Female choice for male immunocompetence: when is it worth it?

Shelley A. Adamo[1] and Raymond J. Spiteri[2,3]

Corresponding Author:

1. Department of Psychology

Dalhousie University

Halifax, Nova Scotia, Canada, B3H 4J1

Phone: 902 494-8853 Fax: 902 494-6585 e-mail: sadamo@dal.ca

2. Department of Mathematics and Statistics and Faculty of Computer Science

Dalhousie University

Halifax, Nova Scotia, Canada, B3H 3J5

3. Present address: Department of Computer Science

University of Sackatchewan

Saskatoon, Saskatchewan, Canada, S7N 5C9

Phone: 306-966-2909 Fax: 306-966-4884 e-mail: spiteri@cs.usask.ca

Running head: Female choice for male immunocompetence

Disease resistance is not determined by any single immune component. Nevertheless, female choice for individual immune components could produce more disease-resistant offspring. Using a mathematical model, we tested whether female choice for male immune responsiveness was maintained or lost in simulated populations. We divided immunity into 3 different components: 2 different types of immune responsiveness (inducible immunity and constitutive immunity) and the ability to recognize pathogens. When the pathogen prevalence fluctuated from generation to generation, female choice for inducible or constitutive immunity was usually lost. Female choice for constitutive immunity was often lost even when choosiness carried no fitness penalty. Choosing for constitutive or inducible immunity produced a fitness advantage, when compared to non-choosers, during some generations, but not for others, depending on the identity of the most prevalent pathogens. Choosing for inducible or constitutive immunity led to high mortality when pathogens sensitive to the non-chosen component became prevalent in the population, giving non-choosers the advantage. Given that most animals experience fluctuating pathogen pressure, our model suggests that there may be little selection for female choice for male constitutive and/or inducible immunity in some species. We discuss the implications of our results for the study of female choice for male disease resistance.

Key words: ecological immunology, sexual selection, invertebrate, specific immunity, mate choice

Introduction

In many species, females actively choose their mates despite the potential costs of being choosy (Andersson, 1994). Mate choice may benefit females by allowing them to select males capable of bestowing 'good genes' on their offspring. By increasing offspring quality, females could enhance their own fitness enough to offset the costs of choice. Disease can drastically reduce female fitness by destroying susceptible offspring. Disease resistance appears to be heritable in a wide variety of species (e.g. Ryder and Siva-Jothy, 2001). Therefore, if females could choose disease-resistant males, more of their offspring would survive. This selection pressure should favour females capable of choosing males based on their disease resistance (i.e. immunocompetence) (Møller et al., 1999).

In this paper we develop a mathematical model to examine the selection pressure on female choice for superior male immune responses. Immune responsiveness refers to the ability of the immune system to produce cells and/or molecules capable of neutralizing invaders after a foreign antigen has been identified. Virtually all empirical papers testing for female choice for male disease resistance do so by correlating measures of immune responsiveness with sexually selected traits (e.g. Møller et al., 1999). There are good reasons to suspect that females may prefer males with superior immune responses (Kurtz and Sauer, 1999). Increased immune responsiveness (e.g. increased lysozyme production) could increase disease resistance (e.g. Adamo, 2004a). Different types of immune responses are heritable (Pinard-van der Laan et al., 1998). Therefore females may be able to increase the disease resistance of their offspring by selecting males with superior immune responses.

However, there are two issues that may limit the evolution of female choice for enhanced male immune responses. The first is that the immune system is composed of a diverse array of biochemical and cellular components (Roitt et al., 2001; Gillespie et al., 1997). No single immune component can predict disease resistance (Luster et al, 1993; Keil et al., 2001; Adamo, 2004b), partly because the relative strengths of different immune components are not necessarily positively correlated (see Westneat and Birkhead, 1998; Boa-Amponsen et al., 1999; Mallon et al., 2003; Adamo, 2004a, b). For example, there is evidence that some immune responses are negatively correlated with the ability to recognize pathogens (e.g. Mallon et al., 2003). Therefore, female choice for one aspect of immunity, such as the ability to form antibodies, may not result in the selection of males who are superior in other aspects of immunity (e.g. ability to recognize a pathogen). The lack of positive correlation between different immune components may decrease selection for female choice for male immune responsiveness. However, it is possible that female choice for this trait could produce offspring that would be more disease-resistant than would be produced from mating randomly with any male, even though superior immune responsiveness may not always be equivalent to superior disease resistance. Selection would then favour choosy females. We use our model to test this hypothesis.

We use the same mathematical model to examine a second difficulty for the evolution of female choice for superior male immune responsiveness. Different pathogens require different types of immune responses (Table 1). If a female knew which pathogens were going to pose the greatest threat to her offspring, she could select for males who had the immune responses that would give her offspring the greatest

protection. Therefore, whether a female would benefit from selecting a male based on his immune responsiveness may depend on the dynamics of the pathogen population. We hypothesize that when females live in an environment in which the important pathogens are predictable, they are more likely to benefit from female choice for enhanced male immune responsiveness than when they are exposed to fluctuating pathogen populations.

Methods

To examine selection for female choice for enhanced male immune responsiveness, we developed a mathematical model similar to that of Kokko and Lindström (1996). We based our model on the invertebrate immune system because of its relative simplicity. Nevertheless, the model is general enough to apply to both vertebrates and invertebrates (see below). To ensure that we used biologically meaningful parameter estimates in our model, we used literature values for Orthopteran species whenever possible (Table 1). We assumed our model Orthopteran had one generation per year, no parental care, and no overlap in generations.

We modelled the immune system as having three basic components: 2 types of immune responsiveness (constitutive immunity and inducible immunity) and the ability to recognize pathogens. Constitutive immunity is composed of the immune factors that an animal produces continuously, even without an immune challenge. Inducible immunity is composed of factors produced only during an immune challenge (see Schmid-Hempel and Ebert, 2003). Vertebrates and invertebrates have both constitutive and inducible immunity (Roitt et al., 2001; Gillespie et al. 1997). We divided immune responsiveness

in this way because inducible immunity is important for defence against bacteria and fungi in insects (Gillespie et al., 1997) but appears to be less important against other types of pathogens (e.g. viruses, Evans and Entwhistle (1987); Table 1). Although we are dividing immune responsiveness into constitutive and inducible immunity, the model can accept other ways of dividing the immune system as long as the separate components are independent.

Individual insects in our model were assigned normally distributed randomly chosen values with a mean of 1/2 and a variance of 1/9 and truncated to the interval [0, 1] for constitutive immunity (CI), inducible immunity (IN), and the ability to recognize 7 different pathogens (Table 1). At least some immune components (e.g. lysozyme-like activity and phenoloxidase activity) are normally distributed in real populations (e.g. the cricket *Gryllus texensis*, Adamo, 2004a). A score of 0 denoted individuals having no disease resistance and 1 denoted perfect resistance. The scores for CI, IN, and the 7 recognition scores were chosen independently (i.e. scores were not required to be either positively or negatively correlated). We justify the lack of enforced correlation between our scores because some immune components are known to be independent of one another (e.g. Ferrandon et al., 1998; Khush et al., 2001; Gottar et al., 2002). Moreover, the ability to recognize different pathogens, which differ in their antigens, is not necessarily correlated (Franc and White, 2000). Therefore, in our model, it is possible for an animal to have robust immune responses but still die of an infection if it lacks the ability to recognize that particular pathogen. In this model we examine the case in which females are able to assess male immune responsiveness (CI and IN), but not recognition. We omitted modelling female choice for recognition primarily because we wished to test

how fluctuating pathogen prevalence and the lack of positive correlation within the immune system might impact current studies on female choice for male immunocompetence. Virtually all current studies rely on measures of immune responsiveness (Adamo, 2004b). We assumed that females were able to assess CI and IN accurately.

