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Selection for Chaperone-Like Mediated Genetic Robustness at Low Mutation Rate: Impact of Drift, Epistasis and Complexity

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ABSTRACT

Genetic robustness is defined as the constancy of a phenotype in the face of deleterious mutations. Overexpression of chaperones, to assist the folding of proteins carrying deleterious mutations, is so far one of the most accepted molecular mechanisms enhancing genetic robustness. Most theories on the evolution of robustness have focused on the implications of high mutation rate. Here we show that genetic drift, which is modulated by population size, organism complexity, and epistasis, can be a sufficient force to select for chaperone-mediated genetic robustness. Using an exact analytical solution, we also show that selection for costly genetic robustness leads to a paradox: the decrease of population fitness on long timescales and the long-term dependency on robustness mechanisms. We suggest that selection for genetic robustness could be universal and not restricted to high mutation rate organisms such as RNA viruses. The evolution of the endosymbiont Buchnera illustrates this selection mechanism and its paradox: the increased dependency on chaperones mediating genetic robustness. Our model explains why most chaperones might have become essential even in optimal growth conditions.

MUTATIONAL (or genetic) robustness is defined as the constancy of a phenotype in the face of deleterious mutations (Sanjuan *et al.* 2007). Selection drives populations to adapt to their environment by the fixation of successive advantageous mutations. However, in approaching a fitness optimum—*i.e.*, a genotype that is maximally adapted—they have to cope with an increasing proportion of deleterious mutations and, when at the optimum, they experience only neutral and deleterious mutations (Silander *et al.* 2007). Therefore any mechanism that would reduce the effect of deleterious mutations, *i.e.*, increase mutational robustness, could be favored by natural selection when at, or near, an optimum of fitness. Indeed, the general observation that for a large range of organisms, mutations have little effect on fitness, suggests that selection for robustness is pervasive (Melton 1994; Winzeler *et al.* 1999). Three main mechanisms that are not mutually exclusive could explain how genetic robustness has arisen. First, in the "intrinsic hypothesis" (de Visser *et al.* 2003) robustness could simply be a by-product of some biologically relevant functions. Second, mutational robustness could be a by-product of the selection for nongenetic perturbations such as environment changes or intrinsic noise (Wagner 2005). Third, mutational robustness could be selected for because it is adaptive in itself. In the following we restrict our attention to this "adaptive hypothesis" (de Visser *et al.* 2003).

Chaperone proteins, proteins that help other proteins to fold properly, have been shown to buffer the effect of deleterious mutations in diverse organisms (Rutherford 2003). In lineages that have accumulated deleterious mutations, the overexpression of the chaperone GroESL in *Escherichia coli* (Fares *et al.* 2002) or *Salmonella typhymurium* (Maisnier-Patin *et al.* 2005) resulted in an improved fitness. However, such robustness appears to come at a cost, as the buffering was visible only in carbon-rich media (Fares *et al.* 2002), and it is also known that GroESL-mediated refolding of proteins is ATP dependent. Chaperones can also buffer against environmental perturbations (such as heat shock); however, the observation that *groESL* evolved under positive selection and is overproduced in obligate intracellular endosymbionts (Moran 1996; Fares *et al.* 2004), for which environmental perturbations are assumed to be very weak, suggests that genetic robustness could be the direct target of selection.

Selection for a modifier of genetic robustness, *i.e.*, a gene modulating the effect of mutations, has been mainly studied in the context of high mutation rates, as the effect of the modifier allele affects the fitness of mutants (Wagner 2005). Under some theoretical frameworks, it has been suggested that the intensity of selection acting on a modifier of robustness would be of the order of the mutation rate (Gardner and Kalinka 2006). Therefore it has been presumed that selection

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for genetic robustness is relevant only in very large populations having a high mutation rate, such as RNA virus populations. In agreement with these ideas, artificial life experiments (Wilke and Adami 2001; Azevedo *et al.* 2006) and experimental data on viruses (Montville *et al.* 2005; Sanjuan *et al.* 2007) have shown that robustness varies between organisms and that it can be selected for under high mutation rates. It has also been shown by Krakauer and Plotkin (2002) that drift, *i.e.*, stochastic effects due to the finite size of populations, can promote selection for robustness even when more robust alleles are costly, as suggested in the case of chaperone overexpression. However, again this effect was examined only under high mutation rates.

