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Recombination and the evolution of mutational robustness

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Abstract

Mutational robustness is the degree to which a phenotype, such as fitness, is resistant to mutational perturbations. Since most of these perturbations will tend to reduce fitness, robustness provides an immediate benefit for the mutated individual. However, robust systems decay due to the accumulation of deleterious mutations that would otherwise have been cleared by selection. This decay has received very little theoretical attention. At equilibrium, a population or asexual lineage is expected to have a mutation load that is invariant with respect to the selection coefficient of deleterious alleles, so the benefit of robustness (at the level of the population or asexual lineage) is temporary. However, previous work has shown that robustness can be favoured when robustness loci segregate independently of the mutating loci they act upon. We examine a simple two-locus model that allows for intermediate rates of recombination and inbreeding to show that increasing the effective recombination rate allows for the evolution of greater mutational robustness.

Keywords: Canalization; Epistasis; Linkage disequilibrium; Multilocus methodology; Mutation–selection balance

1. Introduction

It has long been observed that many developmental traits display a high degree of phenotypic robustness, that is, the phenotype is remarkably immune to environmental and genetic perturbations (Waddington, 1940; Schmalhausen, 1949). Waddington (1942) described the phenomenon as 'canalization', and proposed an adaptive explanation. He reasoned that traits under stabilising selection towards some intermediate optimum should benefit from any mechanism that prevents deviation from that optimum due to either genetic or environmental perturbations. Within the class of genetic perturbations are those that are due to deleterious mutations. In recent years, mutational robustness has attracted renewed interest, on both theoretical and empirical fronts (Wagner et al., 1997; Wilkins, 1997; Rutherford and Lindquist, 1998; van Nimwegen et al., 1999; Kawecki, 2000; Wagner, 2000; Wilke, 2001; Wilke et al., 2001; Queitsch et al., 2002; Wilke and Adami, 2003; de Visser et al., 2003; Proulx and Phillips, 2005). Most attention has been given to adaptive explanations, although some researchers have speculated that mutational robustness is a by-product of adaptation against environmental perturbations (Wagner et al., 1997; Burch and Chao, 2004) or simply an emergent property of genetic systems (Kacser and Burns, 1981; von Dassow et al., 2000; Meir et al., 2002; Shen-Orr et al., 2002).

All genetic models for the evolution of robustness require some form of gene interaction or epistasis between the loci involved. In this respect, a distinction can be drawn between the two different models of robustness that are commonly discussed; whether the epistasis is exclusively between the loci involved in the trait or between the trait loci and an unrelated locus (a 'modifier'). There is evidence for the former in RNA and protein folding where there is often extensive degeneracy between the primary sequence and the secondary or tertiary structure (Maynard Smith, 1970; Lau and Dill, 1990; Schuster et al., 1994;

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van Nimwegen et al., 1999; Wilke, 2001) and in metabolic and developmental pathways in prokaryotes and eukaryotes, where distributed network architectures facilitate robustness via internal pathway degeneracy (Edwards and Palsson, 1999, 2000a, b; von Dassow et al., 2000; Meir et al., 2002; Shen-Orr et al., 2002; Ingolia, 2004; Wagner, 2005). Wagner (2005) refers to this form of robustness as 'distributed robustness' and argues, based on empirical evidence, for the primacy of its role in mutational robustness in favour of gene redundancy arising from gene duplicates (Wagner 2000, 2001, 2005). Conversely, there is evidence that heat shock proteins, such as *Hsp*90 in *Drosophila* (Rutherford and Lindquist, 1998) and *Arabidopsis* (Queitsch et al., 2002), and *GroEL* in *Escherischia coli* (Fares et al., 2002), behave as modifiers of mutational robustness. For the remainder of this article, we will restrict attention to the modifier view of robustness.

The evolution of mutational robustness is conceptually similar to the adaptive evolution of dominance proposed by Fisher (1928). In both cases it is the heritable deviation from the wild type that is being buffered, and the selective advantage of the modifier is of the order of the mutation rate (Wright, 1929). Fisher believed that although the selective advantage is weak, in a large population with a number of recessive mutations the accumulated selective pressure would drive the evolution of dominance. Wright took the view that dominance emerged as an intrinsic property of metabolic pathways and proposed an alternative 'physiological' theory of dominance (Wright, 1934). Kacser and Burns (1981) provided considerable support for Wright's argument with a model of a multienzyme system that showed that the flux of the enzyme pathway is insensitive to concentration changes in the enzymes involved, suggesting that dominance is an inevitable property of such systems. More recently, however, Bagheri and Wagner (2004) have shown that this may only be the case when one neglects nonlinear enzyme interactions. Currently, empirical evidence appears not to support Fisher's adaptationist hypothesis (Orr, 1991), although it may be relevant in situations involving strong selection (Haldane, 1956; Mayo and Burger, 1997). The debate continues. Another related phenomenon that has received much attention is the evolutionary transition from haploidy to diploidy. A benefit may be afforded by an extended diploid phase due to the masking of recessive or partially recessive deleterious mutations (Crow and Kimura, 1965). Here, the adaptationist view appears to have a plausible theoretical foundation (Kondrashov and Crow, 1991; Perrot et al., 1991) although, interestingly, it is incompatible with Fisher's view of dominance since it requires that newly arisen deleterious mutations are always (at least partially) recessive (Perrot et al., 1991). Together with the evolution of mutational robustness, these scenarios involve evolutionary modification of the genetic system itself driven by the immediate benefit of alleviating the effects of deleterious mutations, which are of course a ubiquitous evolutionary phenomenon.

A classic result that motivates the present study is that at equilibrium the mutation load (L^*) of the population is invariant with respect to the fitness consequences of deleterious alleles. Assuming fitnesses combine multiplicatively across loci, an allele which arises by recurrent irreversible mutation at rate μ and incurs a fitness decrement s will equilibrate at frequency μ/s in a haploid population (mutation–selection balance). Hence the average fitness contributed by this locus is $(1-\mu/s)\times 1+(\mu/s)\times(1-s)=1-\mu$; the mutation load at this locus is then μ, and not a function of s (Haldane, 1937). The result has been generalized for all loci in the genome, giving a population load of mutations $L*=1-e^{-U}$ (Kimura and Maruyama, 1966; Kondrashov, 1988), where U is the per genome per generation mutation rate, and hence the decrement to fitness due to individual mutations is again irrelevant. The reason for this is intuitive: if mutations are more harmful they are more readily removed from the population by selection. Those mutations with large deleterious effects are held at low frequency at mutation–selection balance, and thus cause the same decrement to the mean fitness of the population as less harmful, and hence more frequently encountered, mutations.