For insect *i*, CI, IN, and recognition factors for pathogen *j*, where *j*=1,2, …, 7 are given by expressions of the form:

$$x_{i,j}=\max(\min(N(1/2,1/9), 1), 0),$$

where *N* (1/2, 1/9) is a number taken from a normal distribution with a mean 1/2 and variance 1/9.

The immunocompetence score I(*i*,*j*) of insect *i* with respect to pathogen *j* is determined according to the formula:

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

where recog(*i*,*j*), CI(*i*), IN(*i*) are the recognition factor, CI, and IN of insect *i* with respect to pathogen *j*, and w1(*j*), w2(*j*) are the weights for pathogen *j* (see Table 1), where w1 represents the importance of CI for resistance to pathogen *j,* and w2 represents the importance of IN for resistance to pathogen *j*.

Fitness was modelled as being a product of lifespan and fecundity:

Fitness=ideal fecundity*cost of immunity*ideal lifespan*survival

We take ideal fecundity = 1.

Fecundity is reduced by the cost of immunity. The cost of immunity remains controversial (e.g. Zuk and Stoehr, 2002). However, increased immune function decreases fecundity in insects, and we use literature values to estimate costs of CI and IN (Kraaijeveld et al., 2001; Ahmed et al., 2002; Koella and Boëte, 2002; Freitak et al., 2003; Jacot et al., 2004). We assume that constitutive immunity (CI) is more costly than inducible immunity (IN) (Rolff and Siva-Jothy, 2003). We omit costs due to recognition factors because these costs are uncertain in insects, and if they do have costs they are likely to be low (Wedekind, 1994b).

$$\text{Cost of immunity} = \text{cost of CI} + \text{cost of IN}$$

$$= 0.2*\text{CI}(i) + 0.02*\text{IN}(i)$$

Ideal lifespan = 1

Lifespan (ideal lifespan * survival) is modelled here as being entirely dependent on immunocompetence (I). This formulation increases the selection pressure for female choice for this trait. Low immunocompetence reduces fitness by decreasing lifespan. The decrease in lifespan due to disease is calculated by estimating the individual's risk of death for each of the 7 pathogens in a given year. The risk of death is determined by the virulence for each pathogen (Table 1) and the pathogen prevalence, which in our simulations can be set to a constant value for all generations or which can fluctuate from generation to generation. For the development of realistic fluctuations in pathogen prevalence, we relied on the long-term field study of Smith (1965) that recorded the incidence of parasitoids and nematodes as well as Carruthers et al., (1997) for *Entomophaga grylli* and Fuxa and Tanada (1987) for other organisms. The pathogens chosen are broadly representative of the different types of pathogens an insect encounters

(Fuxa and Tanada, 1987). Figure 3A shows the fluctuating pathogen pressure (pathogen virulence x pathogen prevalence) for seed 1 (one of the 100 randomly generated populations).

Each pathogen population was assumed to have a cycle of 18 years. This period is somewhat longer than that estimated by Anderson and May (1981) (but see Smith, 1965), but a longer pathogen frequency cycle should bias toward female choice (Hamilton and Zuk, 1982).

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

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

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

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. 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. 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:

$$\mathrm{Pmax}(j)*Pj(\mathrm{Pindex}(j))*\mathrm{Vbar}(j),$$

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*.

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

$$D(i,j)=\min(\text{Prevalence of pathogen } j^* \text{ Virulence of pathogen } j *(1/I(i,j) - 1), 1).$$

The survival of insect *i* is given by

$$s(i)=\prod_{j=1}^{7}(1-D(i,j));$$

therefore the fitness of insect *i* is given by

$$w(i)=(1 - 0.2*CI(i) - 0.02*IN(i))*s(i).$$

In each generation there were 500 females and 500 males. Each female was ranked by her fitness score to determine 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 females to replace the original population with one mating, 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, and recognition were inherited from the father (for both male and female offspring). Because this is a haploid model of inheritance (from the male), female choice had an immediate effect on the fitness of the female's offspring. 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 according to the formula:

$$x_i \rightarrow \min(N(1, 0.00255)x_i,1),$$

where x_i = CI, IN, or recognition. This procedure maintained variability in CI, IN, and recognition in the population (pers. obs). Because changes were chosen from a normal distribution, most mutations caused little change, as might be expected for a polygenic trait (Beck and Powell, 2000) such as immunity. Rare mutations may cause large

changes, however. Because CI, IN, and recognition may vary slightly every generation, this rate is somewhat higher than might be expected in a wild population (see Kokko and Lindström, 1996, for a discussion). However, Kokko and Lindström (1996) found that higher mutation rates favour the evolution of female choice. We also ran simulations with mutation rates at 1/10 our standard level. The method we used to create mutations biased mutations so that without selection, values for CI, IN, and recognition tended to decrease. This negative bias also increases the likelihood of selection for female choice (Iwasa et al., 1991; Pomiankowski et al., 1991).

Each population began with 50% choosy females and 50% non-choosy females. Choosy females mated only with males who were above average for the criterion of choice (i.e. CI, IN, CI + IN, fitness (w) or survival (s)). We ran simulations allowing females to choose for fitness (w) as an example of the strongest selection we could expect for choice. In our model, we expected that selection for choice for fitness would be more likely to fix in the population than choice for any individual component of fitness. We allowed females to choose for survival (s) to test whether selection for choice would be stronger for a general trait that is influenced by environmental conditions than it is for immune responsiveness. Non-choosy females mated randomly with any living male.

For choosy females, there was an additional cost of choice. The cost of choosing varies greatly between species, and in some animals appears to be close to 0 (Gibson and 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%. This value is used by other modellers (e.g. Kokko and Lindström, 1996; Beck and Powell, 2000).

Female fitness for insect *i* was modelled by

$$w(i)=(1-0.2*CI(i) - 0.02*IN(i))*s(i)*(1-\text{choosiness penalty } (i))$$

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.

We also estimated the effect of choice on fitness by calculating the average difference in fitness between choosers and non-choosers for each generation. We made these calculations for females choosing for constitutive immunity (CI), inducible immunity (IN), and fitness for the first 6 seeds.

Simulations were run using Matlab version R13 for 100 different populations for 1800 generations. Values for CI, IN, and the 7 recognition values were recorded at this time point. In some cases, choosiness had not fixed to 100% or 0% by 1800 generations. In these cases we ran the simulations for up to 18,000 generations only to determine whether they fixed to choosiness. By 18,000 generations, all simulations had fixed to either 0% or 100%. We also ran some simulations (10 populations) using a larger number of individuals (10,000).