When mutations are very rare, populations experience at the most the presence of a single mutant. In such conditions, the population fitness at equilibrium does not depend on the mutation rate but only on drift (Sella and Hirsh 2005; Tenaillon *et al.* 2007). Two factors modulate how drift affects fitness:

i. Epistasis, defined here as a local property of the adaptive landscape, describes how the selective effects of mutations depend on the genetic background in which they arise. Epistasis is negative (positive) if two mutations have a lower (higher) fitness when simultaneously present within a genome than expected if they did not interact. Negative epistasis increases selection against mutation-loaded individuals and therefore reduces the effect of drift on population fitness (Charlesworth 1990; Tenaillon *et al.* 2007).
ii. Phenotypic complexity, defined as the number of independent mutable traits that contribute to fitness (Orr 2000; Tenaillon *et al.* 2007), also affects population fitness in finite populations: complex organisms are more sensitive to the action of drift (Hartl and Taubes 1998; Poon and Otto 2000; Tenaillon *et al.* 2007).

In this article, we attempt to further clarify the role of drift on the evolution of chaperone-like genetic robustness and to decouple the effect of drift from the effect of the mutation rate. We use Fisher's geometric model of adaptation (Fisher 1930), to map phenotype to fitness under an assumption of a vanishing mutation rate and extract exact analytical solutions for the genetic properties of the population at mutation–selection–drift equilibrium (MSDE). We examine how these genetic properties change under various population sizes and epistasis parameters and in organisms ranging in phenotypic complexity.

MODEL

A geometric model of adaptation: Fisher's geometric model of adaptation (FGM) was first introduced by Fisher in 1930 (Fisher 1930) to suggest that adaptation occurs primarily through small-effect mutations. This model has received a lot of attention in the last decade (Hartl and Taubes 1996, 1998; Orr 1998, 2000, 2006; Poon and Otto 2000; Welch and Waxman 2003; Waxman and Welch 2005; Martin and Lenormand 2006; Martin *et al.* 2007; Tenaillon *et al.* 2007) and provides a good system to study the evolution of complex systems. It relies on a simple description of an organism based on independent phenotypes under stabilizing selection contributing to fitness. These independent phenotypes define a Euclidian space in which an organism is placed according to its set of phenotype values. Accordingly, a mutation affecting the phenotype of an organism results in a change of position in the geometric space. These assumptions make FGM fairly simple as it relies on a small number of parameters:

The dimensionality of phenotype space, *i.e.*, the number of independent mutable phenotypic traits: We refer to this parameter as the phenotypic complexity of the organism (Tenaillon *et al.* 2007). It is equivalent to the reduced number of axes explaining variance in fitness due to mutational perturbations in a principal components analysis.

The mutation process: In this article, we assume that mutations are random vectors affecting all traits and that mutations are symmetrical (*i.e.*, the mutation from an allele a to an allele a^* and the corresponding back mutation from a^* to a are equiprobable). In simulations mutations are random Gaussian vectors.

The fitness function: As all traits are under stabilizing selection, a single fitness optimum exists. Here for simplicity we assume that fitness is dependent only on the distance to that optimum (fitness isoclines are spherical).

As such, FGM, which is a phenotypic model, has provided some interesting predictions on the distributions of mutational effect sizes (Fisher 1930; Kimura 1962; Orr 1999, 2000, 2006; Welch and Waxman 2003; Waxman and Welch 2005; Martin and Lenormand 2006; Martin *et al.* 2007) and the fitness costs associated with reduced population size (Hartl and Taubes 1998; Poon and Otto 2000; Tenaillon *et al.* 2007) that have been supported by experimental work (Burch and Chao 1999; Silander *et al.* 2007; Tenaillon *et al.* 2007).

To study the selective pressure acting on genetic robustness, we have to add to the model an additional evolvable trait value: the robustness that affects the way fitness is derived from phenotypes. Robustness itself can evolve, but is not part of the Euclidian phenotypic space; such a locus is a classical modifier locus (Feldman *et al.* 1980).

