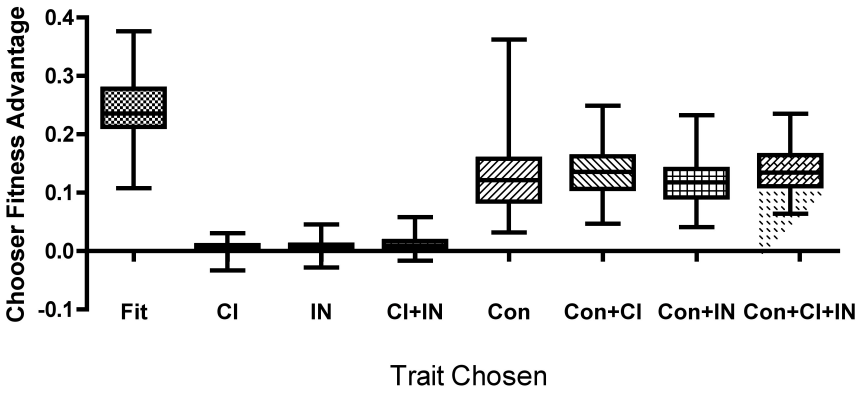
[14] The value of w1 reflects the relative importance of constitutive immunity (CI) in the defense against each pathogen. Although CI is important against bacteria and fungi (e.g. Gillespie et al. 1997), studies have shown that without inducible immunity (IN) insects die from these pathogens (e.g. Gottar et al. 2002), and this motivates our weighting. We ran preliminary simulations with CI weighted 0.45 and IN weighted 0.55 for bacterial and fungal pathogens. We found the same general results as described below (unpublished observations); i.e. female choice was lost in most populations selecting for CI or IN.

[15] The value of w2 reflects the relative importance of inducible immunity in the defense against each pathogen. The role of inducible immunity in the defense against some pathogens is still under study, and, therefore, instead of 0 we assigned a small value to w2 for these pathogens.

Table 2. Percent of Populations (*N*=100) that Lose Choice for SP or NSP

Immune Factor[1]	Fluctuating Pathogens	Constant Pathogens
SP1	82	33
SP2	88	73
SP3	90	67
SP4	85	46
SP5	86	50
SP6	93	67
SP7	85	63
AgSp 1-7	1	0
NSP	0	0

1. See Table 1 for virulence and prevalence of each of the 7 pathogens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Trait Chosen

Fig. 1

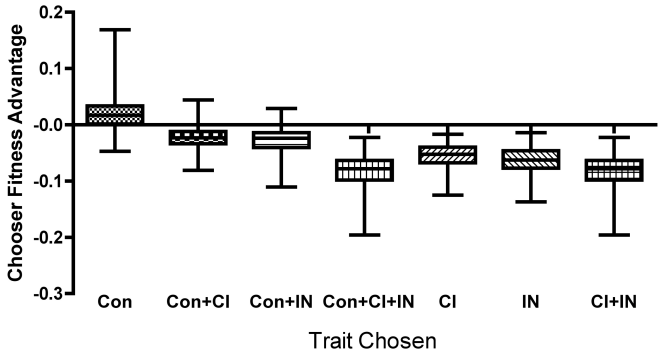


Figure 2a

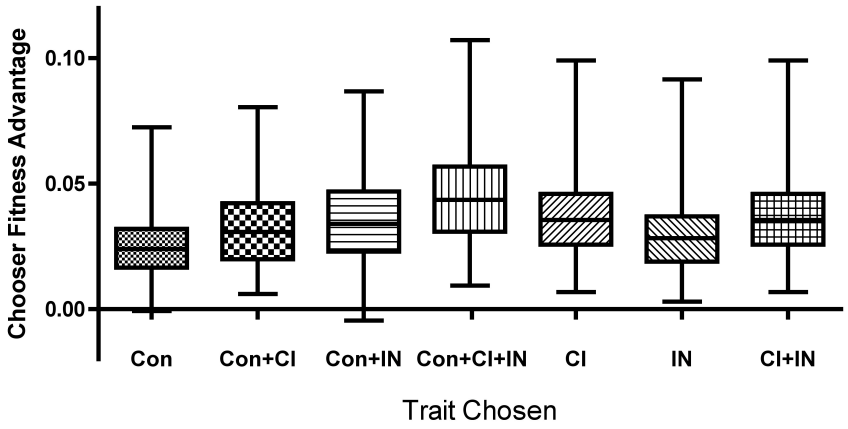
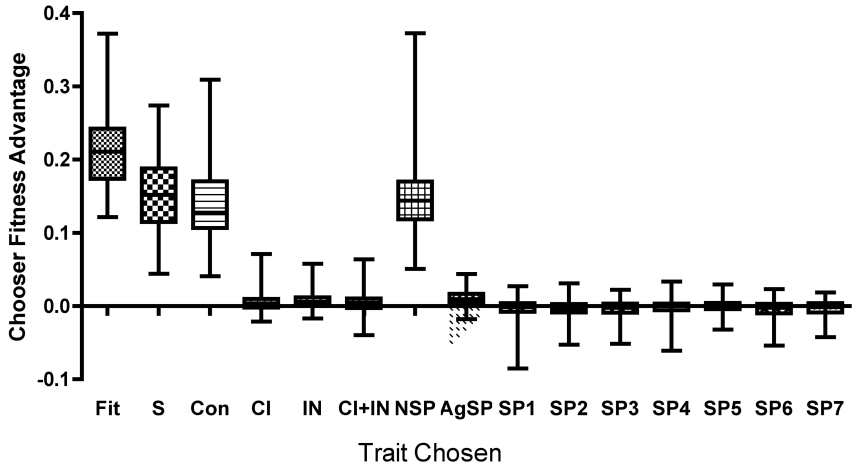


