



Immune Response to Parasitic Attack: Evolution of a Pulsed Character

STEVEN A. FRANK*

*Department of Ecology and Evolutionary Biology, University of California, Irvine,
CA 92697-2525, U.S.A.*

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Pulsed characters are temporary biochemical, cellular, or structural changes produced in response to environmental or biotic challenge. For example, response to parasitic attack develops as a pulse of defensive chemicals or cells that typically decay after the parasite has been controlled. Almost all theories for the genetic variability of characters assume measurements on static characters. This paper presents theoretical tools to examine optimal control variables for pulsed characters and the expected level of genetic variability in those control variables. The example of host immune response to parasitic attack is used to develop the theory.

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Introduction

Many characters form pulsed responses to environmental or biotic challenge. For example, individuals frequently express potent immune factors at a low level until faced with a direct challenge. Parasitic attack starts a dynamic cascade of recognition and amplification.

Four challenges arise when thinking about how natural selection shapes a pulsed, dynamically regulated response. First, the functional character is a time-varying rise and fall of a factor, such as killer cells that can fight an infection. The dynamical path of killer cell abundance is shaped by the benefits of defense and the costs of host resources or collateral host tissue damage. Dynamical paths form a more complex notion of a character than the usual

static measurements that provide the basis for most theories of quantitative characters.

Second, component parts of the overall response have their own dynamics and can be measured as separate characters. For example, immune cells may be stimulated to divide by an increase in the concentration of a molecule secreted by other cells that recognize parasitic invaders.

Third, the dynamical path that maximizes fitness arises from an optimal set of control variables that govern the dynamical response of each system component. Technically, this means that mathematical analysis requires dynamic optimization. Certain types of dynamic optimization have been applied to problems in ecology and behavior (Clark & Mangel, 2000), but, in general, analysis of quantitative characters has focused on static measures.

Fourth, optimization shows the direction of natural selection on the value of the variables that govern dynamical response. The next step is to analyse the strength of selection on each

*Tel.: +1-949-824-2244; fax: +1-949-824-2181.
E-mail address: safrank@uci.edu (S. A. Frank).

component in order to understand which characteristics of populations are likely to be variable and which are likely to be nearly constant.

An evolutionary framework for dynamic response characters can aid conceptual understanding and the development of testable predictions about genetic variability. As a first step, I formulate a dynamical model for a simple immune response against parasitic attack. I study how natural selection shapes the variables that control the dynamics of the immune response. In particular, I compare optimal control variables under changing assumptions, and I measure the strength of natural selection on each variable. The strength of selection provides an indication of the genetic variability in control variables and in response components that would be maintained in natural populations under a balance between mutation and selection.

I have chosen the immune response as my example of a pulsed, dynamically regulated character. Many other responses have properties of dynamic regulation, including aspects of development (Wearing *et al.*, 2000), wound healing (Wearing & Sherratt, 2000), and biochemical responses such as the regulation of PDE5 in muscle cells (Rybalkin *et al.*, 2002). The determination of sex in *Caenorhabditis elegans* follows from several dynamically regulated components (Goodwin & Ellis, 2002), as does apoptosis (Krakauer & Payne, 1997; Martinou, 1999; Shimizu *et al.*, 1999).

The main contribution of this paper is to call attention to dynamically regulated or pulsed characters as an important topic for quantitative genetic and evolutionary study. To provide some insight into the nature of these characters and how they can be analysed, I introduce a simple model.

Empirical Background

Preliminary data show widespread genetic variability in several components of the vertebrate immune response (Frank, 2002). In pigs, polygenic control has been observed for several traits including: antibody response, with an important contribution of non-MHC loci; proliferative and cytokine responses of mononuclear blood lymphocytes, such as T cells, B cells, and

natural killer (NK) cells; T cell mediated inflammatory response to innocuous antigens (delayed-type hypersensitivity); and the total number and relative proportions of the various kinds of blood-borne immune cells (Edfors-Lilja *et al.*, 1998). High heritabilities have been estimated for several of these traits.

Linkage studies of mice have begun to map locations of genes that influence quantitative variability in components of immunity (Puel *et al.*, 1995; Wu *et al.*, 1996). Many studies of humans report nucleotide polymorphisms in promoters of cytokines and other immune regulatory loci (Daser *et al.*, 1996; Mitchison, 1997; Cowell *et al.*, 1998; Mitchison *et al.*, 2000; Terry *et al.*, 2000; Smirnova *et al.*, 2001).