We define fitness classically as

$$f(\delta, \alpha) = A(\alpha)e^{-\alpha\delta^Q}$$

(supporting information, File S1, Appendix A), where δ is the distance to the optimum, Q is an epistasis parameter, and α and $A(\alpha)$ are linked to robustness and

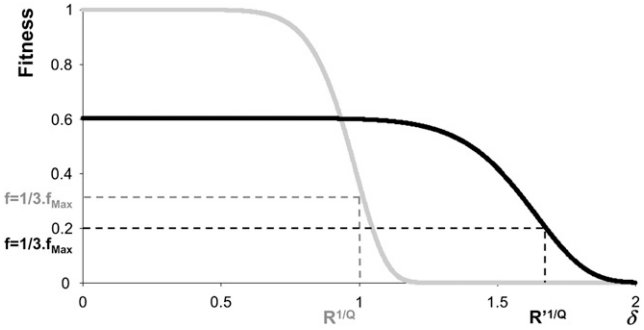

FIGURE 1.—Influence of robustness R on fitness. The fitness function *vs.* the distance to optimum (δ) is plotted for two robustness values (R). If R is small (shaded lines), the maximal fitness is high but fitness decreases rapidly as the distance to optimum increases. A higher value R' (solid lines) allows distant phenotypes to have a high fitness even though maximal fitness is reduced through the cost of robustness. In the stability threshold model (dashed lines) unstable proteins ($\delta > R^{1/Q}$) cannot fold. Even if costly, a chaperone can artificially allow some unstable proteins ($R'^{1/Q} > \delta > R^{1/Q}$) to still fold properly by artificially increasing the stability cutoff value.

its associated cost (see below). We define robustness R as the inverse of the mean effect on log-fitness of a mutation occurring in an individual initially at the optimum ($\delta = 0$),

$$\frac{1}{R} = \left\langle \mathrm{Log}\left(\frac{f(0,\,\alpha)}{f(\delta,\,\alpha)}\right)\right\rangle = \alpha\langle\delta^Q\rangle,$$

such that robustness R is proportional to $1/\alpha$. The proportionality constant depends on the particular distribution of the phenotype perturbation caused by one mutation. The following results are mostly independent of this distribution such that we can assume this constant is equal to one. Without loss of generality we can thus redefine fitness as

$$f(\delta,\,R) = \frac{1}{C(R)}\,e^{-(\delta^Q/R)}, \qquad (1)$$

where R is robustness and $C(R)$ is its associated cost [$C(R)$ increases when R increases]. In addition, with this definition, R has a more qualitative meaning: $R^{1/Q}$ reflects the distance at which fitness will be $\sim\frac{1}{3}$ of its maximal value (Equation 1, Figure 1).

The parameter Q, set to 2 in classical quantitative genetics, defines the sign of average epistasis among pair of mutations: null when $Q = 2$ (MARTIN *et al.* 2007) and positive (negative) if it is smaller (larger) than 2 (GROS *et al.* 2009). Q affects the sharpness of the transition from high to low fitness value around the distance $R^{1/Q}$ that always has fitness equal to $\sim\frac{1}{3}C(R)$ (Figure 2). When Q becomes very large, fitness is 1 if distance to the optimum is $<R^{1/Q}$ and fitness is 0 if distance is $>R^{1/Q}$. An analogous model has been used to study how protein stability modulates the effect of mutations (BLOOM *et al.* 2005). If the protein stability is below a given threshold, the protein is folded and

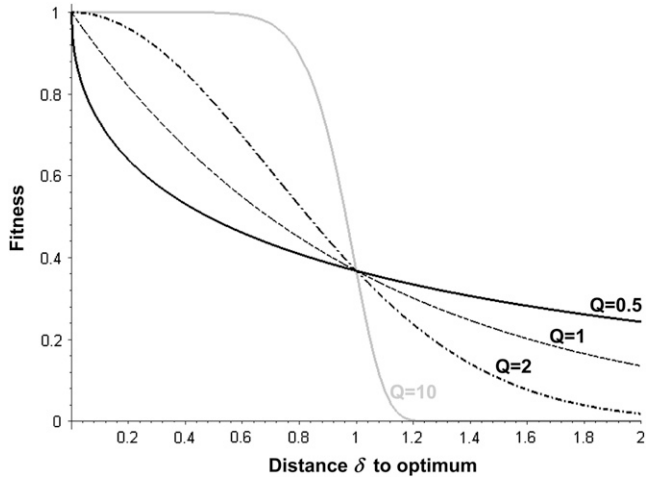

FIGURE 2.—Influence of Q on fitness. Fitness is plotted against the distance δ to optimum for various values of the epistasis parameter Q. As Q increases, fitness changes from concave up to concave down and decreases more rapidly as the distance to optimum increases such that the mean epistasis becomes less positive and finally more negative.