Figure 2b


Chooser Fitness Advantage
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Fit
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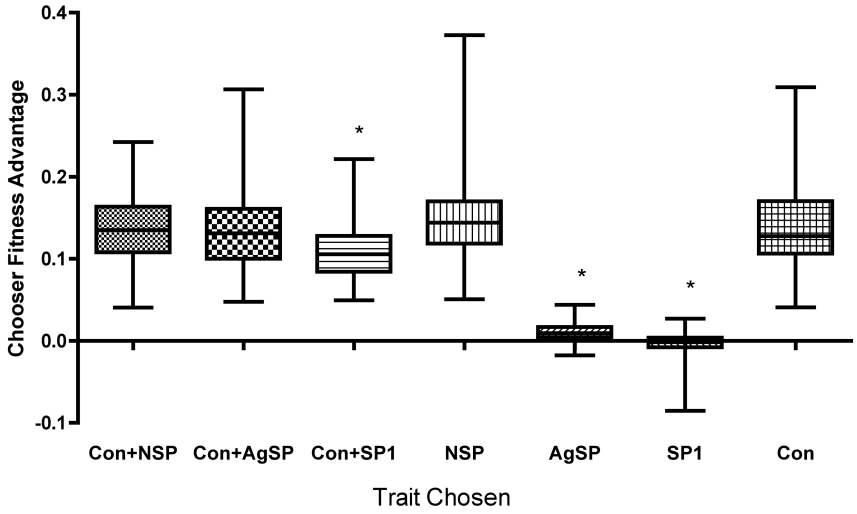
SP7
Trait Chosen

Figure 3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SP1
Con
Trait Chosen

Figure 4

Figure 5a

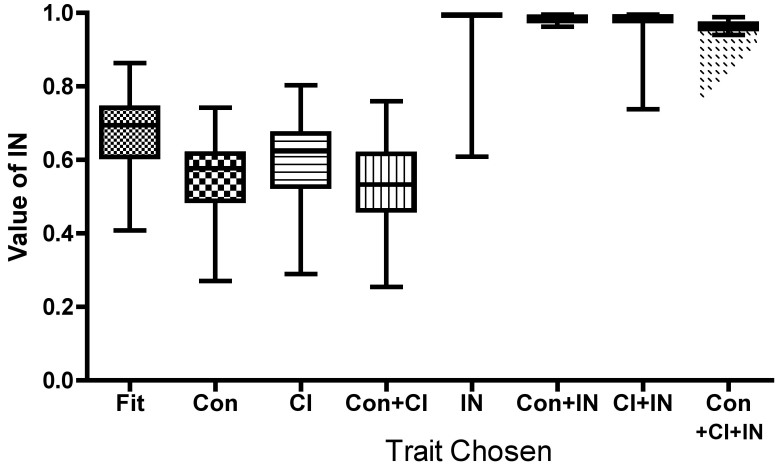

Figure 5b

Appendices:

A. Mathematical Details

Individual insects in our model were assigned normally distributed randomly chosen values with a mean of 1/2, a variance of 1/9, and truncated to the interval [0, 1] for constitutive immunity (CI) and inducible immunity (IN). The non-specific recognition (NSP) and the specific recognition (SP) values for the 7 different pathogens were chosen with a mean of 0.5, a variance of 0.0225 and truncated to the interval [0,1]. Condition was assigned normally distributed values with a mean of 1, a variance of 0.0225, and truncated to the interval [0, 2]. Condition was given a greater range of values because it influences a wide variety of fitness parameters, including fecundity, and therefore it was given a higher weighting than individual immune components. However, we also ran simulations in which the range of values for condition was the same as that for immune function (Results section). A score of 0 denoted individuals having no ability for that particular function, and a score of 1 (or a score of 2 in the case of condition) denoted maximal ability.