The action of mutational robustness is to reduce the magnitude of a mutation's fitness effect. Whilst it may be temporarily advantageous to reduce the selection coefficient associated with the deleterious mutation, this leads to the accumulation of mutations that would otherwise have been cleared by selection, and so a closed population (i.e. no flow of genetic material between populations) with enhanced robustness does not improve its equilibrium mutation load. Thus there is no long-term benefit for being robust, at the level of the closed population. This mutational decay of robust systems has received only limited attention (Frank, 2003). If robustness has an intrinsic cost, such as the energetic cost of synthesizing the robustness gene product, then in the long term it will cause a net disadvantage for the population. Therefore, in an asexual population, we predict eventual loss of robust lineages. However, robustness might be favoured in a sexual population. Since the benefit of robustness (a reduced impact of mutations) accrues to the robust lineage, yet the cost (an increased equilibrium frequency of mutations) is shared by the whole population, robust lineages may have a relative advantage. It seems that this will increasingly be the case as the rate of recombination (in particular, between robustness genes and those genes undergoing mutational perturbation) is increased. This has received some attention, and the hypothesis is supported by contrasting the predictions of models of complete linkage, in which costly robustness is never favoured (Hermisson et al., 2002, p. 26), with those which assume free recombination, in which costly robustness can evolve (Wagner et al., 1997; Dawson, 1999). However, results for robustness evolution with intermediate recombination rates are lacking (de Visser et al., 2003, p. 1962).

We examine a simple model that captures the essence of this problem. The dynamics of the system are described

using a multilocus methodology (developed by Barton and Turelli, 1991; Kirkpatrick et al., 2002) that highlights allele frequencies and linkage disequilibria, which is more natural than following genotype frequencies. Also, it provides a general notation that neatly partitions the various causes of evolutionary change, and allows for arbitrary complexity so that the model is readily extensible within this single framework. Specifically, we develop exact analytical recursions describing the dynamics of a costly robustness modifier and its association with a mutating locus, and from this we generate an invasion condition to determine when this modifier will increase in frequency when vanishingly rare. We then make an assumption of minor robustness variants to examine how the robustness phenotype evolves in the longer term, moving from multilocus population genetics to an evolutionary game theoretic analysis.

2. Model and analysis

2.1. Two-locus model

We consider a simple model which captures all the important features of this problem—a large population of sexual haploids, with a life cycle which involves (i) selection, followed by (ii) mutation, and finally (iii) mating to form diploid zygotes, which undergo meiosis to form the next generation of haploid individuals. All notation used in this article are summarized and defined in Table 1. A locus i suffers recurrent, irreversible mutation, from the wild-type allele with value $X_i = 0$ to mutant allele with value $X_i = 1$, at a rate μ. The fitness of the wild type is 1, and the fitness of the mutant is $1-s$ in the absence of robustness. The frequency of the mutant is denoted p_i, and thus the frequency of the wild type is $q_i = 1 - p_i$. A second locus j controls the expression of the deleterious mutant, when it occurs at the first locus: with robustness k, the fitness contributed by the first locus is $1-(1-k)s$. Two alleles, with varying robustness effect, are present. The 'resident' allele has value $X_j = 0$ and robustness effect k_x, and the 'variant' allele has value $X_j = 1$ and robustness effect k_y. The robustness locus also incurs a direct (intrinsic) cost, with the resident contributing $1-c_x$, and the variant $1-c_y$, to an individual's fitness. The frequency of the variant is denoted p_j and the frequency of the resident is $q_j = 1 - p_j$. We will assume that the direct effects of the loci multiply to give genotype fitness. The four genotype fitnesses are summarized in Table 2. The effects of linkage and inbreeding are described by an effective rate of recombination parameter, r_e.

Following the above model, an individual's fitness may be written in the form:

$$\begin{aligned} w &= (1-X_i)(1-X_j)w_{00} + X_i(1-X_j)w_{10} + (1-X_i)X_j w_{01} \\ &\quad + X_i X_j w_{11} = (1-X_i)(1-X_j)(1-c_x) \\ &\quad + X_i(1-X_j)(1-(1-k_x)s)(1-c_x) \\ &\quad + (1-X_i)X_j(1-c_y) + X_i X_j(1-(1-k_y)s)(1-c_y), \end{aligned} \tag{1}$$

Table 1
Summary of notation used in this article

Notation	Definition
μ	Deleterious mutation rate at a single locus
s	Selection coefficient associated with deleterious mutation
U	Per genome per generation mutation rate
L^*	Equilibrium mutation load
i	Locus under recurrent mutation
j	Locus controlling robustness
$\mathbf{i}$, $\mathbf{j}$	A generic gene position
$\mathbf{A}$, $\mathbf{B}$	A generic set of gene positions
$\mathbf{W}$	The set of all gene positions contributing to fitness
$X_\mathbf{i}$	Allelic value for gene position $\mathbf{i}$ (0 or 1)
$p_\mathbf{i}$	Frequency of the $X_\mathbf{i} = 1$ allele
$q_\mathbf{i} = 1 - p_\mathbf{i}$	Frequency of the $X_\mathbf{i} = 0$ allele
$\zeta_\mathbf{i} = X_\mathbf{i} - p_\mathbf{i}$	Allelic deviation for gene position $\mathbf{i}$
$\zeta_\mathbf{A} = \prod_{\mathbf{i}\in\mathbf{A}}\zeta_\mathbf{i}$	Allelic deviation for a set $\mathbf{A}$ of gene positions
$D_\mathbf{A} = E[\zeta_\mathbf{A}]$	Association for set $\mathbf{A}$ of gene positions
w	Fitness of an individual
$\bar{w}$	Population mean fitness
$a_\mathbf{A}$	Multilocus selection coefficient for set $\mathbf{A}$ of gene positions
z	A generic robustness strategy
x	Resident robustness strategy
$y = x + \delta x$	Variant robustness strategy
x^*	Equilibrium robustness strategy
k_z, $k[z]$	Robustness effect associated with strategy z
c_z, $c[z]$	Cost of robustness associated with strategy z
r_e	Effective rate of recombination
$\lambda = 1 + \delta\lambda$	Invasion fitness of robustness variant; its asymptotic rate of increase

Table 2
Genotype fitness (w) as a function of allelic value ($X = 0$, 1) at the mutating locus (i) and the robustness locus (j).

		X_j	
		0	1
X_i	0	$1-c_x$	$1-c_y$
	1	$(1-(1-k_x)s)(1-c_x)$	$(1-(1-k_y)s)(1-c_y)$

where $w_{X_iX_j}$ is the fitness of the (X_i, X_j) genotype (see Table 2). This fitness function is analogous to Eq. (7) in Barton and Turelli (1991).