Results and Discussion

Selection pressure for female choice for male immune responsiveness and pathogen population dynamics

The likelihood that female choice for male immune responsiveness was maintained in a population was dependent on the population dynamics of the pathogens. When pathogen prevalence was constant, but with the same average prevalence as the fluctuating pathogen populations, choosiness for constitutive immunity (CI), inducible

immunity (IN), CI+IN, fitness, or survival fixed to 100% of the population in all simulations (n=100). However, under conditions of fluctuating pathogen prevalence, choosiness for CI, IN, or CI+IN fixed to 0% in more than 1/2 of the 100 simulated populations (Fig. 1, Fig. 2). Given that most animals experience fluctuating pathogen pressure (e.g. Anderson and May, 1981), our model suggests that there may be little selection for female choice for male constitutive and/or inducible immunity in some species.

Our results do not support the hypothesis that female choice for immune responsiveness will have a selective advantage over random mating in the presence of fluctuating pathogen populations. Choosing for fitness led to a dramatic decline in mortality in subsequent generations (Fig. 3). However, choosing for either CI or IN led to oscillating levels of mortality, with mortality declining during generations in which pathogens relying on the selected response were prominent, followed by increased mortality when pathogens requiring the non-selected response became more important (Fig. 3). Choosing for the sum of CI and IN resulted in low scores for recognition (Fig. 4). Females choosing for fitness always had a fitness advantage over non-choosy females (Fig. 5). However, females choosing for CI or IN had a variable fitness advantage, depending on the pathogen pressure (Fig. 5). When pathogens sensitive to the non-selected immune component were prevalent in the population, females typically were less fit than non-choosers, and choosiness was often lost at this time. Averaged over the first 6 seeds, choosing for fitness gave an average fitness advantage (i.e. fitness score of choosers – non-choosers) that was almost 10X greater (0.0720 ± 0.0085 units) than it was

than when choosing for either A (0.0075 ± 0.0010), B (0.0071 ± 0.0060), or A+B (0.0142 ± 0.0008).

Reducing pathogen prevalence to 0 led to 0% mortality and 0% choosiness in all populations whether choosing for CI, IN, CI+IN, fitness, or survival. Once choice fixed at 0%, and with no mortality, the values of CI, IN, and all 7 recognition values declined. The starting means for CI, IN, and the 7 recognition values were approximately 0.5. By 1,800 generations, all values were less than 0.00001. This decline occurred because our mutation equation was biased such that scores for recognition, CI, and IN gradually declined without selection. This bias occurred because the mutation is based upon a *percentage* increase or decrease in the quantity x_i and not an absolute amount. .Mathematically, it can be shown that this process is related to a random walk with a non-positive bias for the logarithm of x_i . Using Jansen's inequality (see e.g. Feller, 1971), it can be shown that this implies that the logarithm of x_i drifts to negative infinity with probability 1; hence x_i drifts to 0 with probability 1.

The larger populations of 10,000 individuals took longer to fix to either choosiness or non-choosiness. Under fluctuating pathogen prevalence, choosing for fitness still fixed at 100% of the population in all simulations (n=10). Choosing for survival fixed at 100% of the population for almost all simulations (9/10). Choosiness for CI was lost in 80% of the simulations, consistent with the results based on the smaller population. Choosing for IN fixed at 0% in 1/10 simulations. The other 9 simulations resulted in no fixation even after 18,000 generations.

Selection for female choice for male immune responsiveness depends on costs

The cost of CI and IN was important in determining whether choosiness would be lost. If the cost of CI and IN was reduced, choosiness was more likely to fix at 100% of the population (Fig. 6). In some species, males form leks, and it has been suggested that leks can reduce the cost of female choice (Höglund and Alatalo, 1995). Species in which the cost of choice is low are more likely to have evolved choice for male immune responsiveness. However, even with no cost to immunity, choosiness was lost in some populations when choosing for CI or IN (Fig. 6).

When the penalty for choosiness was reduced to zero, female choice for IN, CI+IN, fitness, or survival fixed at 100% of the population in all simulations. However, when selecting for CI, choosiness still decreased to 0% of the population in 52% of simulations. This result demonstrates that choosing for a single immune component can be a worse strategy than mating randomly.

Selection for female choice for male immune responsiveness and genetic variability in males under conditions of fluctuating pathogen prevalence

Lowering the mutation rate by an order of magnitude decreased the selection for choice. When choosing for CI or IN under these conditions, choosiness was lost in 100% of simulations. If the mutation equation was altered to remove the tendency of scores to move towards 0 when there is no selection, choice fixed to 100% of the population in all simulations when choosing for fitness. However, choice was lost in all populations choosing for CI, in 93% of populations choosing for IN, and in 95% of populations choosing for CI+IN.

Removing mutation from the model led to a loss in variability between males for CI, IN, and recognition and a subsequent loss of female choice for any parameter. Evolutionary biologists have long sought solutions to the problem of how female choice for 'good' genes can be maintained when this selection should reduce variability among males to zero (see Höglund and Alatalo, 1995). Hamilton and Zuk (1982) suggested that fluctuating cycles of parasite prevalence could maintain enough variability in the population to maintain female choice for disease resistance. Using the parameters in our model, fluctuating pathogen prevalence was not sufficient to maintain choice without mutation; however further research with the type of model presented here may shed light on this problem.

The importance of variability in immune responsiveness within a population for the evolution of female choice for this trait should be explored more fully. Even though mutation exists in real populations, the amount of biologically significant variability in immune responsiveness may be small between healthy males (Adamo, 2004a, b). For example, Lazzaro et al. (2004) found that variability in resistance to the bacterium *Serratia marcescens* among individual *Drosophila melanogaster* was associated with polymorphisms in genes corresponding to pattern recognition, not immune responsiveness. Our results show that if there is little immunologically significant variability in immune responsiveness between males, female choice for traits correlated with individual immune components are unlikely to evolve.

Effect of female choice on immune responsiveness and immune recognition

Female choice for the different criteria gave rise to different values of CI, IN, and the 7 recognition values. Interestingly, choosing for CI, IN, or CI+IN resulted in significantly lower recognition values after 1800 generations than when choosing for fitness or survival (Fig. 4; Kruskal-Wallis, 378.3, $p<0.0001$, Dunn's multiple comparisons, $p<0.001$). Choosing for CI led to significantly lower levels of IN than when selecting for IN, fitness, or survival (Fig. 4; Kruskall-Wallis, 323.2, $p<0.0001$, Dunn's multiple comparisons, $p<0.001$). Choosing for IN led to significantly lower levels of CI than when selecting for IN, fitness, or survival (Fig. 4; Kruskall-Wallis, 362.4, $p<0.0001$, Dunn's multiple comparisons, $p<0.001$).