Genomic analysis of SNPs and other markers for QTLs will bring new data measuring genetic components of complex, polygenic traits (Wright *et al.*, 1999). Biomedical research will likely pay particular attention to genetic variability in the immune response and in the response to drugs that alter dynamical aspects of the immune response. To be useful for understanding evolutionary process, those techniques must be informed by a clear theoretical framework for dynamically regulated characters.

The Model

The model has three response variables that change with time: X is the concentration of a molecule that increases directly in response to parasitic attack, I is the concentration of immune cells that can kill the parasite, and P is the concentration of parasites within the host. Cells that act as sentinels directly recognize the parasite—those recognition sentinels secrete X in response to attack. X stimulates division of immune cells, I , and I kills P . In particular,

$$\frac{dX}{dt} = a + (b + d)X \left(\frac{P}{k + P} \right) - dX - bKX^2, \quad (1a)$$

$$\frac{dI}{dt} = \alpha + (\beta + \delta)I \left(\frac{X}{s + X} \right) - \delta I - \beta KI^2, \quad (1b)$$

$$\frac{dP}{dt} = P \left(r - m \left(\frac{I}{v + I} \right) - rKP \right). \quad (1c)$$

The four terms on the right side of dX/dt can be read as follows: a is the constitutive production of X ; maximal stimulation by parasites increases X at a rate $(b + d) - d = b$, with parasite concentration k defining one-half maximal stimulation; X decays at rate d ; and the final term with K provides an upper bound on the concentration of X . The terms for dI/dt have similar structure, but I is stimulated by X rather than P . For dP/dt , parasites increase at their intrinsic rate of increase, r , minus the mortality, m , caused by the killing immune cells, I . Parasite concentration is controlled to a maximum carrying capacity of $1/K$. Concentration units are the number of parasites, cells, or molecules per ml. Time is measured in days.

DYNAMICS AND FITNESS

Dynamics begin at time zero with X and I equilibrated in the absence of parasites, $P = 0$. The equilibrium resting values of X and I are approximately of order $\tilde{X} = a/d$ and $\tilde{I} = \alpha/\delta$, given the bounds on values described below. At $t = 0$, the system is initialized with $P = 1$ parasites. The dynamics end at $T = 50$ days.

Fitness depends on three factors

$$w = (1 - c\tilde{I}) \left(1 - \sigma \int_0^T (I - \tilde{I}) dt \right) \left(1 - \gamma \int_0^T P dt \right). \quad (2)$$

The first term decrements fitness by the standing density of immune cells, \tilde{I} , weighted by the cost for baseline immunity, c . The second term lowers fitness by the total increase in immune cells, $I - \tilde{I}$, in response to parasitic attack—this measures costs in terms of resources and collateral tissue damage caused by the spike in immunity. The factor σ weights the immune response costs. The third term measures damage caused by parasites as the total density of parasitemia, weighted by γ .

I follow convention and use the word “fitness” for w , but “performance” is more accurate for this model for two reasons. First, w contributes only a component to overall fitness. This is important because I will later analyse how much change in the control variables must be made to reduce w by 10%. That would be a large change

in total fitness but perhaps a relatively small change in the performance of a component of fitness. Changes in scale for w do not affect the results, thus one only needs to keep in mind that a particular percentage change in w maps to some smaller percentage change in total fitness.

The second important aspect of w is that it may take on negative values. Optimization simply finds the highest value of w , so negative scores do not necessarily influence the outcome. However, the fact that one or more of the three separate components of eqn (2) may be negative distorts the shape of the fitness surface. The optimization procedure is sensitive to such distortions. To simplify the optimization search, if at least one component was negative, I set fitness to the largest (least negative) of the negative components. This approach provides a reasonable interpretation of biological components of fitness, in which severe danger in one component creates mortality risks that override other components of fitness.

RESPONSE VARIABLES, CONTROL VARIABLES, AND PARAMETERS

Table 1 lists the definitions for all values in eqns (1) and (2). It is important to distinguish among the response variables, control variables, and parameters.

The dynamically changing components of immunity and infection, X , I , and P , form the response variables. The host controls its immune response through six control variables, a , b , d , α , β , and δ . I separate out these particular control variables from other values that influence dynamics because, in this model, these control variables evolve in response to the conditions set by the extrinsically fixed constraints and parameters.