2.2. Multilocus population statistics

The multilocus framework of Kirkpatrick et al. (2002) describes individuals and populations according to deviations from average values. An allelic deviation ($\zeta_\mathbf{i} = X_\mathbf{i} - p_\mathbf{i}$) is defined for a generic gene position $\mathbf{i}$, and describes the deviation of the allelic value ($X_\mathbf{i} = 0$ or 1) from the population average ($p_\mathbf{i}$) at that position. Thus, the population average allelic deviation for a single gene position is zero. A corresponding deviation term ($\zeta_\mathbf{A} = \prod_{\mathbf{i}\in\mathbf{A}}\zeta_\mathbf{i}$) may be assigned to a set $\mathbf{A}$ of gene positions, and is the product of the allelic deviations for all the positions in

that set. Note that the average deviation for a set of two gene positions (**i** and **j**) is equal to the allelic covariance between these positions ($E[\zeta_{\mathbf{ij}}] = E[(X_{\mathbf{i}} - p_{\mathbf{i}})(X_{\mathbf{j}} - p_{\mathbf{j}})] = Cov[X_{\mathbf{i}}, X_{\mathbf{j}}]$), and thus is equivalent to the linkage disequilibrium ($D_{\mathbf{ij}}$) between these gene positions. In general, the population average deviation for a set **A** of gene positions will be denoted $D_{\mathbf{A}}$. Thus, the population composition with respect to a set of gene positions **B** may be fully described by the set of allele frequencies ($p_{\mathbf{i}}$, $\mathbf{i} \in \mathbf{B}$) at these positions, and the statistical associations ($D_{\mathbf{A}}$, $\mathbf{A} \subseteq \mathbf{B}$) between these positions. If an association term corresponds to a set of gene positions in which a particular position features several times, for example $D_{\mathbf{iiA}}$, then a reduction formula may be applied to re-express this as $p_{\mathbf{i}}q_{\mathbf{i}}D_{\mathbf{A}} + (1-2p_{\mathbf{i}})D_{\mathbf{iA}}$, as outlined by Kirkpatrick et al. (2002).

We may now describe how sets of gene positions impact upon an individual's fitness. Making the substitution $X_{\mathbf{i}} = \zeta_{\mathbf{i}} + p_{\mathbf{i}}$ into the fitness function (1), this may be rearranged into the form

$$\frac{w}{\bar{w}} = 1 + a_i(\zeta_i - D_i) + a_j(\zeta_j - D_j) + a_{ij}(\zeta_{ij} - D_{ij}), \tag{2}$$

where $a_{\mathbf{A}}$ is the contribution of the deviation for a set of gene positions **A** to relative fitness, $w/\bar{w}$. This is analogous to Eq. (6) of Barton and Turelli (1991) and Eq. (7) of Kirkpatrick et al., (2002). The $a_{\mathbf{A}}$ terms provide selection coefficients for a multilocus analysis (Kirkpatrick et al., 2002). For the present model, we have

$$\begin{aligned} a_i &= -s(1 - ((1-p_j)(k_x + c_x(1-k_x)) \\ &\quad + p_j(k_y + c_y(1-k_y))))/\bar{w}, \\ a_j &= (s\,p_i((k_y + c_y(1-k_y)) - (k_x + c_x(1-k_x))) \\ &\quad - (c_y - c_x))/\bar{w}, \\ a_{ij} &= s((k_y + c_y(1-k_y)) - (k_x + c_x(1-k_x)))/\bar{w}. \end{aligned} \tag{3}$$

Mean fitness is found by taking an average of w over the population:

$$\begin{aligned} \bar{w} = 1 &- sp_i(1-k_x)(1-c_x) - (p_jc_y + (1-p_j)c_x) \\ &- s((1-k_y)(1-c_y) - (1-k_x)(1-c_x))(p_ip_j + D_{ij}). \end{aligned} \tag{4}$$

Having extracted multilocus population fitness statistics from the model, we can now use them to make some remarkably elegant statements about how selection moulds the allele frequencies and linkage disequilibrium of this system. After selection we will consider mutation and then transmission.

2.3. Selection

The multilocus methodology provides simple recursion expressions for the change in allele frequencies and genetic associations due to selection. The basic equation is

$$\Delta_{\mathrm{S}} D_{\mathbf{A}} = \sum_{\mathbf{B} \subseteq \mathbf{W}} a_{\mathbf{B}}(D_{\mathbf{AB}} - D_{\mathbf{A}}D_{\mathbf{B}}), \tag{5}$$

where **W** is the set of all gene positions contributing to fitness. For the case of a single gene position ($\mathbf{A} = \mathbf{i}$), we may use expression (5) to describe the change in allele frequency ($p_{\mathbf{i}}$) due to selection:

$$\Delta_{\mathrm{S}} p_{\mathbf{i}} = \sum_{\mathbf{B} \subseteq \mathbf{W}} a_{\mathbf{B}} D_{\mathbf{iB}}. \tag{6}$$

A complication arises in that the association after selection ($D'_{\mathbf{A}} = D_{\mathbf{A}} + \Delta_{\mathrm{S}} D_{\mathbf{A}}$) is described with respect to allele frequencies before selection. It will usually be helpful to correct for this, and the procedure is described in Kirkpatrick et al. (2002). No correction is necessary for the expressions describing allele frequency change. In the context of the present model, the change in the frequency of the deleterious mutation that is due to selection is described by

$$\begin{aligned} p'_i &= p_i + a_iD_{ii} + a_jD_{ij} + a_{ij}D_{iij} = p_i + a_ip_iq_i + a_jD_{ij} \\ &\quad + a_{ij}(1-2p_i)D_{ij}. \end{aligned} \tag{7}$$

This notational framework makes clear the causes of evolutionary change: here we see that the response to selection ($\Delta_S p_i = p'_i - p_i$) is given by the product of the strength of selection operating directly on the focal locus (a_i) and the variation at that locus (p_iq_i), plus the product of selection operating directly on the other locus (a_j) and the association between the two loci (D_{ij}), plus the product of selection due to the epistatic interaction between the two loci (a_{ij}) and the appropriate association ($(1-2p_i)D_{ij}$). Similarly, the change in allele frequency, due to selection, at the robustness locus is given by

$$\begin{aligned} p'_j &= p_j + a_iD_{ij} + a_jD_{jj} + a_{ij}D_{ijj} = p_j + a_iD_{ij} \\ &\quad + a_jp_jq_j + a_{ij}(1-2p_j)D_{ij}. \end{aligned} \tag{8}$$

From expression (5), the change in the association between the loci is described by

$$\begin{aligned} D'_{ij} &= D_{ij} + a_iD_{iij} + a_jD_{ijj} + a_{ij}(D_{iijj} - D_{ij}^2) - (p'_i - p_i)(p'_j - p_j) \\ &= D_{ij} + a_i(1-2p_i)D_{ij} + a_j(1-2p_j)D_{ij} \\ &\quad + a_{ij}(p_iq_ip_jq_j + (1-2p_i)(1-2p_j)D_{ij} - D_{ij}^2) \\ &\quad - (p'_i - p_i)(p'_j - p_j), \end{aligned} \tag{9}$$

where the trailing term corrects for change in allele frequency (Kirkpatrick et al., 2002).