Limitations of the model

Our model has several limitations, but none of these are likely to alter our general conclusions. The model was biased in favour of selection for female choice. We used model values that favoured the development of female choice (e.g. high mutation rates). Our model was formulated to increase selection pressure for choice (e.g. survival was determined solely by disease resistance, and female choice determined female reproductive success because offspring inherited the male's immune system scores). Our assumptions were also biased towards selecting for female choice. For example, we assumed that reliable signs indicating the value of CI and IN exist in males. We ignored how such signs would evolve or be maintained. There is a large literature discussing the conditions necessary for the evolution of such indicator traits in males, whether they are ornaments or metabolic by-products (e.g. Kokko et al., 2003). Difficulties in maintaining a reliable detection system would only decrease the selection for female choice for this

trait. Therefore, our model should be conservative in its estimate of how often female choice will be lost in a population. Moreover, the model is robust. The results are qualitatively similar even if the parameters are changed. For example, we altered the pathogen generation time from 18 years to 14 and 8 years. Changing the cycle time alters the pattern of prevalence for every pathogen. Choice for constitutive immunity was still lost in more than 1/2 of the simulations at the two different generation times. In other words, our results are correct for a range of model values. The model does oversimplify both invertebrate and vertebrate immunity (e.g. see Natori, 1997; Lavine and Strand, 2002; Roitt et al., 2001). However, a more realistic model, with an increased number of interacting factors, immunological memory, etc., is unlikely to increase selection pressure for female choice for enhanced male immune responsiveness. We also neglect other complexities in the evolution of female choice for male immune responsiveness (e.g. choosing the most vigorous immune response may not be the best strategy; Wedekind, 1994a; females select males for other traits in addition to disease resistance; e.g. Blais et al., 2004) that would reduce the selection pressure for female choice for superior male immune responses.

In our model, choosiness for individual immune components spread throughout the population when the costs of choice and immunity were low. The costs of superior recognition abilities are probably not zero (Webster and Woolhouse, 1999), but they may be low (Wedekind, 1994b). Therefore, female choice for recognition may be easier to select for than female choice for immune responsiveness. There is empirical evidence that females can select mates on the basis of recognition factors like the major

histocompatibility complex (MHC) (e.g. Reusch et al., 2001); however the relationship between MHC factors and disease resistance is complex (Penn and Potts, 1999).

Female choice, immune responsiveness, and disease resistance

Most researchers studying female choice for male immune responsiveness assume that females are using this information to select disease resistant males (see Møller et al, 1999, but see Faivre et al., 2003; Saks et al., 2003). However, disease resistance may be difficult to assess, requiring information about both immune responsiveness (including that of local immunity) and pathogen recognition ability. Evolving the ability to assess (or signal) several immune features simultaneously may be rare. Moreover, given that some immune traits may be negatively correlated, it remains unclear how disease resistance could be determined (see also Schmid-Hempel, 2003; Adamo, 2004b). If determining disease resistance directly is not possible, females may have no choice but to base their decision about male disease resistance on the immune components that they can assess. In the case of immune responsiveness, this information may not provide them with a selective advantage unless they can predict the pathogens that will be important for their offspring. However, females may be able to assess disease resistance in males indirectly, without requiring that signals correlate with individual immune components. For example, choosing for survival was much more likely to evolve than female choice for individual, or even combined, components of immunity (Fig. 1). The best estimate of disease resistance may be simple survival (e.g. age) as opposed to the robustness of individual immune components.

There is substantial evidence that females choose males on the basis of traits that reflect health and vigour (reviewed by von Schantz et al., 1997; for Orthoptera: Scheuber et al., 2003). By selecting for current health, females may be able to find immunocompetent males even if there is little selection pressure for females to evolve the ability to assess male immune responsiveness *per se*. However, choosing for current health may not necessarily select for the most disease-resistant males for two reasons. First, unless an animal becomes ill, there may be no outward signs of an inferior immune system (e.g. Faivre et al., 2003). Unless challenged by pathogens and parasites, all males, including those with little disease resistance, may look the same. The second problem with selecting for health and vigour as a way of finding the most disease-resistant mate is that in an environment of fluctuating pathogen prevalence, current health may not be the best predictor of future disease resistance (Pomiankowski, 1987). Individuals resistant to some diseases can be susceptible to others (e.g. Adamo, 2004b). A resistant male may produce resistant offspring only if his offspring will be facing the same pathogens that he faced. If pathogen prevalence fluctuates rapidly, a male's current health may be a poor predictor of his offspring's future disease resistance.

Therefore, in some species, present health and vigour may not necessarily correlate with offspring disease resistance, and, in species that fit the assumptions of this model, immune responsiveness is not a good predictor of offspring disease resistance. If these results apply widely, why do females of many species choose traits that seem to correlate with one or both of these male attributes?

By choosing healthy, vigorous males, females could acquire other indirect benefits in addition to the possibility of disease-resistant offspring. For example, healthy,

vibrant males are likely to be superior in many ways, and some of these traits could be heritable (Getty, 2002). Testing whether females care about male immune abilities *per se* is necessary before concluding that studies demonstrating a correlation between a trait of general health and female choice is really female choice for disease resistance. The same difficulty exists in interpreting correlations between sexually selected traits and measures of immune responsiveness. Immune responsiveness probably positively correlates with a number of other physiological measures important for health, and it is itself affected by condition (e.g. Rantala et al., 2003; Westneat et al., 2003). Without direct manipulation of these positively correlated traits, it is difficult to determine to what extent each of them may be driving female choice (see Kokko et al., 2003).

Moreover, by choosing healthy, vigorous males, females probably also accrue direct benefits by avoiding infection from a sick mate (Able, 1996). In some species, secondary sexual traits appear to signal present health status as opposed to male immune responsiveness (Faivre et al. 2003, but see Masvaer et al., 2004). This direct benefit may be a more important pressure driving female choice for healthy males than the possible indirect benefits provided by selecting mates with enhanced immune responsiveness.

Acknowledgements

This work was supported by NSERC (Natural Science and Engineering Research Council of Canada) grants to S. Adamo and R. Spiteri.

References

Able D, 1996. The contagion indicator hypothesis for parasite-mediated sexual selection.

Proceedings of the National Academy of Sciences USA 93:2229-2233.

Adamo SA, 1998. The specificity of behavioral fever in the cricket *Acheta domesticus*. Journal of Parasitology 84:529-533.

Adamo SA, 2004a. Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket Gryllus texensis. Journal of Insect Physiology 50:209-216.

Adamo SA, 2004b. How should behavioural ecologists interpret measurments of immunity? Animal Behaviour 68:1443-1449.

Adamo SA, Robert D, Perez J, Hoy R, 1995. The response of an insect parasitoid, *Ormia ochracea* (Tachinidae), to the uncertainty of larval success during infestation. Behavioral Ecology and Sociobiology 36:111-118.

Ahmed A, Baggott S, Maingon R, Hurd H, 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos 97:371-377.

Anderson R, May R, 1981. The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society, Series B 291:451-524.

Andersson M, 1994. Sexual Selection. Princeton, New Jersey: Princeton University Press.

Beck C, Powell L, 2000. Evolution of female choice based on male age: are older males

better mates? Evolutionary Ecology Research 2:107-118.

Benz G, 1987. Environment. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 177-214.

Blais J, Rico C, Bernatchez L, 2004. Nonlinear effects of female mate choice in wild threespine sticklebacks. Evolution 58:2498-2510.

Boa-Amponsem K, Larsen C, Dunnington E, Siegel P, 1999. Immunocompetence and resistance to marble spleen disease of broiler- and layer-type pure lines of chickens. Avian Pathology 28:379-384.

Carruthers R, Ramos M, Larkin T, Hostetter D, Soper R, 1997. The *Entomophaga grylli* (Fresenius) Batko species complex: Its biology, ecology and use for the biological control of pest grasshoppers. Memoirs of the Entomological Society of Canada 171:329-353.