The parameters for the dynamics in eqns (1) are k , K , s , r , m , and v . In addition, all control variables are constrained to be positive and I impose an upper limit of 2.5 on b , d , β , and δ . This upper bound sets the maximum doubling rate and minimum half-life of immune signals and cells as $\ln(2)/2.5 = 0.28$ days or about 6.7 hr. This matches the roughly 3–4 doublings per day that appear to be the upper limit on cell division rates.

TABLE 1
Variables and parameters of the model

Symbol	Description
X	Concentration of molecular signal of parasitic attack
I	Concentration of immune cells
P	Concentration of parasites in the host
a	Constitutive production rate of X
b	Maximal production rate of X in response to parasites
d	Decay rate of X
α	Constitutive production rate of I
β	Maximal production rate of I in response to X
δ	Decay rate of I
r	Intrinsic rate of increase of parasites
m	Maximum mortality rate of parasites caused by immune cells
$1/K$	Upper bound on parasite concentration, bound limiting X and I
k	Value of P at which X achieves one-half maximal rate of increase
s	Value of X at which I achieves one-half maximal rate of increase
v	Value of I at which P suffers one-half maximal rate of mortality
c	Fitness cost for baseline level of immune cells, I
σ	Fitness cost for immune cell concentration above baseline level
γ	Fitness cost imposed by parasites over total parasitemia
T	End time for measuring immune-parasite dynamics
P_0	Initial concentration of parasites at time zero

The parameters that control fitness also set key conditions that influence the evolution of the control variables. These fitness parameters are T , c , σ , and γ .

EXPERIMENTAL DESIGN

In each computer run, I set all of the parameters and searched for the optimal control variables that maximize fitness. The next section describes the search method for optimization.

I fixed seven of the parameters as constants in all runs: the initial concentration of parasites at time zero, $P_0 = 1$; the time period over which to study the dynamics of infection and measure fitness, $T = 50$; the upper bound on parasite concentration and a bound limiting host immune factor concentrations, $1/K = 10^8$; the intrinsic rate of parasite increase, $r = 2.5$; maximal parasite mortality imposed by host immunity, $m = 5$; the concentration of parasites at which X achieves one-half of its maximal rate of increase, $k = 10^4$; and the concentration of X at which immune cells, I , achieve one-half of their maximal rate of increase, $s = 10^4$.

I varied four parameters in a factorial design with $3 \times 5^3 = 375$ combinations, each combination repeated three times for a total of 1125 runs.

The parameter v is the concentration of immune cells at which parasite mortality is one-half of its maximum. I varied v over three levels such that $\log_{10}(v)$ ranged from 4 to 6 in steps of one. The parameter c is the fitness cost for the resting level of immune cells. I varied c over five levels such that $\log_{10}(c)$ ranged from 2 to 4 in steps of one-half. The parameter σ is the fitness cost for concentration of immune cells above the normal resting level, in other words, the host damage caused by the immune response. I varied σ over five levels such that $\log_{10}(\sigma)$ ranged from 8 to 10 in steps of one-half. The parameter γ is the fitness cost imposed by the parasites. I varied γ over five levels such that $\log_{10}(\gamma)$ ranged from 7 to 9 in steps of one-half.

METHOD FOR DYNAMIC OPTIMIZATION

Many numerical methods search for optimal control variables of a dynamic process (Newhauser *et al.*, 1989; Horst & Pardalos, 1995). I chose the differential evolution (DE) optimization algorithm (Storn & Price, 1997). This heuristic search technique provides a generic, reasonably fast, and reasonably robust approach. Briefly, this method encodes vectors of candidate control variables into linear

“chromosomes”. A population of candidate chromosomes competes following a simple, discrete-time life cycle with measurement of fitness by eqn (2) for each chromosome, mating and recombination between chromosomes, and mutation to perturb the population.

The life cycle and genetics of DE are designed to give good results in searching a complex response surface for an optimum value rather than to mimic biologically realistic genetics. The idea is simply that evolution by natural selection is a useful method to search for good solutions, the trick being to formulate the evolutionary process to enhance the speed and probability of convergence to the best outcome.

STRENGTH OF SELECTION ON CONTROL VARIABLES

Optimization finds values of the control variables that maximize fitness. An optimum, by itself, does not give any information about the strength of natural selection to maintain the optimum against the inevitable influx of mutations and other perturbations.