2.4. Mutation

The change in frequency of the deleterious allele after mutation is described by

$$p''_i = p'_i + \mu(1 - p'_i). \tag{10}$$

Since the j locus does not undergo mutation, $p''_j = p'_j$. From Kirkpatrick et al. (2002), the change in the linkage disequilibrium due to mutation is given by

$$D''_{ij} = (1-\mu)D'_{ij}. \tag{11}$$

2.5. Transmission

Transmission—the union of gametes, crossing over, and fair meiosis—does not alter the allele frequencies in this model (so $p_i''' = p_i''$ and $p_j''' = p_j''$, where triple primes denote the variable is measured after transmission), but it does impact on the linkage disequilibrium. This is reduced by a fraction equal to the effective rate of recombination (Crow and Kimura, 1970), and so we have

$$D_{ij}''' = (1 - r_e)D_{ij}''. \tag{12}$$

2.6. Invasion analysis

We have obtained recursions describing the change in the frequencies of the deleterious mutation (p_i) and robustness modifier (p_j) and the linkage disequilibrium (D_{ij}) over a single generation incorporating selection, mutation and transmission. We now consider that the variant robustness allele is vanishingly rare ($p_j \to 0$, and hence $D_{ij} \to 0$), and examine the conditions under which this rare allele will increase in frequency (invasion). We will assume that μ is sufficiently small for us not to have to worry about fixation of the deleterious mutation i.e. $\mu < (1 - k_x)s$. We will assume that the deleterious mutation is initially at its equilibrium point, $p_i^* = \mu/((1 - k_x)s)$. While the variant is rare, the evolutionary dynamics at the j locus has vanishing impact on dynamics at the i locus, so in any generation we may express the frequency of the deleterious mutation as $p_i = p_i^* + \delta p_i$, where $\delta p_i \to 0$. Making this substitution, and summarising the changes in the allele frequency at the robustness locus and linkage disequilibrium due to selection, mutation and recombination, obtains $p_j''' = \alpha_1 p_j + \alpha_2 D_{ij} + O(\delta p_i^2, p_j^2, D_{ij}^2)$ and $D_{ij}''' = \alpha_3 p_j + \alpha_4 D_{ij} + O(\delta p_i^2, p_j^2, D_{ij}^2)$, where

$$\begin{aligned}
\alpha_1 &= \frac{(1 - c_y)(1 - k_x - \mu(1 - k_y))}{(1 - \mu)(1 - c_x)(1 - k_x)}, \\
\alpha_2 &= -\frac{s(1 - c_y)(1 - k_y)}{(1 - \mu)(1 - c_x)}, \\
\alpha_3 &= \frac{(1 - r_e)(k_y - k_x)(1 - c_y)\mu((1 - k_x)s - \mu)}{(1 - c_x)(1 - k_x)^2 s(1 - \mu)}, \quad \text{and} \\
\alpha_4 &= \frac{(1 - r_e)(1 - c_y)((1 - k_x)(1 - (1 - k_y)s) - \mu(k_y - k_x))}{(1 - \mu)(1 - c_x)(1 - k_x)}.
\end{aligned} \tag{13}$$

Neither of these recursions are functions of δp_i, therefore we need not explicitly follow the frequency of the deleterious mutation, so long as we assume it is close to its equilibrium. The asymptotic rate of increase of the rare variant, its 'invasion fitness', is given by the leading eigenvalue for the above system. This is the solution λ to the characteristic equation $(\alpha_1 - \lambda)(\alpha_4 - \lambda) - \alpha_2\alpha_3 = 0$ that has the largest magnitude. The condition for invasion of the robustness variant is $\lambda > 1$.

2.7. Evolution of robustness

We have obtained a condition for the invasion of a given resident population by a given variant robustness allele. We now ask the following questions: (1) is there a resident allele that cannot be invaded by any robustness variant? (2) Will the population converge on this evolutionarily stable state? In other words, we are interested in identifying the endpoint of robustness evolution in the longer term. To address this, we will now consider a continuum of robustness strategies (z), from zero robustness ($z = 0$) to full robustness ($z = 1$), each encoded by an allele at the j locus. The cost and effect of robustness parameters from the previous sections are now considered as functions of the robustness strategy ($c[z]$ and $k[z]$; where $c[0] = k[0] = 0$ and $\mathrm{d}c/\mathrm{d}z$, $\mathrm{d}k/\mathrm{d}z > 0$ for all z). The resident allele encodes the robustness strategy $z = x$, and the variant encodes $z = y$. Thus, $c_x = c[x]$, $k_x = k[x]$, $c_y = c[y]$ and $k_y = k[y]$. For ease of analysis, we will consider only local stability, restricting our attention to $y = x + \delta x$ where $\delta x \to 0$. Since this represents near-neutrality, the invasion fitness of the variant will be of the form $\lambda = 1 + \delta\lambda$, where $\delta\lambda \to 0$. Upon this assumption, we may solve the characteristic equation from earlier to obtain $\delta\lambda \to ((1 - \alpha_1)(1 - \alpha_4) - \alpha_2\alpha_3)/(\alpha_1 + \alpha_4 - 2)$ as $\delta x \to 0$, or

$$\delta\lambda \approx \frac{\mu r_e(1 - c[x])k'[x] - (1 - k[x])(r_e + (1 - r_e)(1 - k[x])s - \mu)c'[x]}{(1 - c[x])(1 - k[x])(r_e + (1 - r_e)(1 - k[x])s - \mu)}\,\delta x, \tag{14}$$

where the primes denote derivatives evaluated at the resident robustness strategy, i.e. $c'[x] = \mathrm{d}c[z]/\mathrm{d}z\big|_{z=x}$ and $k'[x] = \mathrm{d}k[z]/\mathrm{d}z\big|_{z=x}$. Marginal invasion fitness is given by $\partial\lambda/\partial y|_{y=x} = \delta\lambda/\delta x$. Setting $r_e = 0$, marginal invasion fitness reduces to $\partial\lambda/\partial y|_{y=x} = -c'[x]/(1 - c[x])$, which is negative for all x: over the whole range of resident strategies, selection favours variants with reduced robustness. Hence, the only equilibrium point in the absence of recombination is at $x^* = 0$. This means that when the effective rate of recombination is zero, costly robustness cannot evolve (Hermisson et al., 2002). We now ask, for $r_e > 0$, what is the end point of robustness evolution? We are therefore looking for a strategy that is both evolutionarily stable once attained (an ESS; Maynard Smith and Price, 1973; Maynard Smith, 1982), and is also attainable (i.e. convergence stable, so that when x is close to x^*, y closer to x^* will invade; Eshel and Motro, 1981; Taylor, 1996). A strategy that is both an ESS and is convergence stable is termed a 'continuously stable strategy' (CSS; Eshel, 1983; Christiansen, 1991). If a strategy x^* is evolutionarily stable, then it must satisfy $\partial\lambda/\partial y|_{y=x=x^*} = 0$. Thus,

$$\frac{\mathrm{d}}{\mathrm{d}r_e}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right] = \frac{\partial}{\partial r_e}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right] + \frac{\partial}{\partial x^*}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right]\frac{\mathrm{d}x^*}{\mathrm{d}r_e} = 0, \tag{15}$$

which can be re-arranged to give

$$\frac{\mathrm{d}x^*}{\mathrm{d}r_e} = -\frac{\partial/\partial r_e[\partial\lambda/\partial y|_{y=x=x^*}]}{\partial/\partial x^*[\partial\lambda/\partial y|_{y=x=x^*}]}. \tag{16}$$