Carruthers R, Soper R, 1987. Fungal Diseases. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 357-416.

Evans MR, Entwistle P, 1987. Viral Diseases. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 257-322.

Faivre B, Pr´eault M, Salvadori F, Th´ery M, Gaillard M, C´ezilly F, 2003. Bill colour and immunocompetence in the European blackbird. Animal Behaviour 65:1125-1131.

Ferrandon D, Jung A, Criqui M, Lemaitre B, Uttenweiler-Joseph S, Michaut L, Reichhart

J, Hoffmann JA, 1998. A drosomycin-GFP reporter transgene reveals a local immune response in *Drosophilia* that is not dependent on the *Toll* pathway. EMBO Journal 17:1217-1227.

Franc N, White K, 2000. Innate recognition systems in insect immunity and development: new approaches in *Drosophila*. Microbes and Infection 2:243-250.

Freitak D, Ots I, Vanatoa A, Ho˜rak P, 2003. Immune response is energetically costly in white cabbagge butterfly pupae. Proceedings of the Royal Society of London B (Supplement) 270:S220-S222.

Fuxa JR, Tanada Y, 1987. Epizootiology. New York: Wiley and Sons.

Getty T, 2002. Signaling health versus parasites. American Naturalist 159:363-371.

Gibson R, Bachman G, 1992. The costs of female choice in a lekking bird. Behavioral Ecology 3:300-309.

Gillespie JP, Kanost MR, Trenczek T, 1997. Biological mediators of insect immunity. Annual Review of Entomology 42:611-643.

Gottar M, Gobert V, Michel T, Belvin M, Duyk G, Hoffmann JA, Ferrandon D, Royat J, 2002. The *Drosophila* immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature 416:640-644.

Gray D, 1999. Intrinsic factors affecting female choice in house crickets: time cost, female age, nutritional conditions, body size and size-relative reproductive investment. Journal of Insect Behavior 12:691-700.

Hamilton W, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites? Science 218:384-387.

Hoffmann JA, Reichhart J, Hetru C, 1996. Innate immunity in higher insects. Current Opinion in Immunology 8:8-13.

Holzer B, Jacot A, Brinkhof M, 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. Behavioral Ecology 14:353-359.

Höglund J, Alatalo R, 1995. Leks. Princeton, NJ: Princeton University Press.

Iwasa Y, Pomiankowski A, Nee S, 1991. The evolution of costly mate preferences II. The "handicap" principle. Evolution 45:1431-1442.

Jacot A, Scheuber H, Brinkhof M, 2004. Costs of an induced immune response on the sexual display and longevity in field crickets. Evolution 58:2280-2286.

Johnson D, Dolinski M, 1997. Nosematidae and other Protozoan agents for control of grasshoppers and locusts: Current status and prospects. Memoirs of the Entomological Society of Canada 171:375-389.

Kaya H, 1987. Diseases caused by nematodes. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 453-470.

Keil D, Luebke R, Pruett S, 2001. Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. Journal of Immunology 167:4543-4552.

Khush R, Leulier F, Lemaitre B, 2001. *Drosophila* immunity: two paths to NF-□B. Trends in Immunology 22:260-264.

Koella J, Boe¨te C, 2002. A genetic correlation between age at pupation and melanization immune response of the yellow fever mosquito *Aedes aegypti*. Evolution 56:1074-1079.

Kokko H, Brooks R, Jennions M, Morley J, 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society of London B 270:653-664.

Kokko H, Lindström J, 1996. Evolution of female preference for old mates. Proceedings of the Royal Society of London B 263:1533-1538.

Kraaijeveld A, Limentani E, Godfray H, 2001. Basis of the trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. Proceedings of the Royal Society of London B 268:259-261.

Krieg A, 1987. Diseases caused by bacteria and other prokaryotes. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 323-355.

Kurtz J, Sauer K, 1999. The immunocompetence handicap hypothesis: testing the genetic predictions. Proceedings of the Royal Society of London, B 266:2515-2522.

Lavine M, Strand M, 2002. Insect hemocytes and their role in immunity. Insect Biochemistry and Molecular Biology 32:1295-1309.

Lazzaro B, Sceurman B, Clark A, 2004. Genetic basis of natural variation in D. melanogaster antibacterial immunity. Science 303:1873-1876.

Luster M, Portier C, Pait D, Rosenthal G, Germolec D, Corsini E, Blaylock B, Pollock P, Kouchi Y, Craig W, White K, Munson A, Comment C, 1993. Risk assessment in immunotoxicology: II. Relationships between immune and host resistance tests. Fundamental and Applied Toxicology 21:71-82.

Maddox J, 1987. Protozoan diseases. In: Epizootiology of insect diseases (Fuxa JR, Tanada Y, eds). New York: Wiley and Sons; 417-452.

Mallon E, Loosli R, P S-H, 2003. Specific versus nonspecific immune defense in the bumblebee, *Bombus terrestris* L. Evolution 57:1444-1447.

Masvaer M, Liljedal S, Folstad I, 2004. Are secondary sex traits, parasites and immunity related to variation in primary sex traits in Arctic charr? Proceedings of the Royal Society of London B 271:S40-S42.

Moret Y, 2003. Explaining variable costs of the immune response: selection for specific versus non-specific immunity and faculative life history change. Oikos 102:213-216.

Møller AP, Christe P, Lux E, 1999. Parasitism, host immune function, and sexual selection. Quarterly Review of Biology 74:3-20.

Natori S, 1997. Relation between insect defense proteins and development of the flesh fly, *Sarcophaga peregrina*. In: Molecular mechanisms of immune responses in insects (Brey PT, Hultmark D, eds). London: Chapman and Hall; 245-260.

Penn DJ, Potts WK, 1999. The evolution of mating preferences and major

histocompatibility complex genes. American Naturalist 153:145-164.

Pinard-van der Laan MH, Siegel PB, Lamont SJ, 1998. Lessons from selection experiments on immune response in chicken. Poultry and Avian Biology Reviews 9:125-141.

Pomiankowski A, 1987. The costs of choice in sexual selection. Journal of Theoretical Biology 128:195-218.

Pomiankowski A, Iwasa Y, Nee S, 1991. The evolution of costly mate preferences 1. Fisher and biased mutation. Evolution 45:1422-1430.

Rantala MJ, Kortet R, Kotiaho J, Vainikka A, Suhonen J, 2003. Condition dependence of pheromones and immune function in the grain beetle *Tenebrio molitor*. Functional Ecology 17:534-540.

Reusch T, Haaberli M, Aeschlimann P, Milinski M, 2001. Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature 414:300-302.

Roitt I, Brostoff J, Male D, 2001. Immunology, Sixth ed. London: Mosby.

Rolff J, Siva-Jothy M, 2003. Invertebrate ecological immunology. Science 301:472-475.

Ryder JJ, Siva-Jothy MT, 2001. Quantitative genetics of immune function and body size in the house cricket, *Acheta domesticus*. Journal of Evolutionary Biology 14:646-653.

Saks L, Ots I, Horak P, 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. Oecologia 134:301-307.

Scheuber H, Jacot A, Brinkhof M, 2003. Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. Animal Behaviour 65:721-727.