I measured the strength of selection on each control variable. For each variable, I first set all the control variables to their optimum values. I then changed the target variable until I found values above and below the optimum that caused a 10% decline in fitness. The distance from the optimum to the values that reduce fitness by 10% provides a measure of the strength of natural selection. Short distances imply strong selection and long distances imply weak selection. For b , d , β , and δ , upper values above the limit of 2.5 were set to 2.5.

I did not calculate the strength of selection when optimum fitness was negative because the calculation of fitness differed for negative values, changing the shape of the fitness surface. That change makes it difficult to compare the strength of selection between cases with positive and negative optimum fitness.

Results

DYNAMICS OF INFECTION AND IMMUNITY

Figure 1 shows the dynamics of the three response variables over the course of an infection. The top panel illustrates the outcome for

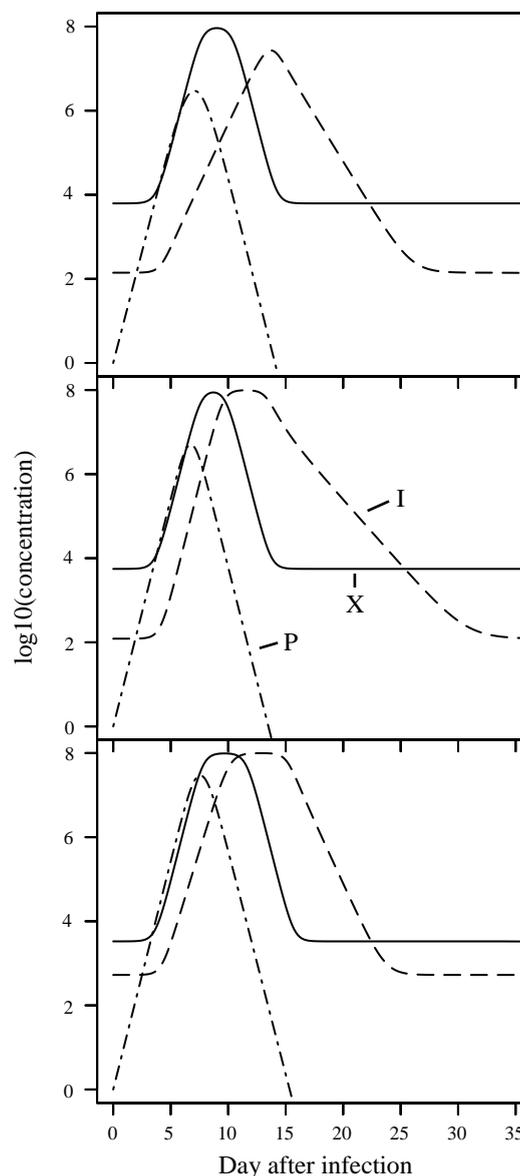


FIG. 1. Dynamics of infection and host response. The top panel shows the smallest parameter values used in the computer runs, $v = 10^4$, $c = 10^{-4}$, $\sigma = 10^{-10}$, and $\gamma = 10^{-9}$. The middle panel uses parameters in the middle of the ranges, $v = 10^5$, $c = 10^{-3}$, $\sigma = 10^{-9}$, and $\gamma = 10^{-8}$. The bottom panel uses the highest values, $v = 10^6$, $c = 10^{-2}$, $\sigma = 10^{-8}$, and $\gamma = 10^{-7}$.

the smallest parameter values used in the computer analyses, the middle panel has parameter values from the middle of the range, and the lower panel has the largest parameters used (see figure legend).

The basic shape of the dynamics does not change much in spite of the different weightings for costs and benefits (c , σ , and γ) and the

changing threshold, v , for stimulation of immune cells, I . Rather, the parasite rate of increase, r , and the constraint on maximum rates for the growth and decay of host responses, b , d , β , and δ , set the basic shape of the dynamics. Within this basic shape, the various control parameters do evolve in response to changing extrinsic parameters, as discussed below.

To the eye, the shape of the dynamics varies only in small ways when plotted on a logarithmic scale. However, peak concentrations of the parasite vary by an order of magnitude in Fig. 1. The date of clearance, when the parasite concentration drops below one, varies from about day 13 to day 16. To an infected organism, these visually small differences may have a significant impact on the consequences of infectious disease.