fitness is maximal, if it is above the threshold the protein is unfolded and fitness is null. As most mutations destabilize proteins, the closer the stability of the protein is to the threshold ($R^{1/Q}$ in our formalism), the larger the fraction of mutations that result in unfolded proteins. Overexpression of a chaperone will allow some unfolded proteins to be folded properly and will therefore result in a shift of R to a higher value. The cost $C(R)$, an increasing function of R, reflects the increased energy requirement of enhanced chaperone-assisted folding.

METHODS

Theoretical results were confirmed by simulations using an individual-based model of evolution as previously described in TENAILLON *et al.* (2007). Briefly, a population of N individuals evolves under a Wright–Fisher mode of sampling. Each discrete generation, each individual gives birth, on average, to $Npf\langle f\rangle^{-1}$ descendants to produce the next generation, where p is its frequency in the population, f is its fitness, and $\langle f\rangle$ is the mean population fitness. Its descendants can then mutate at rate $0.01/N$ (multiple mutations are allowed) with a probability drawn from a Poisson distribution. The mutation step is drawn from an isotropic Gaussian distribution centered on the individual position in the n-dimensional space. In simulations in which robustness evolves, the additive mutation of R is sampled from a uniform distribution between −0.01 and +0.01.

RESULTS

Population at mutation–selection–drift equilibrium: FGM provides an interesting framework to study the

behavior of populations at MSDE, *i.e.*, finite size populations evolving under the joint effect of mutations, selection, and genetic drift. Exact solutions can be found (Sella and Hirsh 2005), in the limit of a small mutation rate, when populations are monomorphic unless a single mutant occurs and either reaches fixation and replaces the previous population or is lost. Sella and Hirsh (2005) showed that at MSDE, the probability density of fitness f is

$$p(f) = \frac{f^{v}\rho(f)}{\int f^{v}\rho(f)df},$$

where $\rho(f)$ is the density of genotypes with fitness f and v is related to the population size and depends on the exact mode of sampling of the model; for a Wright–Fisher mode of sampling and a haploid population of size N, $v = 2N - 2$. Therefore, for a given value of robustness R the probability density of the distance δ to the optimum is

$$p(\delta \mid R) = \frac{f^{v}(\delta, R)\rho(\delta)}{\int f^{v}(\delta, R)\rho(\delta)d\delta},$$

where, with a slightly abusive notation, $\rho(\delta)$ is the density of genotypes at distance δ and is proportional to δ^{n-1} (*i.e.*, to the surface area of a hypersphere with radius δ in an n-dimensional Euclidian space).

We finally have

$$p(\delta \mid R) = \frac{1}{A(Q, v, n, R)}\delta^{n-1}e^{-v(\delta^{Q}/R)} \tag{2}$$

with the integration constant

$$A(Q, v, n, R) = \int_0^{+\infty} \delta^{n-1}e^{-v(\delta^{Q}/R)}d\delta = \left(\frac{R}{v}\right)^{n/Q}\frac{\Gamma(n/Q)}{Q}.$$

This results in a mean distance to the optimum

$$\overline{\delta(R)} = \frac{\Gamma((n+1)/Q)}{\Gamma(n/Q)}\left(\frac{R}{v}\right)^{1/Q} \approx (\overline{\delta^{Q}})^{1/Q} \approx \left(\frac{R}{4N_e}\right)^{1/Q} \tag{3}$$

with

$$N_e(n, Q, N) = N\frac{Q}{2n}. \tag{4}$$

Similarly the fitness distribution can be computed and results in a mean logarithm of fitness

$$\begin{aligned}\overline{\mathrm{Log}(f(R))} &= \int_0^{+\infty} \mathrm{Log}(f(\delta, R))p(\delta \mid R)d\delta \\ &= -\frac{1}{4N_e(n, Q, N)} - \mathrm{Log}(C(R))\end{aligned} \tag{5}$$

and a mean fitness

$$\overline{f(R)} = \int_0^{+\infty} fp(\delta \mid R)d\delta = \frac{1}{C(R)}\left(\frac{v}{v+1}\right)^{n/Q}.$$