Schmid-Hempel P, 2003. Variation in the immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London B 270:357-366.

Schmid-Hempel P, Ebert D, 2003. On the evolutionary ecology of specific immune defense. Trends in Ecology and Evolution 18:27-32.

Smith R, 1965. A field population of *Melanoplus sanguinipes* and its parasites. Canadian Journal of Zoology 43:179-201.

von Schantz T, Wittzell H, G¨oransson G, Grahn M, 1997. Mate choice, male condition-dependent ornamentation and MHC in the pheasant. Hereditas 127:133-140.

Webster JP, Woolhouse MEJ, 1999. Cost of resistance: relationship between reduced fertility and increased resistance in a snail-schistosome host-parasite system. Proceedings of the Royal Society of London B 266:391-396.

Wedekind C, 1994a. Mate choice and maternal selection for specific parasite resistances before, during and after fertilization. Philosophical Transactions of the Royal Society, Series B 346:303-311.

Wedekind C, 1994b. Handicaps not obligatory in sexual selection for resistance genes.

Journal of Theoretical Biology 170:57-62.

Westneat DF, Birkhead TR, 1998. Alternative hypotheses linking the immune system and mate choice for good genes. Proceedings of the Royal Society of London, B 265:1065-1073.

Westneat DF, Hasselquist D, Wingfield J, 2003. Tests of association between humoral immune response of red-winged blackbirds (Agelaius phoeniceus) and male plumage, testosterone, or reproductive success.

Zelazny B, Goettel M, Keller B, 1997. The potential of bacterial for the microbial control of grasshoppers and locusts. Memoirs of the Entomological Society of Canada 171:147-156.

Zuk M, Stoehr AM, 2002. Immune defense and host life history. American Naturalist 160:S9-S22.

Figure Legends

Figure 1. Percentage of simulated populations in which female choice is lost. For each of the five choice criteria, bars denote the percentage of simulations in which choice falls to 0% of the population.

Figure 2. Time required for female choice to fix to 100% or 0% in a simulated population (seed 1). A) Female choice for fitness. B) Female choice for CI (constitutive immunity). C) Female choice for IN (inducible immunity). D) Female choice for CI+IN. Choice for CI+IN fixes at 0 at by generation 550.

Figure 3. Pathogen pressure and mortality in a simulated population (seed 1). A) Pathogen pressure fluctuates over the18 year cycle. B) Mortality over the generations when selecting for fitness. C) Mortality over the generations when selecting for CI. D) Mortality over the generations when selecting for IN. Note that there is always a spike of mortality in the first generation as the most susceptible animals are lost. Pathogen pressure is pathogen virulence x pathogen prevalence.

Figure 4. The effect of different choice criteria on parameter values for CI, IN, and recognition. Bars denote the average parameter value. The error bars indicate the standard deviation.

Figure 5. The relative fitness of choosers vs. non-choosers. The solid line represents relative fitness. When the value is above 0, choosers have a fitness advantage over non-

choosers. The dashed line represents pathogen pressure. The identity of the pathogen making the largest contribution to pathogen pressure changes over time. A) Female choice for male fitness. Choice for fitness fixes to 100% by generation 13 and therefore only the first 12 generations are shown. B) Female choice for constitutive immunity (CI). C) Female choice for inducible immunity (IN). Pathogen pressure is pathogen virulence x pathogen prevalence.

Figure 6. Percentage of simulated populations that lose female choice when the cost of immunity is reduced. For each of the five choice criteria, the bars denote the percentage of simulated populations that have lost female choice. (0) indicates that no populations lost female choice.

Table 1. Values used to model the effect of 7 different pathogens

Pathogen	Virulence*	Maximum Prevalence**	w1[13,14]	w2[13,15]
Virus				
a) Cricket Paralytic Virus[1]	0.80	0.55	0.95	0.05
Bacteria				
a) *Serratia marcescens*[2,3,4]	0.90	0.02	0.05	0.95
b) *Ricketsiella grylli*[4, 5]	0.80	0.15	0.05	0.95
Fungi				
Entomophaga grylli[6,7]	0.98	0.40	0.05	0.95
Protozoan				
Nosema locustae[8,9,12]	0.90	0.38	0.95	0.05
Metazoan				
a) *Mermithidae*[10,12]	0.98	0.21	0.95	0.05
b) *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 and May, 1981 (Table 6); Carruthers et al., 1997).

[1]. Evans and 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 and

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 defence against each pathogen. Although constitutive immunity is important against bacteria and fungi (e.g. 13), studies have shown that without inducible immunity insects die from these pathogens (e.g. Gottar et al, 2002) and this explains our weighting. We ran preliminary simulations with CI weighted 0.45 and IN 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) in the defence against each pathogen. The role of inducible immunity in the defence against some pathogens is still under study, and, therefore, instead of 0 we assigned a small value to w2 for these pathogens.

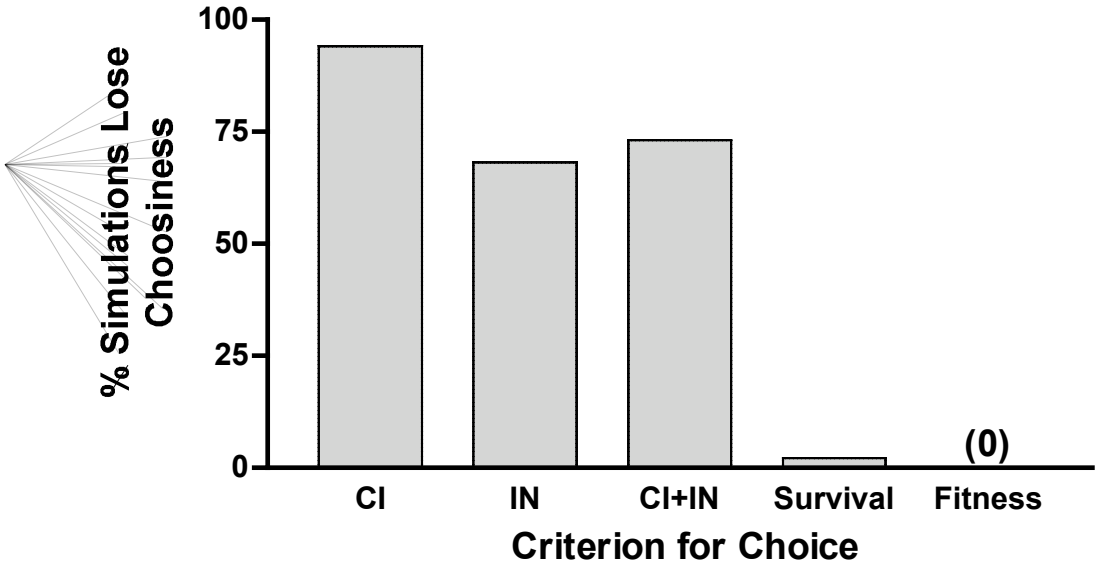
% Simulations Lose Choosiness
100
75
50
25
0
(0)
CI
IN
CI+IN
Survival
Fitness
Criterion for Choice