Noting that convergence stability implies $\partial\left[\partial\lambda/\partial y|_{y=x=x^*}\right]/\partial x^* < 0$ (Taylor, 1996), the CSS satisfies

$$\operatorname{sgn}\left[\frac{\mathrm{d}x^*}{\mathrm{d}r_e}\right] = \operatorname{sgn}\left[\frac{\partial}{\partial r_e}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right]\right] \tag{17}$$

(Pen, 2000), where the function sgn returns the sign, or sense, of its real argument, i.e. positive or negative or zero. The partial derivative on the RHS is

$$\frac{\partial}{\partial r_e}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right] = \frac{\mu((1-k[x^*])s-\mu)k'[x^*]}{(1-k[x^*])(r_e+(1-r_e)(1-k[x^*])s-\mu)}. \tag{18}$$

Since RHS of (18) >0, it follows from (16) that the CSS x^* (when it exists) is a monotonically increasing function of r_e. Thus, we expect the endpoint of evolution to be a greater degree of robustness the higher the effective rate of recombination. Applying the same procedure to the selection coefficient (s) and mutation rate (μ) obtains

$$\frac{\partial}{\partial s}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right] = -\frac{\mu r_e(1-r_e)k'[x^*]}{(r_e+(1-r_e)(1-k[x^*])s-\mu)^2} < 0 \tag{19}$$

and

$$\frac{\partial}{\partial \mu}\left[\left.\frac{\partial\lambda}{\partial y}\right|_{y=x=x^*}\right] = \frac{r_e(r_e+(1-r_e)(1-k[x^*])s)k'[x^*]}{(1-k[x^*])(r_e+(1-r_e)(1-k[x^*])s-\mu)^2} > 0 \tag{20}$$

i.e. the CSS x^* is a decreasing function of s and an increasing function of μ, so we expect the endpoint of evolution to be a greater degree of robustness as we decrease the magnitude of the deleterious effect of mutations and as we increase the mutation rate. From (14), the exact value of the CSS x^* can be found by solving the equation

$$\mu r_e(1-c[x])k'[x] - (1-k[x])(r_e+(1-r_e)(1-k[x])s-\mu)c'[x] = 0. \tag{21}$$

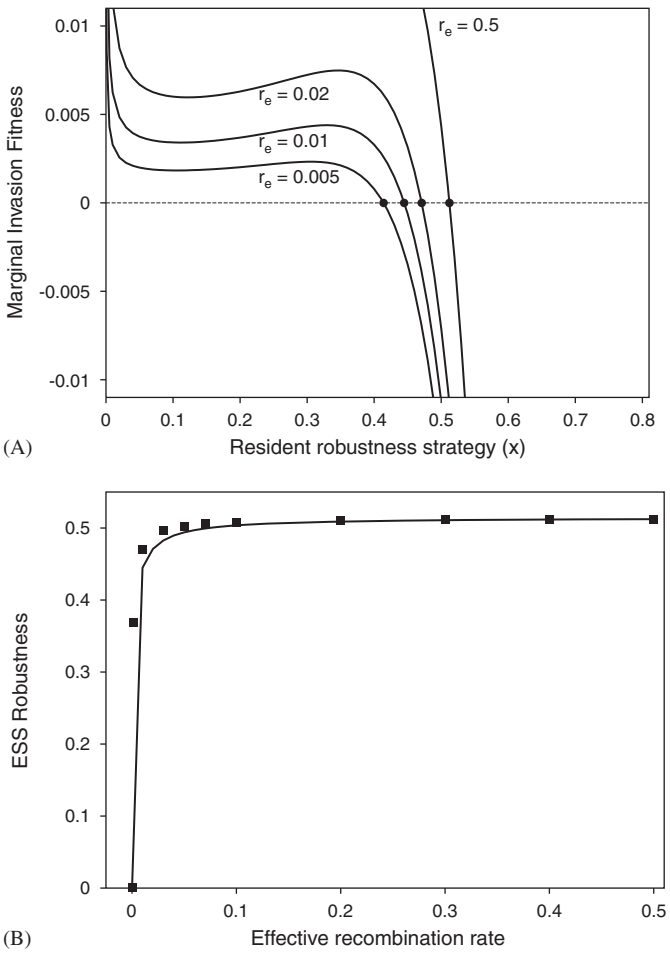

Fig. 1. (A) Marginal invasion fitness $\partial\lambda/\partial y|_{y=x}$ as a function of the resident robustness strategy (x) and the effective recombination rate (r_e), assuming $\mu = 0.01, s = 0.1, c[z] = z^{10}, k[z] = z^{1/2}$. The sign of marginal invasion fitness determines the direction of selection; if it is positive then variant strategies increasing robustness are favoured, and if it is negative then variant strategies reducing robustness are favoured. The convergence stable robustness strategy (marked by a filled circle) is an increasing function of the effective rate of recombination. (B) For the same model, simulation results (squares) confirm the analytical prediction (line) that robustness increases with the effective rate of recombination.

Some representative numerical examples are given in Figs. 1A and 2A. The assumption of vanishing variation is somewhat artificial, and so we have used simulations to test the predictions using a similar two-locus model that allows for continuum alleles which are simultaneously extant (simulation results are presented in Figs. 1B and 2B). We find that numerical solutions to the analytical prediction given by (21) and the results of the simulations are generally in good agreement. Depending on the choice of parameters and robustness functions, there may be: (1) a single internal equilibrium, which is a CSS (Fig. 1A); (2) an unstable equilibrium in addition to the CSS (Fig. 2A, e.g. lines for $r_e = 0.1$, 0.05, 0.01); or (3) no internal equilibria (Fig. 2A, e.g. $r_e = 0.5$). The simulations confirm that a population initialized at close to zero robustness will ultimately find itself trapped at the CSS, where this exists (Figs. 1B and 2B). Due to the assumption that mutation–selection balance holds the deleterious mutation at intermediate frequency (i.e. $\mu < (1-k_x)s$, which may also be written as $k_x < 1-\mu/s$) the present analysis does not allow for examination of the evolution of almost complete robustness ($k_x = 1$).