EVOLUTION OF CONTROL VARIABLES: INTRODUCTION

This section provides a sample of the results, which may be sufficient for readers wishing only a qualitative overview of the analysis. The following section presents detailed results for the evolution of each control variable.

Two issues must be considered in studying the evolution of the variables that control the immune response. First, what are the optimum values? Second, what is the shape of the fitness surface? The optimum sets the idealized target toward which selection will push the population. The shape of the fitness surface determines how much variation will be maintained for each control variable caused by mutation, drift, and other perturbations to the population.

The control variables b and d are favored to increase to their upper bound. These variables control the rates of increase and decay of X , the signal that stimulates the division of immune cells. Not surprisingly, selection favors a rapid increase in the signal that indicates parasitic attack and a rapid decay in the signal as the parasites are cleared. Interestingly, the fitness surface tends to be rather flat for many parameter combinations, suggesting that large deviations in these control variables may not affect performance significantly and that these variables are likely to be highly variable in populations.

The control variable α determines the constitutive production rate of immune cells, influencing the baseline level of immunity in the absence of parasites. Selection of α depends strongly on the fitness scaling factor, σ , which sets the cost for a spike in immunity. High σ penalizes an immune spike, favoring a higher baseline level of immunity and causing more intense selection on α and lower population variability.

The control variable β sets the maximal rate at which immune cells can increase in abundance. Higher σ causes a large decline in β because of the higher penalty on spikes in immunity. The parameter v sets the immune cell concentration required to control parasites. Lower v causes a decline in β because immune cells do not have to build up to high levels to control the parasite. Lower v also weakens selection on β , admitting greater population variability in β .

EVOLUTION OF CONTROL VARIABLES: DETAILS

This section presents a series of plots that show the optimum value for each control variable for different parameter combinations. I show the parameter combinations that explain the most variation for each control variable. Each of the control variable figures also illustrates the strength of selection as $\pm 10\%$ in fitness for perturbation of that control variable (see above).

Before turning to the six control variables, I show the distribution of optimum fitness values in Fig. 2. This figure helps to interpret the following plots because I do not calculate the strength of selection for cases in which fitness is negative, as explained earlier. In particular, some of the plots illustrating the strength of selection look a bit odd for those parameters that sometimes yield negative fitness, because only the subset with positive fitness is used. In Fig. 2, a decline in fitness occurs as v rises, where v is the threshold of immune cells needed to kill at one-half the maximum rate. Higher costs for immune cell and parasite concentrations, σ and γ , also decrease fitness.

Figure 3 shows the optimum values and 10% perturbation values for a , the constitutive production level for the host's signal of parasite attack, X . This control variable influences the

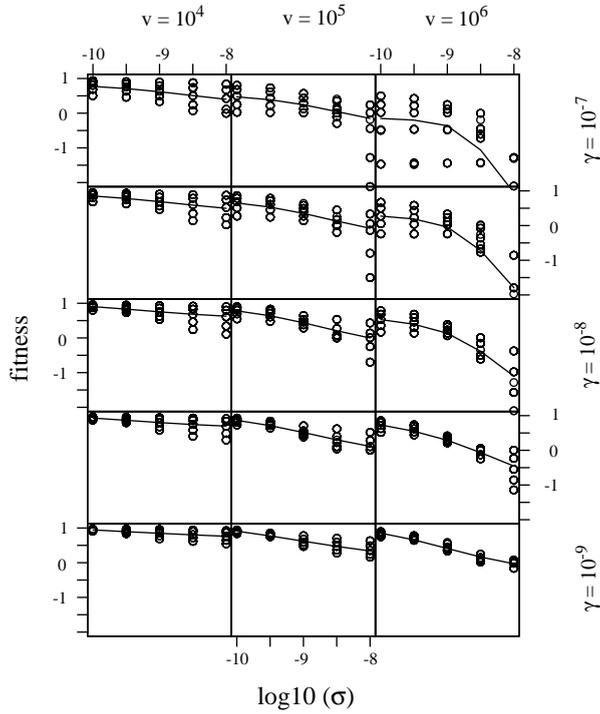


FIG. 2. Optimal fitness for various parameter combinations. For parameters yielding negative fitness, I did not calculate perturbations of control variables to obtain a 10% reduction in fitness.

resting level of X , which determines the speed of the host's response to attack. The optimum values of a do not vary greatly. An increase of two orders of magnitude in σ , the intensity of selection on the spike in immune cells, I , during an immune response, typically causes a decline of less than one order of magnitude in the optimal value of a . The lower a value reduces the total intensity of the immune response, for example, the lower peak level of I in the top vs. the bottom panel of Fig. 1. The strength of selection increases for higher cost of parasite load, γ , probably because high γ requires greater speed and intensity of the immune response, which benefits from a higher starting point in X . A similar argument applies to stronger selection at higher values of v , the level of immunity required to achieve one-half the maximal rate of parasite killing.