Figure 1

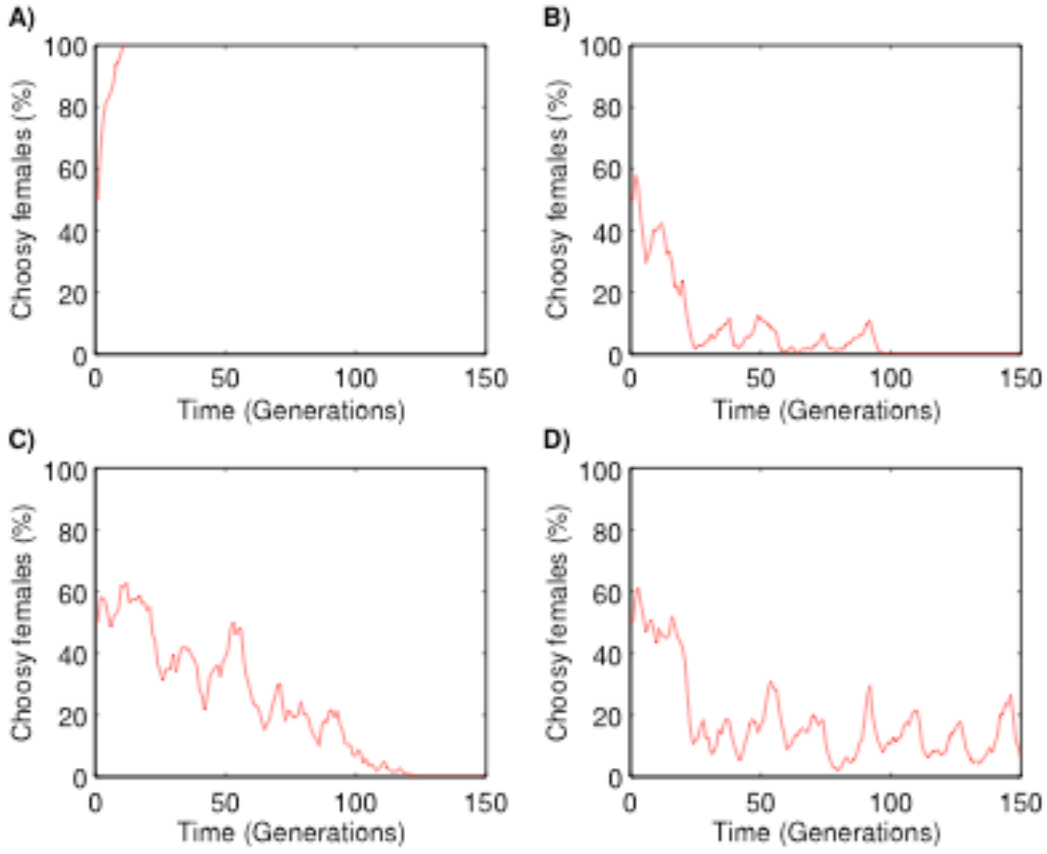
A)
B)
C)
D)
Choosy females (%)
Time (Generations)
0
20
40
60
80
100
0
50
100
150

Figure 2

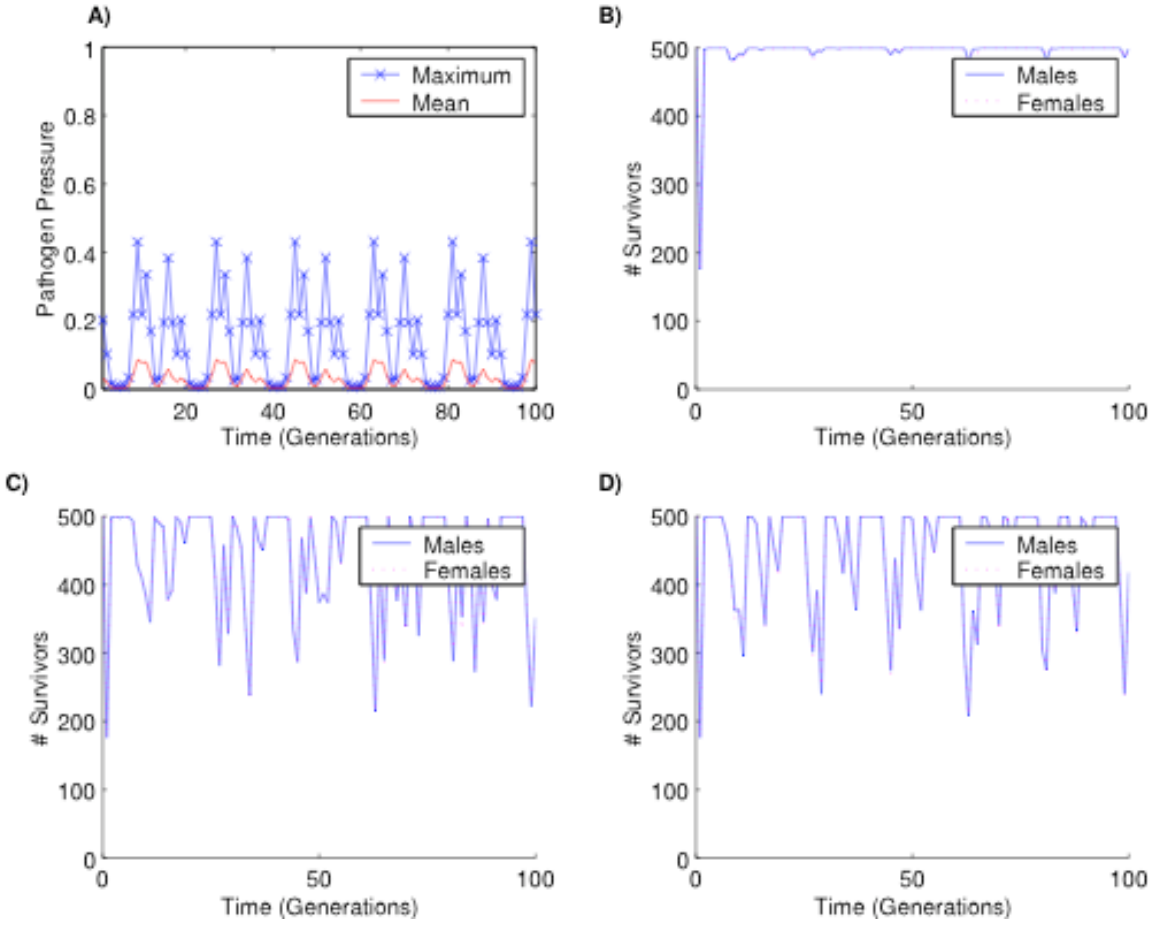
A)
Maximum
Mean
Pathogen Pressure
Time (Generations)
B)
Males
Females
Survivors
Time (Generations)
C)
Males
Females
Survivors
Time (Generations)
D)
Males
Females
Survivors
Time (Generations)