If $v \gg 1$, as is almost always the case in natural populations,

$$\overline{f(R)} \approx \frac{1}{C(R)}e^{-(n/Qv)} \approx \frac{1}{C(R)}e^{-(1/4N_e(n,Q,N))} = e^{\overline{\mathrm{Log}(f(R))}}. \tag{6}$$

We have introduced an effective population size $N_e(n, Q, N)$ that depends on complexity (n), epistasis (Q), and the real population size (N). This definition is justified by two observations:

i. For a hypothetical haploid population of the smallest complexity ($n = 1$) with no average epistasis ($Q = 2$), this effective population size is equal to its real population size: $N_e(1, 2, N) = N$. (Note, however, that *stricto censu* the population size N we consider here is what is classically called the effective population size, which takes into account deviations from the census population size due to non-Poisson distribution of reproductive success. Here, complexity and epistasis modify the value of this classical effective population size N.)
ii. N_e is independent of both the robustness parameter R and the function specifying the cost of robustness ($C(R)$). It is an intrinsic parameter reflecting how far from the optimum the population is (Equation 3) and, consequently, how much finite population size affects the fitness of the population in combination with the phenotypic complexity (n) and the epistasis parameter (Q) (Equations 4–6). Thus $1/N_e$ quantifies the intensity of drift in this model. This last property of $N_e(n, Q, N)$ becomes obvious when we look at the genetic load. The genetic load, defined by

$$L = \frac{f_{\mathrm{Max}} - \bar{f}}{f_{\mathrm{Max}}},$$

quantifies the proportion of fitness that is lost when selection fails to maintain the population concentrated in the fittest genotype. In our treatment, since we are working in the limit of small mutation rates, the only pressure that prevents selection from maintaining the population at the optimum is drift, and the drift load is given by

$$L_D = 1 - e^{-(1/4N_e)}, \tag{7}$$

which again is completely determined by N_e. Furthermore, we can show that if the mutation rate increases, the total load becomes

$$L = 1 - e^{-(1/4N_e)-\mu} \tag{8}$$

(File S1, Appendix B), such that if $N_e(n, Q, v) \to \infty$, we recover the classical mutation load when drift is absent