Although the simulations and analytical predictions have a very good fit, they are not perfect. In particular, the simulations tend to give an end-point of robustness evolution that is higher than predicted from the game theoretic approach. There is reason to suspect that the invasion analysis underestimates the benefits of robustness. For example consider a variant robustness allele with associated cost such that its asymptotic rate of increase is exactly 1. Initially, this allele will increase in frequency, and will eventually settle at a neutral equilibrium. To some

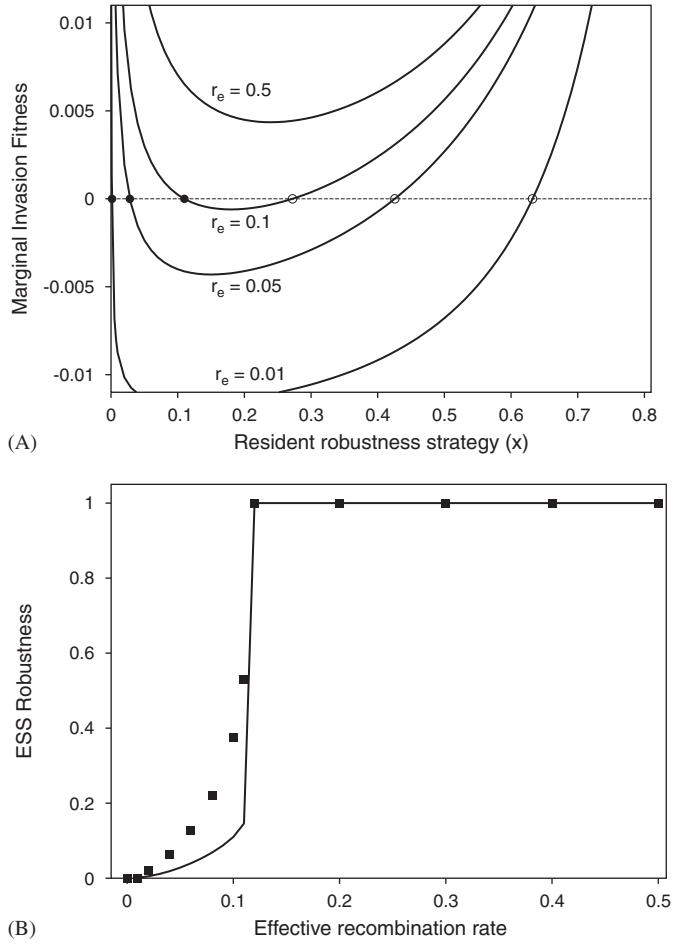

Fig. 2. (A) Marginal invasion fitness $\partial\lambda/\partial y|_{y=x}$ as a function of the resident robustness strategy (x) and the effective recombination rate (r_e), assuming $\mu = 0.01, s = 0.1, c[z] = 0.15z, k[z] = z^{1/2}$. The sign of marginal invasion fitness determines the direction of selection; if it is positive then variant strategies increasing robustness are favoured, and if it is negative then variant strategies reducing robustness are favoured. The convergence stable robustness strategy (where it exists, e.g. for $r_e = 0.01$, 0.05, 0.1, marked by a filled circle) is an increasing function of the effective rate of recombination; there is also sometimes an unstable equilibrium (marked by an empty circle). (B) For the same model, simulation results (squares) confirm the analytical prediction (line) that robustness increases with the effective rate of recombination, and that there is no internal stable end point for $r_e > 0.11$.

extent then, this allele has been favoured, although technically it does not invade. More generally, deviations from the analytical predictions will occur due to slowness in attaining the evolutionary endpoint, as selection acts weakly upon robustness and only a finite number of generations are simulated.

3. Discussion

We have examined the evolution of costly mutational robustness in a simple two-locus model for when recombination (r_e) between the two loci is intermediate. Previously, only the extremes of zero recombination (Hermisson et al., 2002) and freely recombining loci (Wagner et al., 1997; Dawson, 1999) have been considered. A multilocus methodology has been employed to obtain recursions for allele frequencies at the robustness locus and the association between this locus and the locus that is under recurrent mutation. The result is an analytical condition for when the robustness variant invades a population. Restricting attention to minor variants, we have used this condition to determine how the end point of robustness evolution varies with the effective rate of recombination (r_e), the intrinsic deleterious effect of the mutation (s) and the mutation rate (μ). Consistent with previous theory, we find that costly robustness cannot be favoured when the effective rate of recombination is zero. In addition, we show that, where one exists, the internal stable endpoint of robustness evolution is an increasing function of effective recombination rate and the mutation rate, and is a decreasing function of the intrinsic deleterious effect of the mutation. Although the analysis assumes vanishing robustness variation in the population at any time, simulations that relax this assumption reveal the analytical treatment is robust.

Why do we predict enhanced robustness with increasing effective rate of recombination? Recombination favours robustness in two ways: (1) by allowing a robust lineage to discard the excess of deleterious mutations it has accumulated, and (2) these deleterious mutations are inflicted upon non-robust lineages where they cause enhanced damage to fitness, thus increasing the relative fitness of the robust lineages. Put another way, by breaking down the association between the robustness gene and the target of the robustness effect, recombination decouples the immediate benefit of robustness (enhanced fitness in the context of a mutant genotype) from the long-term cost (increased frequency of mutations at mutation–selection equilibrium); the former accruing only to robust individuals, and the latter being paid by the population as a whole. This benefit for robustness is mirrored in the Perrot et al. (1991) model for the evolution of diploidy, which features gene flow between haploids and diploids. With this in mind, the model predicts increased maladaptation in the genomes of sexual, outbred populations, whereas the genomes of asexual or inbred populations should be less afflicted with the mutationally decayed remains of robust networks.

The endpoint of robustness evolution is predicted to be a decreasing function of the selection coefficient associated with deleterious mutations. This is because as the strength of selection upon the mutating locus increases, so does the epistasis between the robustness and mutating loci, which results in a greater association between these. Since it is the build-up of this linkage disequilibrium which acts to disfavour the evolution of robustness, weaker selection against the deleterious mutant favours enhanced robustness. An analogous result emerged from Perrot et al.'s (1991) model for the evolution of diploidy. Interestingly, this is responsible for the run-away selection for robustness observed in some of the simulations (Fig. 2B), because as the population becomes more robust to deleterious

mutations there is reduced selection acting upon the mutating locus, and thus a lower build-up of linkage disequilibrium between the two loci. Intuitively, it would seem that mutational robustness should be increasingly favoured as deleterious mutations become more, rather than less, harmful. Yet it is the mutation load and not the mutation effect that is crucial (Proulx and Phillips, 2005), so the endpoint of robustness evolution is an increasing function of the mutation rate and not the deleterious mutational effect. This is consistent with Wright's (1929) view that selection for mutational robustness will be of the order of mutation rate.