Figure 3

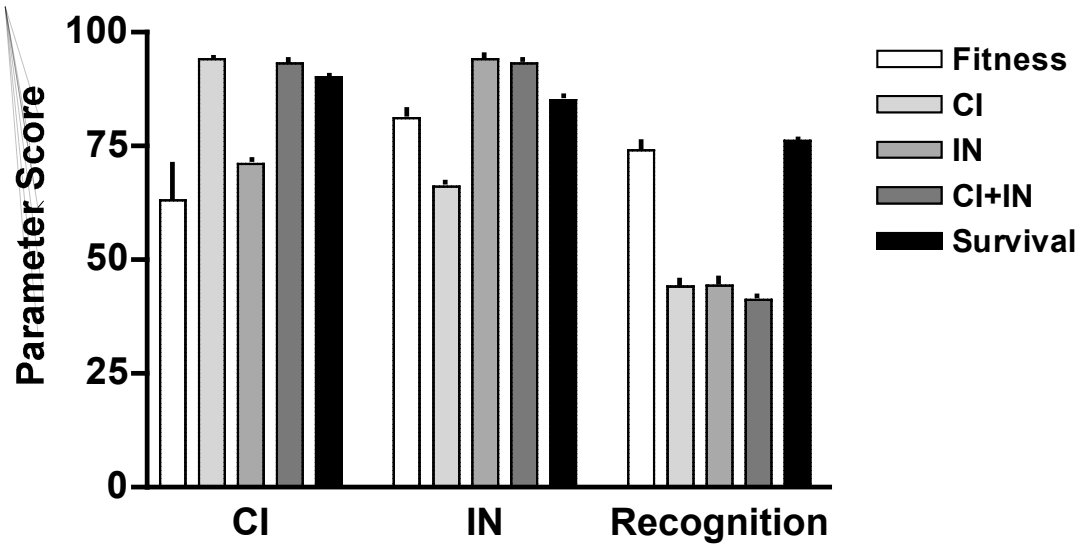
Parameter Score
100
75
50
25
0
CI
IN
Recognition
Fitness
CI
IN
CI+IN
Survival

Figure 4

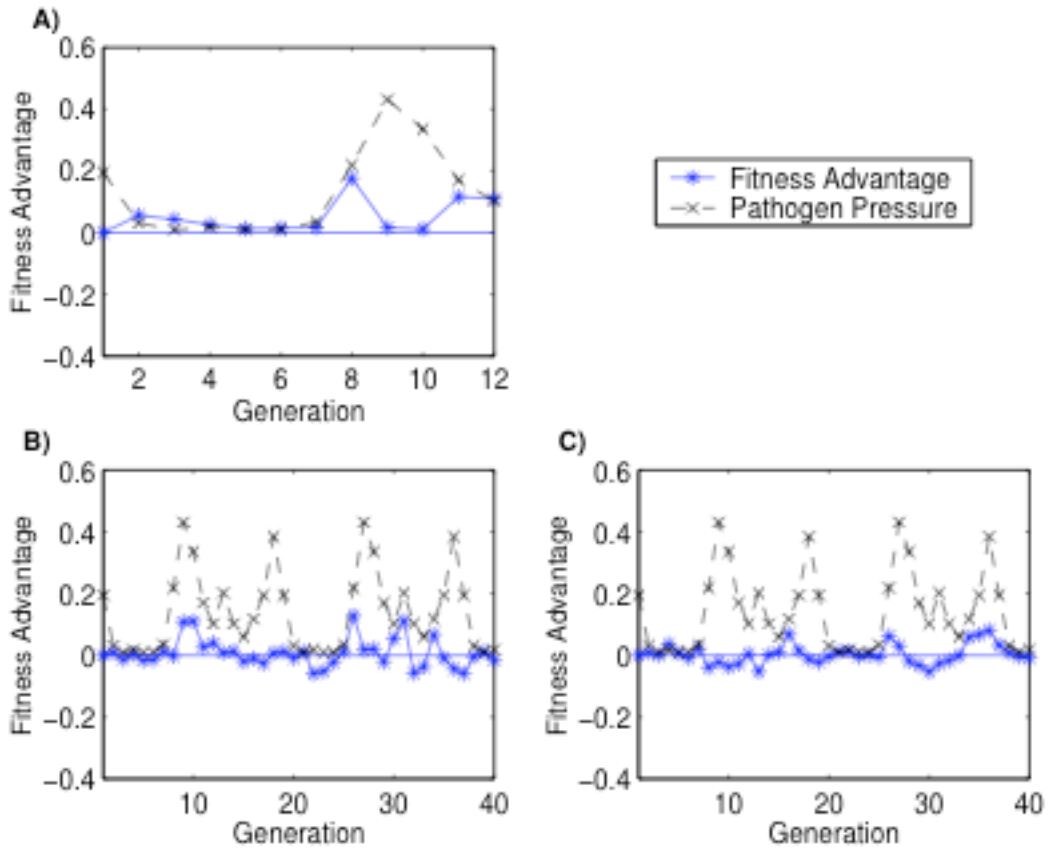
A)
B)
C)
Fitness Advantage
Pathogen Pressure
Fitness Advantage
Generation
0.6
0.4
0.2
0
−0.2
−0.4
2
4
6
8
10
12
10
20
30
40

Figure 5

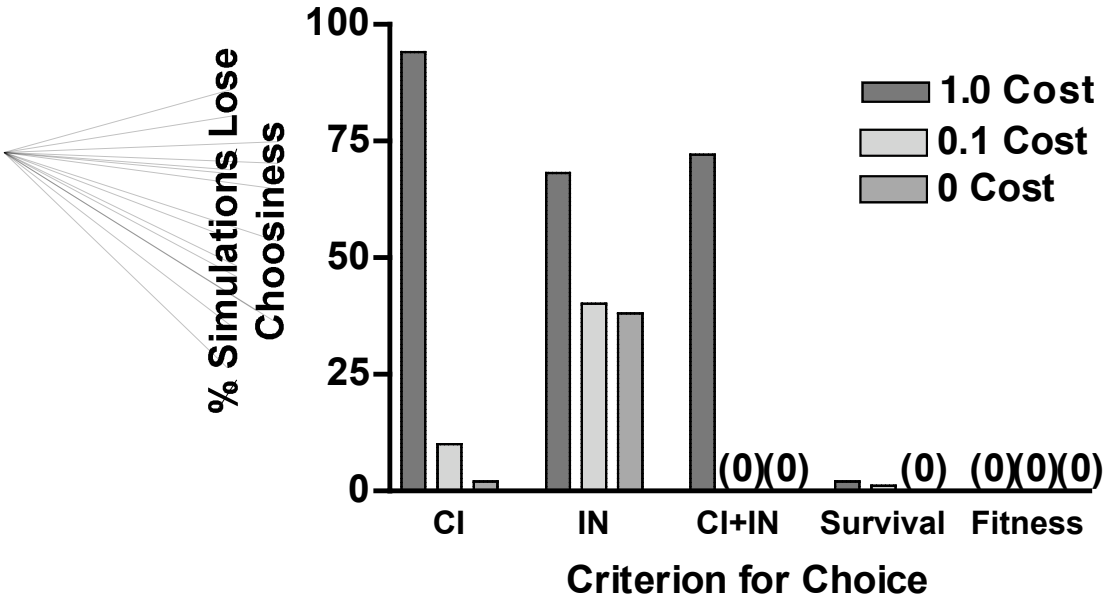
100
75
50
25
0
% Simulations Lose Choosiness
1.0 Cost
0.1 Cost
0 Cost
(0)(0)
(0)
(0)(0)(0)
CI
IN
CI+IN
Survival
Fitness
Criterion for Choice

Fig. 6

1

2

3