Calculating Correlated Values

Correlated values of condition were assigned in the following way. Let r be a number in the interval [-1,1] that represents the correlation between condition and trait *x*, where in our model *x* = CI, IN, or CI+IN. The new value for condition at year t+1 is generated from the old value of condition at year t as a normally distributed random number with mean:

Equation 4. $2*[\text{weight1}*x + \text{weight2}*(\text{old condition})/2 + \text{weight3}*(1-x)]$

and standard deviation

weight2*(0.15),

where weight1 + weight2 + weight3 = 1, with

weight1 = H(r)*r, weight2 = 1-|r|, weight3 = -H(-r)*r,

and H(r) is the Heaviside function H(r)=1 if r>0 and H(r)=0 if r<0.

This formula reduces to the appropriate behaviors at the limit |r|=1, i.e., condition = 2*x when r=1 (condition is perfectly positively correlated with x) or 2*(1-x) when r=-1 (condition is perfectly negatively correlated with x), and if r=0, the new value of condition is randomly generated from the only the old value for condition (no correlation with x).

Pathogen Prevalence

We constructed a canonical cycle of pathogen prevalence according to the following formula:

The prevalence Pj(t) of pathogen j at time t years is given by

Equation 5. $P_j(t)=0.96\exp(-0.7*(\mathrm{mod}(t, 18)-9)^2)+0.02$,

where mod(t, 18) is the remainder left over when dividing t by 18.

The canonical cycle was constructed to have a sharp peak of 0.98 and taper quickly to 0.02 over a period of 9 years on either side of the peak, to give an 18-year cycle. We then scaled the canonical cycle by Pmax(*j*) for pathogen *j*. Also, for a given seed, each pathogen started at a random point on the canonical cycle, meaning that each seed produced a different dynamical pattern among the different pathogens. We denote the sequence of points for pathogen *j* starting from this random point by Pindex(*j*). Therefore, the pressure of pathogen *j* on the population at time t is calculated from:

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Equation 6. Pressure of Pathogen (*j*)=Pmax(*j*)*P*j*(Pindex(*j*))*Vbar(*j*),

where Pmax(*j*) is the maximum prevalence of pathogen *j*, P*j*(Pindex(*j*)) is the canonical prevalence value, and Vbar(*j*) is the mean virulence for pathogen *j*.

Survivorship

The risk of death D(*i*,*j*) of insect *i* due to pathogen *j* is given by

Equation 7. D(*i*,*j*)=min(1/condition*pathogen pressure *j* *(1/I(*i*,*j*) – 1), 1).

In this equation, condition influences how well an individual can withstand its pathogens. Males in good condition can tolerate a higher pathogen load without dying than males in poor condition (see Getty 2002).

The survival of insect *i* is given by

Equation 8. $$s(i)=\prod^{7}(1-D(i,j)).$$

Calculating Offspring Immune Values

Before inheriting values from the father, the values were mutated according to the formula:

Equation 9. $$x \rightarrow \min(\max N(1, 0.0225)x,0),1)^{1},$$

where x = condition, CI, IN, or recognition (NSP and SP) and $N(1, 0.0225)$ is a number chosen from a normal distribution with mean 1 and variance 0.0225. Mutation helps maintain population diversity from one generation to the next, specifically by filtering inherited values through a normal distribution.

Calculating the Cost of Immunity

The cost of immunity for insect i was calculated as

Equation 10. $$\text{cost of immunity}(i) = \text{cost of CI}(i) + \text{cost of IN}(i)$$
$$= \text{conditionFactorCI}(i)*0.2*\text{CI}(i) + \text{conditionFactorIN}(i)*0.02*\text{IN}(i),$$

where conditionFactor$x$ for quantity x is max(2-condition(i),minCostImmunity$x$), x = CI or IN. Condition is included in this equation to ensure that animals in good condition pay proportionately less than animals in poor condition for a high-performing immune system (Getty 2002; Ardia 2005). We also assign a minimum cost to immunity to reflect that even animals in perfect condition pay some cost for their immune system.

When recognition is divided into NSP and SP, the cost of immunity of insect i was calculated as

Equation 11. Cost of immunity(i) = cost of CI(i) + cost of IN(i) + cost of recognition(i),

where cost of recognition(i) = cost of NSP(i)* factor NSP(i) + cost of SP * factor SP(i),

and (recognition) factor x = max(2-condition(i),minCost x), x = NSP(i), SP(i).