It is of interest to compare the present results with previous models for the evolution of mutational robustness. Wagner et al. (1997) investigated the evolution of a modifier of mutational robustness impacting upon a number of loci underlying a quantitative trait under Gaussian stabilising selection. Individual-based simulations showed that the selection coefficient acting on the modifier tends to increase with the intensity of the stabilising selection, a result that was verified in deterministic simulations by Kawecki (2000). This is in contrast to the result we report here where the modifier invades most easily at lower strengths of selection against the deleterious allele because it is here that the modifier experiences weakest linkage disequilibrium with the deleterious allele. Wagner et al. (1997) attribute their result to stronger stabilising selection enabling stronger selection for canalization. Their result crucially depends on the possibility of back mutation, plus high mutation rates, so that back mutation is strong relative to selection. The present analysis assumes no back mutation, and this appears to be the reason for the disparity. The neglecting of back mutation seems reasonable if there are many alleles that give rise to defective gene products and only a few that code for a correctly functioning protein. However, further work is needed to clarify the impact of back mutation on the evolution of robustness.

We note some possibilities for the evolution of synergistic epistasis, where an individual's fitness declines more rapidly with increasing numbers of deleterious mutations than predicted by a multiplicative fitness scheme. In many models, mutational robustness is synonymous with synergistic epistasis (de Visser et al., 2003; Michalakis and Roze, 2004). For example the classic 'neutral network' (van Nimwegen et al., 1999) models of robustness—involving individuals with less than some threshold number of mutations having wild-type fitness, and individuals exceeding that threshold being inviable—presents an extreme form of synergistic epistasis. A substantial amount of theory has been devoted to the evolution of sex and recombination given synergistic epistasis between deleterious mutations (Kimura and Maruyama, 1966; Kondrashov, 1988; Charlesworth, 1990). Inferring from the present analysis, we suggest that synergistic epistasis can be an evolutionary outcome of sex and recombination, insofar as the latter processes promote the evolution of robustness, and synergistic epistasis emerges as a consequence. This hypothesis has some empirical support—there is a general trend towards weak synergistic epistasis between deleterious mutations among eukaryotes, but no trend in prokaryotes (de Visser et al., 1997; Elena and Lenski, 1997; de Visser and Hoekstra, 1998; Elena, 1999; Burch and Chao, 2004). This is beyond the scope of the present analysis, which has maximally one deleterious mutation in each individual, though it presents an interesting problem for the future.

Currently, no convincing empirical evidence has been published that demonstrates that genetic robustness exists as an adaptation. One reason for this is that, while it is possible to demonstrate that heritable variation is buffered in particular organisms, it is not easy to determine whether genetic robustness is the primary function, merely a side-effect of evolution for environmental robustness (Rutherford and Lindquist, 1998; Ancel and Fontana, 2000; Queitsch et al., 2002; Burch and Chao, 2004), or perhaps simply an emergent property of gene networks (Kacser and Burns, 1981; von Dassow et al., 2000; Edelman and Gally, 2001; Meir et al., 2002; Shen-Orr et al., 2002). A closely related problem is that the selection coefficient for a modifier of genetic robustness will be very weak, typically of the order of the mutation rate itself. However, the evolution of genetic robustness as a primary function may be plausible if there is migration between subpopulations in a heterogeneous environment (Mayr, 1963; Otto and Bourguet, 1999; Stearns, 2002). Migration rates can be much higher than mutation rates and therefore provide a stronger selective pressure for the buffering of (locally) maladapted alleles. Additionally, genetic robustness may evolve when selection fluctuates over time (Kawecki, 2000) and when selective sweeps take a population out of equilibrium (Mayo and Burger, 1997). It is with a view to extending the analysis to more complicated multilocus models that we have employed the methodology of Kirkpatrick et al. (2002), which permits arbitrary complexity within a single notational framework.

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References

Ancel, L.W., Fontana, W., 2000. Plasticity, evolvability and modularity in RNA. J. Exp. Zool. 288, 242–283.

Bagheri, H.C., Wagner, G.P., 2004. Evolution of dominance in metabolic pathways. Genetics 168, 1713–1735.

Barton, N.H., Turelli, M., 1991. Natural and sexual selection on many loci. Genetics 127, 229–255.

Burch, C.L., Chao, L., 2004. Epistasis and its relationship to canalization in RNA virus phi 6. Genetics 167, 559–567.

Charlesworth, B., 1990. Mutation–selection balance and the evolutionary advantage of sex and recombination. Genet Res. 55, 199–221.