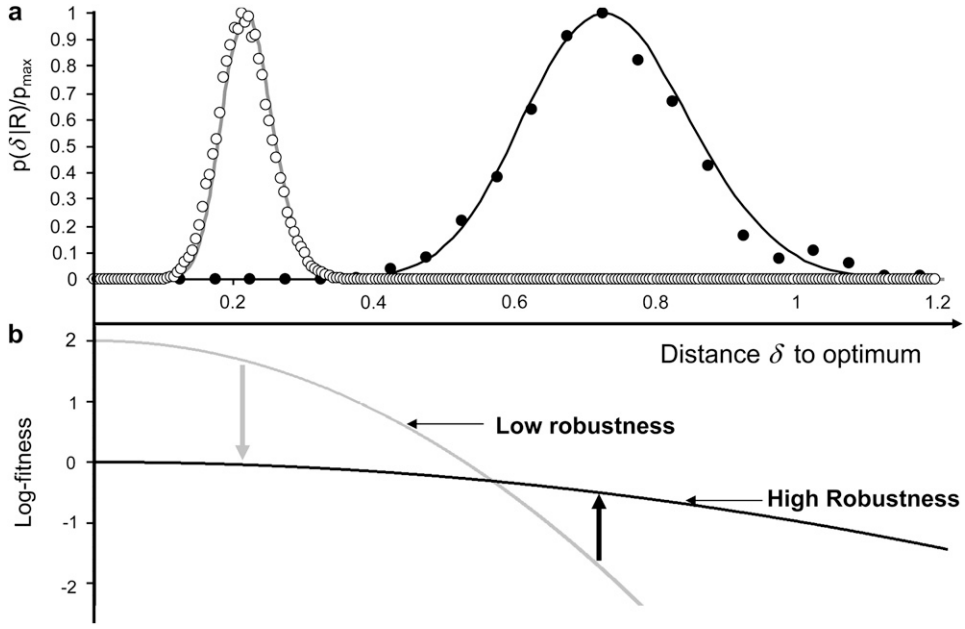

FIGURE 3.—(a) Distance probability density at equilibrium (normalized to its maximum) and (b) log-fitness as a function of distance. (a) For a strong drift intensity the population is delocalized from the optimum (solid circles and solid line, $N_e \sim 0.5$), whereas it is closer for a low drift intensity (open circles and shaded line, $N_e \sim 5$). Symbols are simulated data, lines are theoretical curves ($n = 20$, $Q = 2$, $N = 10$ or 100, $R = 1$). (b) Increasing R (*i.e.*, robustness) is deleterious in the case of low drift (shaded arrow) whereas it is advantageous in the case of strong drift (solid arrow).

(HALDANE 1937). $1/N_e$, which quantifies the drift pressure, is thus analogous to μ, which quantifies the mutational pressure. We therefore refer to $1/N_e$ in the following as the *drift intensity*.

Selection for robustness at MSDE: Now that we know how population behaves at MSDE we can study how selection will affect robustness. If the rate at which R mutates is much smaller than the mutation rate affecting the other traits, then the population will reach MSDE before R mutates. We can then study how a mutant carrying a modified value of R, R', will be selected for, in a population at MSDE with the resident value of R. In other words we can identify the evolutionary stable strategy (MAYNARD SMITH 1982) for robustness.

The selective effect of a change in R depends on the phenotype in which it appears, *i.e.*, on the distance to the optimum of the population. At MSDE the probability that the population is at a given distance can be computed (Equation 2); we can therefore compute the mean selective effect of a change in R (*i.e.*, the mean effect of a mutation that changes R to R', when we take into account the probability of the phenotype in which it appears) (File S1, Appendix C). If R' is close to R, this reads

$$\bar{s}_{R\to R'} = \left(\frac{1}{4N_e(n, Q, v)} - \frac{R}{C(R)}\frac{dC(R)}{dR}\right)\left(\frac{R'}{R} - 1\right). \quad (9)$$

What can we say now about the evolution of robustness? Equation 9 shows that the evolutionary behavior will depend on the sign of the difference $D = 1/4N_e - (R/C)(dC/dR)$. If $D < 0$, then antirobustness is advantageous (*i.e.*, the selective effect of the mutation is positive only if $R' < R$), whereas if $D > 0$, then genetic robustness ($R' > R$) becomes advantageous. Interestingly, for a given cost function and a given R value, the selection for or against robustness is determined only by N_e. The shift in selection for robustness with decreasing N_e is shown in Figure 3. As N_e decreases, the average distance to the optimum of the population increases to positions where robustness becomes more advantageous.

Long-term evolution of robustness: The previous result assumes that R does not mutate too often. However, we can relax this assumption if we look at a longer timescale such that the population has reached a global equilibrium for both the fitness influencing phenotypes and the robustness trait. Such equilibrium is completely independent of the rate and distribution of mutations acting either on the phenotypes or on the robustness trait. If robustness R can vary between R_{min} and R_{Max} and, for instance, the cost function is proportional to R^p (*i.e.*, a power function of robustness), we find (File S1, Appendix D) that if drift is intense ($1/N_e \gg p$), $\bar{R}_{1/N_e \gg p} \approx R_{Max}$, and maximal robustness is selected for; conversely, if selection is more efficient than drift ($1/N_e \ll p$), then $\bar{R}_{1/N_e \ll p} \approx R_{min}$ and minimal robustness is selected for. Hence, high phenotypic complexity, positive epistasis, and small population sizes (Equation 4) tend to select for increased robustness (Figure 4a).