Figure 4 shows the optimum values and 10% perturbation values for b , the maximal production rate of X in response to parasites. For the parameters analysed, b is almost always at its upper bound of 2.5. The strength of selection on this control variable declines as each of the three

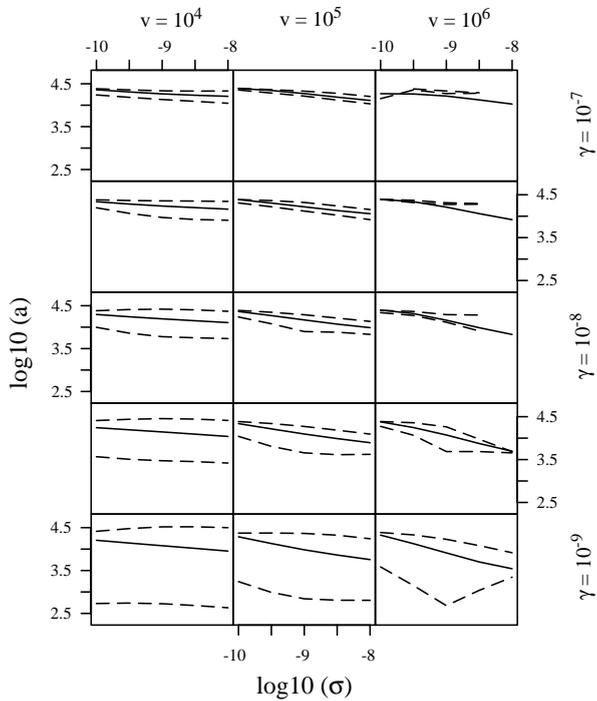


FIG. 3. Optimum values for the control variable a for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

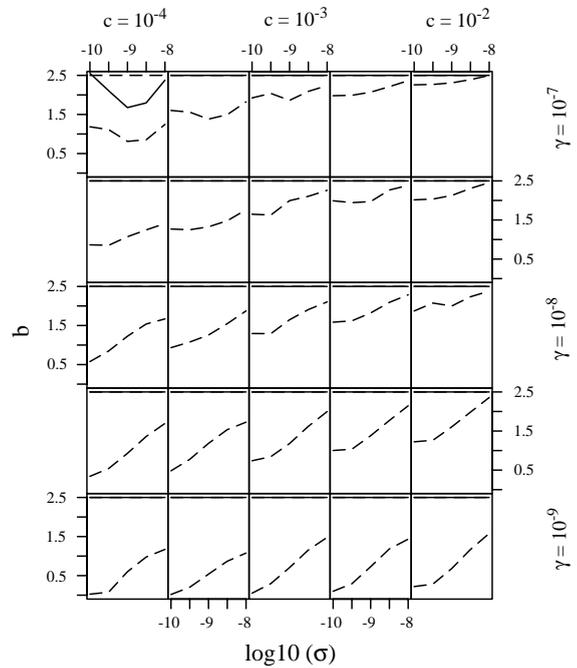


FIG. 4. Optimum values for the control variable b for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

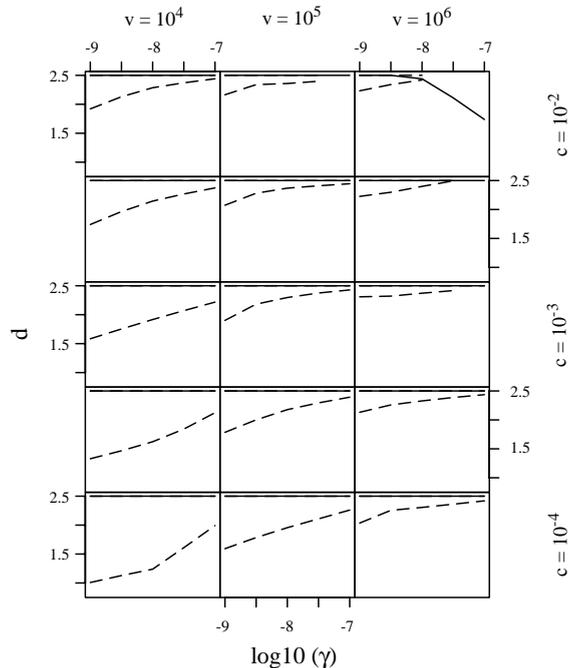


FIG. 5. Optimum values for the control variable d for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

fitness costs, σ , c , and γ , decreases. With weaker cost constraints, this control factor may be highly variable in populations.