Christiansen, F.B., 1991. On conditions for evolutionary stability for a continuously varying character. Am. Nat. 138, 37–50.
Crow, J.F., Kimura, M., 1965. Evolution in sexual and asexual populations. Am. Nat. 99, 439–450.
Crow, J.F., Kimura, M., 1970. An Introduction to population genetics theory. Burgess Publishing Company, Minneapolis, Minnesota.
Dawson, K.J., 1999. The dynamics of infinitesimally rare alleles applied to the evolution of mutation rates and the expression of deleterious mutations. Theor. Popul. Biol. 55, 1–22.
de Visser, J.A.G.M, Hoekstra, R.F., 1998. Synergistic epistasis between loci affecting fitness: evidence in plants and fungi. Genet. Res. 71, 39–49.
de Visser, J.A.G.M., Hoekstra, R.F., van den Ende, H., 1997. Test of interaction between genetic markers that affect fitness in *Aspergillus niger*. Evolution 51, 1499–1505.
de Visser, J.A.G.M., Hermisson, J., Wagner, G.P., Ancel-Meyers, L., Bagheri-Chaichian, H., Blanchard, J.L., Chao, L., Cheverud, J.M., Elena, S.F., Fontana, W., Gibson, G., Hansen, T.F., Krakauer, D., Lewontin, R.C., Ofria, C., Rice, S.H., von Dassow, G., Wagner, A., Whitlock, M.C., 2003. Perspective: evolution and detection of genetic robustness. Evolution 57, 1959–1972.
Edelman, G.M., Gally, J.A., 2001. Degeneracy and complexity in biological systems. Proc. Natl Acad. Sci. USA 98, 13763–13768.
Edwards, J.S., Palsson, B.O., 1999. Systems properties of the *Haemophilus influenzae* Rd metabolic genotype. J. Biol. Chem. 274, 17410–17416.
Edwards, J.S., Palsson, B.O., 2000a. The *Escherichia coli* MG1655 in silico metabolic genotype: its definition, characteristics, and capabilities. Proc. Natl Acad. Sci. USA 97, 5528–5533.
Edwards, J.S., Palsson, B.O., 2000b. Robustness analysis of the *E. coli* metabolic network. Biotech. Progress 16, 927–939.
Elena, S.F., 1999. Little evidence for synergism among deleterious mutations in a nonsegmented RNA virus. J. Mol. Evol. 49, 703–707.
Elena, S.F., Lenski, R.E., 1997. Test of interaction between deleterious mutations in *E. coli*. Nature 390, 395–398.
Eshel, I., 1983. Evolutionary and continuous stability. J. Theor. Biol. 103, 99–111.
Eshel, I., Motro, U., 1981. Kin selection and strong evolutionary stability of mutual help. Theor. Popul. Biol. 19, 420–433.
Fares, M.A., Ruiz-Gonzales, M.X., Moya, A., Elena, S.F., Barrio, E., 2002. GroEL buffers against deleterious mutations. Nature 417, 398.
Fisher, R.A., 1928. The possible modification of the response of the wild type to recurrent mutations. Am. Nat. 62, 115–126.
Frank, S.A., 2003. Genetic variation of polygenic characters and the evolution of genetic degeneracy. J. Evol. Biol. 16, 138–142.
Haldane, J.B.S., 1937. The effect of variation on fitness. Am. Nat. 71, 337–349.
Haldane, J.B.S., 1956. The theory of selection for melanism in *Lepidoptera*. Proc. R. Soc. London B 145, 303–306.
Hermisson, J., Redner, O., Wagner, H., Baake, E., 2002. Mutation–selection balance: ancestry, load, and maximum principle. Theor. Popul. Biol. 62, 9–46.
Ingolia, N.T., 2004. Topology and robustness in the *Drosophila* segment polarity network. PLoS Biol. 2, 805–815.
Kacser, H., Burns, J.A., 1981. The molecular basis of dominance. Genetics 97, 639–666.
Kawecki, T.J., 2000. The evolution of genetic canalization under fluctuating selection. Evolution 54, 1–12.
Kimura, M., Maruyama, T., 1966. The mutational load with epistatic gene interactions in fitness. Genetics 54, 1337–1351.
Kirkpatrick, M., Johnson, T., Barton, N.H., 2002. General models of multilocus evolution. Genetics 161, 1727–1750.
Kondrashov, A.S., 1988. Deleterious mutations and the evolution of sexual reproduction. Nature 336, 435–440.
Kondrashov, A.S., Crow, J.F., 1991. Haploidy or diploidy: which is better? Nature 351, 314–315.
Lau, K.F., Dill, K.A., 1990. Theory for protein mutability and biogenesis. Proc. Natl Acad. Sci. USA 87, 638–642.
Maynard Smith, J., 1970. Natural selection and the concept of a protein space. Nature 225, 563–564.
Maynard Smith, J., 1982. Evolution and the Theory of Games. Cambridge University Press, Cambridge.
Maynard Smith, J., Price, G., 1973. The logic of animal conflict. Nature 246, 15–18.
Mayo, O., Burger, R., 1997. The evolution of dominance: a theory whose time has passed? Biol. Rev. 72, 97–110.
Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge, MA.
Meir, E., von Dassow, G., Munro, E., Odell, G.M., 2002. Robustness, flexibility and the role of lateral inhibition in the neurogenic network. Curr. Biol. 12, 778–786.
Michalakis, Y., Roze, D., 2004. Epistasis in RNA viruses. Science 306, 1492–1493.
Orr, H.A., 1991. A test of Fisher's theory of dominance. Proc. Natl Acad. Sci. USA 88, 11413–11415.
Otto, S.P., Bourguet, D., 1999. Balanced polymorphisms and the evolution of dominance. Am. Nat. 153, 561–574.
Pen, I., 2000. Reproductive effort in viscous populations. Evolution 54, 293–297.
Perrot, V., Richerd, S., Valero, M., 1991. Transition from haploidy to diploidy. Nature 351, 315–317.
Proulx, S.R., Phillips, P.C., 2005. The opportunity for canalization and the evolution of genetic networks. Am. Nat. 165, 147–162.
Queitsch, C., Sangster, T.A., Lindquist, S., 2002. Hsp90 as a capacitor of phenotypic variation. Nature 417, 618–624.
Rutherford, S.L., Lindquist, S., 1998. Hsp90 as a capacitor for morphological evolution. Nature 396, 336–342.
Schmalhausen, I.I., 1949. Factors of Evolution: The Theory of Stabilising Selection. Blakiston, Philadelphia, PA, USA.
Schuster, P., Fontana, W., Stadler, P.F., Hofacker, I., 1994. From sequences to shapes and back: A case study in RNA secondary structures. Proc. R. Soc. London B 255, 279–284.
Shen-Orr, S.S., Milo, R., Mangan, S., Alon, U., 2002. Network motifs in the transcriptional regulation of *E. colii*. Nat. Genet. 31, 64–68.
Stearns, S.C., 2002. Progress on canalization. Proc. Natl Acad. Sci. USA 99, 10229–10230.
Taylor, P.D., 1996. Inclusive fitness arguments in genetic models of behaviour. J. Math Biol. 34, 654–674.
van Nimwegen, E., Crutchfield, J.P., Huynen, M., 1999. Neutral evolution of mutational robustness. Proc. Natl Acad. Sci. USA 96, 9716–9720.
von Dassow, G.E., Meir, E., Munrow, E.M., Odell, G.M., 2000. The segment polarity network is a robust developmental module. Nature 406, 188–192.
Waddington, C.H., 1940. Organisers and Genes. Cambridge University Press, Cambridge.
Waddington, C.H., 1942. Canalization of development and the inheritance of acquired characters. Nature 150, 563–565.
Wagner, A., 2000. Robustness against mutations in genetic networks of yeast. Nat. Genet. 24, 355–361.
Wagner, A., 2001. The yeast protein interaction network evolves rapidly and contains few redundant duplicate genes. Mol. Biol. Evol. 18, 1283–1292.
Wagner, A., 2005. Distributed robustness versus redundancy as causes of mutational robustness. BioEssays 27, 176–188.
Wagner, G.P., Booth, G., Bagheri-Chaichian, H., 1997. A population genetic theory of canalization. Evolution 51, 329–347.
Wilke, C.O., 2001. Selection for fitness versus selection for robustness in RNA secondary structure folding. Evolution 55, 2412–2420.
Wilke, C.O., Adami, C., 2003. Evolution of mutational robustness. Mut. Res. 522, 3–11.
Wilke, C.O., Wang, J.L., Ofria, C., Lenski, R.E., Adami, C., 2001. Evolution of digital organisms at high mutation rates leads to survival of the flattest. Nature 412, 331–333.
Wilkins, A.S., 1997. Canalization: a molecular genetic perspective. BioEssays 19, 257–261.
Wright, S., 1929. Fisher's theory of dominance. Am. Nat. 68, 24–53.
Wright, S., 1934. Physiological and evolutionary theories of dominance. Am. Nat. 68, 25–53.