The cost of robustness: Drift and mutational pressure combine to make populations suffer from several loads; our results suggest that selection for costly robustness can generate an additional load. We have seen that populations with intense drift will select preferentially for genetic robustness. But we have also seen that the mean fitness is proportional to the cost $1/C(R)$ (Equation 6). Therefore, in populations subjected

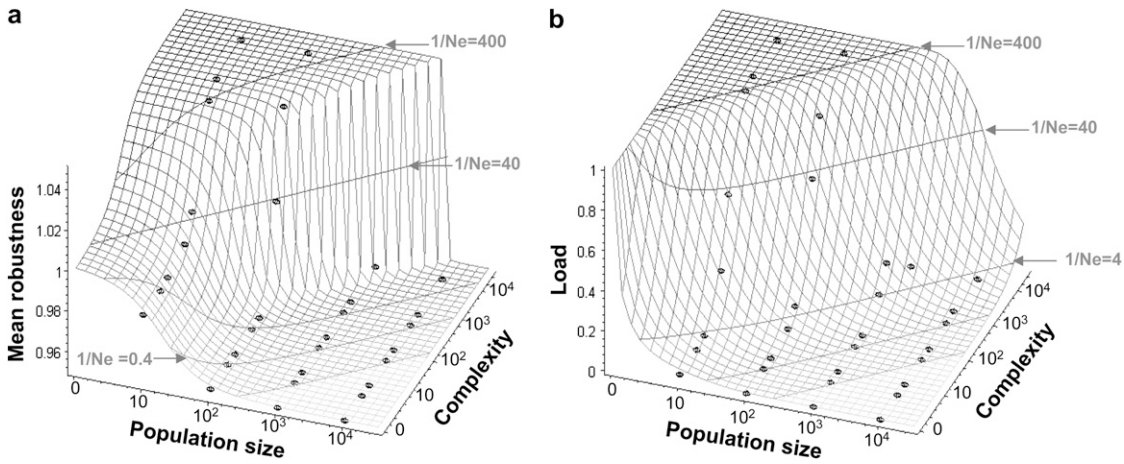

Figure 4.—Long-term equilibrium values of (a) robustness and (b) load as a function of the complexity and the population size. Complex organisms with small population sizes (strong drift intensity, dark shading) tend to be more robust and less fit than simpler organisms with large population sizes (weak drift, light shading). Lines are theoretical expectations (the intensity of shading denotes drift intensity). Dots are simulation results. $C(R) = R$, $R_{\min} = 0.95$, $R_{\max} = 1.05$, $Q = 2$, $\mu = 0.01/N$, $\mu_R = 0.1\mu$.

to high levels of drift, the selection for robust mechanisms will lead to a decrease of the mean fitness. This paradox comes from the two different timescales we use to look at the population fitness: on a short timescale, it is very likely that populations drifting far from the optimum will evolve increased robustness; however, at a larger timescale, since robustness increases the tolerance to mutations, deleterious mutations can fix more easily, and the mean (average in time) fitness slowly decreases in return (Figures 5 and 6). Once additional deleterious mutations have been fixed, returning to lower levels of robustness is highly counterselected. Depending on how R evolves and on its associated cost function, robustness can either increase indefinitely, leading to a vanishing mean fitness, or reach a maximal value where physical constraints impede further evolution of R. Hence, if selection is intense ($1/N_e \ll p$ in our example), the total load L_{long} is reduced to Equation 8, whereas if drift is intense, we have

$$L_{\text{long}} \underset{1/N_e \gg p}{=} 1 - \left(\frac{R_{\min}}{R_{\text{Max}}}\right)^{p} e^{-(1/4N_e(n,Q,\nu))-\mu} \underset{R_{\text{Max}} \text{ or } 1/N_e \to +\infty}{\to} 1$$

(File S1, Appendix E), which tends to the maximal value 1 when R_{Max} increases or when N_e decreases, that is to say, equilibrium fitness converges toward 0 (Figure 4b).

DISCUSSION

Since the beginning of molecular biology and the first mutagenesis experiments, it has become universally accepted that biological organisms are more robust to mutations than what was *a priori* expected (Wagner 2005). This property is called mutational robustness and is hence widely observed in nature. In the last decade, the conditions allowing the promotion of genetic robustness by natural selection have been carefully analyzed (de Visser *et al.* 2003). If genetic robustness is not a by-product of some other processes under selection such as resistance to environmental perturbations, then it has been mainly proposed that genetic robustness would be selected for in large populations experiencing a high mutation rate. Hence, genetic robustness analyses have concentrated on RNA viruses, which present both high mutation rates and large population sizes (Montville *et al.* 2005; Sanjuan *et al.* 2007), while it was implicitly presumed that in other organisms genetic robustness resulted from pleiotropic effects of selection for robustness to environmental perturbations.