Figure 5 shows the optimum values and 10% perturbation values for d , the decay rate of X . This decay rate is particularly important for the speed at which an immune response is shut down after the parasite has been controlled. For the parameters analysed, the optimal value of d is almost always at its upper bound of 2.5. For the strength of selection, a rise in γ imposes stronger cost for parasite burden, which causes a more rapid and intense immune response, for example by higher values of β (see below). The more intense response requires more rapid scaling back of the response as the parasite becomes controlled, imposing stronger pressure on keeping the value of d high. A decline in c lowers the cost for maintaining a high resting level of immunity, so lower values of d can be tolerated because resting immunity rises as d declines. Higher values of v force the intensity of the immune response to higher levels to control parasites, and thus require more rapid decay after clearing the parasites.

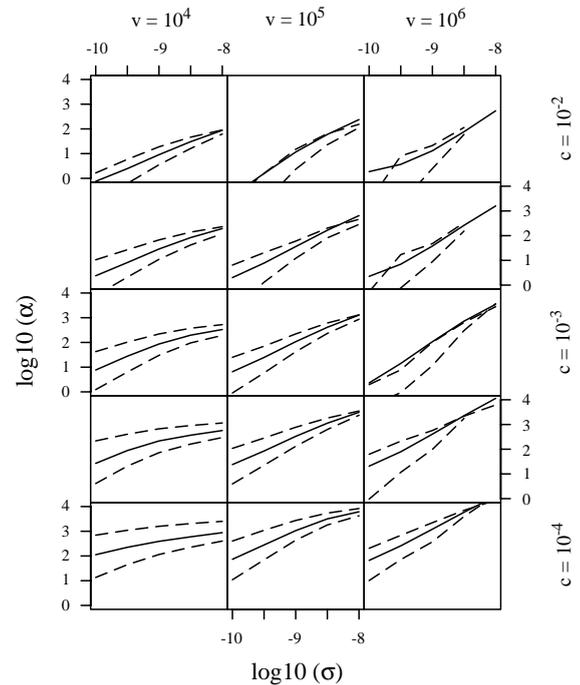


FIG. 6. Optimum values for the control variable α for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

Figure 6 shows the optimum values and 10% perturbation values for α , the constitutive production rate of I . A rise in σ penalizes more heavily the spike in immunity in response to parasites. With higher σ , the optimal value of α rises to give a higher baseline level of immunity that is coupled with slower buildup upon infection (see next paragraph). A rise in c penalizes more heavily the baseline level of immunity, lowering α .

Figure 7 shows the optimum values and 10% perturbation values for β , the maximal production rate of I in response to X . As noted in the last paragraph, a rise in σ penalizes more heavily the spike in immunity in response to parasites. Thus, high σ favors lower β . Higher γ imposes greater cost for parasite load, thus favoring a more rapid response and higher β . Greater v requires higher immune cell concentrations to control parasites, causing a more rapid immune buildup. Note that at the highest v values, fitness is often negative, explaining the anomalous perturbation curves (see above). Lower v levels weaken selection and allow greater variability in β because high values of v