When recognition is not divided into NSP+SP, we assumed that it had no cost.

Evidence is sparse as to the cost of immune surveillance, but we assumed that the relative cost of NSP(i) was 1/20 that of IN(i) and the cost of SP was 1/200 that of IN(i). We note that when recognition was used in the model, the cost of NSP and SP were simply added into the cost of immunity.

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Calculating the Cost of Choosiness

Because choosy females pay a fitness penalty for being choosy, fitness for female insect *i* was modeled by

Equation 12. $w(i)=(1- \text{cost of immunity}(i))*s(i)*(1 - \text{choosiness penalty } (i))$,

where choosiness penalty $(i) = 0.01$ if female *i* was choosy. Even though the individual female's CI and IN values were not inherited by her offspring, they were still used to calculate her individual fitness.

B. Evidence of Model Robustness

The model performed as expected for extreme cases.

Reducing the cost of choice to 0 resulted in an increase in the fitness advantage of choice for all traits (Non-parametric 2 way ANOVA, Meddis 1984; $Z=2.57$, $P<0.01$) and an increase in the number of populations in which females choosing CI reached 100% (CI, Fisher's exact test, CI, $P=0.0002$; IN, $P= 0.01$). However, even with no cost to choice, choice was lost in a small number of populations in which females chose for IN (2/100) or for CI+IN (1/100). When the cost of choice is 0, the advantage to females choosing males resistant to specific pathogens increased (Non-parametric 2 way ANOVA, Meddis 1984; $Z=2.59$, $P<0.01$) and the percentage of populations fixing at 100% choosers for choice for specific pathogen resistance increased (Non-specific test for trends, Meddis 1984, $Z=2.63$, $P<0.01$).

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Reducing the cost of each immune response to 0 increased the fitness advantage for choice for CI (Mann-Whitney: $U=2854$, $P<0.0001$) but not for IN (Mann-Whitney: $U=4909$, $P=0.83$). It also led to an increase in the number of populations fixing at 100%

choice when females chose for CI (Fisher's exact test, $P<0.0002$) but not IN (Fisher's exact test, $P=1.0$). CI is 10 times more costly than IN in this model.

Choice for immune responsiveness (CI and IN) and condition gave females no significant fitness benefit over non-choosers compared with females that chose for condition alone for a wide range of parameter values. For example, changing the cycle duration from 18 years to 8 or 12 years led to no significant increase in the fitness advantage to females for choosing condition + immune responsiveness compared to females choosing for condition alone ($P=0.12$; 8 year cycle, Kruskal-Wallis: $H=588$, $P<0.0001$, Dunn's multiple comparisons $P=0.23$, 12 year cycle, Kruskal-Wallis: $H=639$, $P<0.0001$, Dunn's multiple comparisons). Similarly, choosing males resistant to specific pathogens supplied less of a fitness advantage over non-choosers than choosing a non-specific form of recognition regardless of whether the cycle length was 5 (Kruskal-Wallis: $H=999$, $P<0.001$, Dunn's multiple comparison test, $P<0.001$), 12 (Kruskal-Wallis: $H=980$, $P<0.001$, Dunn's multiple comparison test, $P<0.001$), or 25 years (Kruskal-Wallis: $H=986$, $P<0.001$, Dunn's multiple comparison test, $P<0.001$), or whether condition was correlated at a 0.5 level with immune responsiveness (i.e. CI + IN, Kruskal-Wallis: $H=1259$, $P<0.001$, Dunn's multiple comparison test, $P<0.001$). Also, choosing for resistance against the most virulent pathogen gave the highest fitness advantage over non-choosers compared with choice for the least pathogenic entity under a variety of conditions (cycle length 5; Dunn's multiple comparisons, $P<0.001$; cycle length 12, Dunn's multiple comparisons, $P<0.001$, cycle length 25, Dunn's multiple comparisons, $P<0.001$; condition and CI+IN correlated at 0.5, Dunn's multiple comparisons, $P<0.001$).

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Females choosing for both condition and immune responsiveness (CI + IN) gained no significant fitness advantage relative to non-choosers over females choosing for condition alone regardless of whether there were 1, 3, 5, or 7 pathogens. In fact, choosing for condition and immune responsiveness led to a significantly smaller fitness advantage relative to non-choosers compared with choosing for condition alone when there were 1 (Mann Whitney: U=2582, P<0.0001) or 3 pathogens (Mann Whitney: U=2898, P<0.0001).

Footnotes

1. This formula was used to calculate mutated values in both this paper and in Adamo & Spiteri (2005). The formula given in Adamo & Spiteri (2005) contained a typographical error.