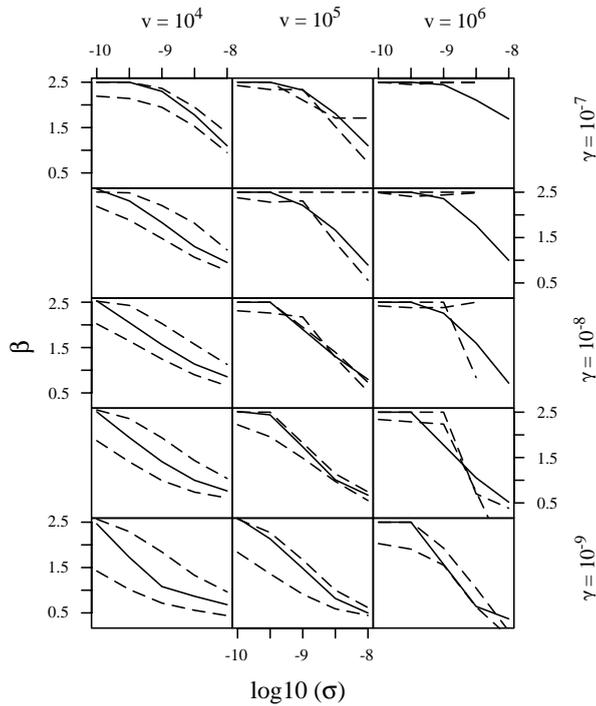


FIG. 7. Optimum values for the control variable β for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

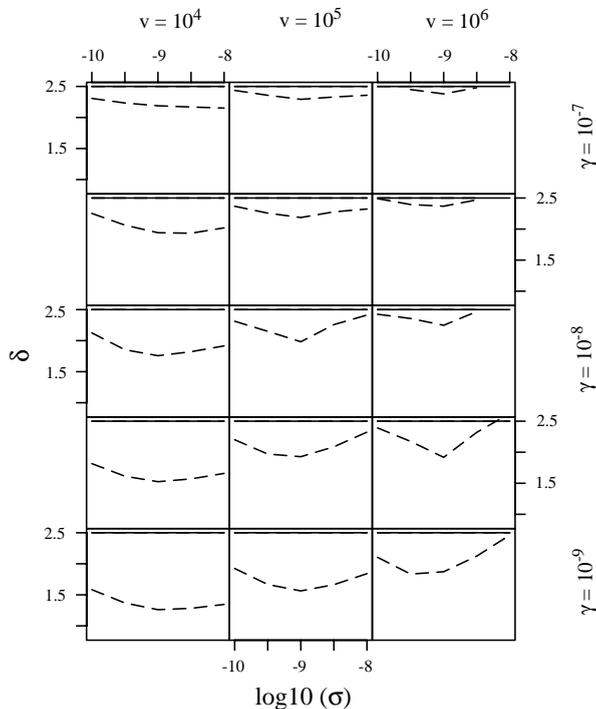


FIG. 8. Optimum values for the control variable δ for different combinations of parameters (solid line) and values that reduce fitness by 10% (dashed lines).

require a fast response to avoid being overwhelmed by the parasites.

Figure 8 shows the optimum values and 10% perturbation values for δ , the decay rate of I . The strong pressure to reduce immunity after clearing the parasites favors a maximal value of 2.5 for all parameter combinations. The strength of selection increases for higher cost of parasite load, γ , probably because high γ demands greater speed and intensity of the immune response, which then requires faster reduction of immunity after clearing the parasites. A similar argument applies to stronger selection at higher values of v , the level of immunity required to achieve one-half the maximal rate of parasite killing.

Discussion

This paper calls attention to pulsed characters and provides an example of how to study natural selection and quantitative variability of such characters. For some parameters, selection was relatively weak for particular components and one would expect rather wide variability in those components. I used the criteria of a 10% reduction in performance to estimate the strength of selection.

In an organism faced with many challenges, the performance of a single character forms only a single component of fitness. Thus, a 10% reduction in performance for a character may translate into a much smaller effect on lifetime fitness. If so, then rather wide variability may be maintained in the individual components of the pulsed character. The more components involved in the response, the more likely that some individuals will carry deviants in two or more components and have a significantly compromised response.

There are not enough data presently available to evaluate this sort of model in detail. At this stage, the theoretical model serves to clarify future research goals and to encourage the search for appropriate empirical models for further study. The theory suggests the following research agenda: find a regulatory network that controls an important trait; understand how perturbations to different components of that network affect performance; map performance to fitness; and then compare the theoretical

selective intensity on individual components with the observed level of variability in those components. Or, in reverse, look at observed levels of variability in components, and predict the sensitivity of perturbations for those components on performance. Laboratory populations of bacteria may be good candidates for initial study.

The theoretical model provides an essential set of hypotheses about the mechanistic interactions between components and the consequences of those interactions for system performance. Without a mechanistic hypothesis, one cannot predict which components are expected to vary or understand why observed variability occurs in some components and not in others.

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