Several mechanisms may promote mutational robustness and their mode of selection could be different. Our model is quite general; however, looking at a particular example may help to substantiate its conclusions. For instance, if we assume that the fitness of an organism increases with the number of functional copies of a given protein, an interesting model is that of Bloom *et al.* (2005) in which a protein is assumed to be properly folded if it is stable enough; *i.e.*, its free energy of folding is lower than a given threshold. In this experimentally validated model, fitness is a function of stability, and epistasis emerges as more stable proteins can tolerate more mutations than less stable ones (Bloom *et al.* 2005; Bershtein *et al.* 2006). Another way to affect the tolerance to mutation is to modulate the threshold position, *i.e.*, to modulate the fraction of proteins that will be properly folded given their free energy of folding. This can be achieved on a local or a global scale. Locally, within a protein, a mutation might facilitate the folding dynamics of the protein but not affect its thermodynamic stability; mutation R120G in TEM1 β-lactamase has been shown to possess such properties (Bershtein *et al.* 2008). Globally, it has been shown with chaperones such as GroESL (Fares *et al.* 2002) in bacteria or HSP90 in eukaryotes (Rutherford and

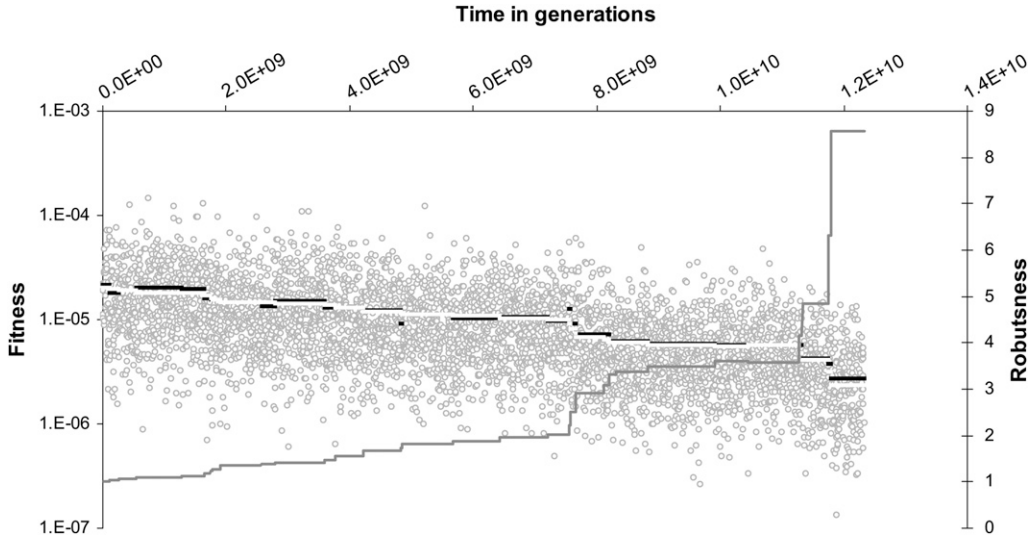

FIGURE 5.—The paradox of robustness. When robustness is selected for (shaded line, right axis) the mean population fitness decreases on a long timescale (open circles, left axis). $n = 200$, $Q = 1$, $C(R) = R$, $N = 10$, $\mu = 0.01/N$, $\mu_R = 1E\text{-}6\mu$. Simulated (solid line) mean fitness and theoretical (open line, Equation 6) mean fitness for each fixed value of R (*i.e.*, time averages over windows of given population robustness R) are represented.

LINDQUIST 1998) that chaperone proteins can assist in the folding of some destabilized proteins on the basis of the exposure of hydrophobic residues and result in a higher tolerance to mutation. Such chaperone-associated robustness is likely to be costly to the cell, as it requires synthesizing a new protein that itself needs many ATPs to function. This would result in a phenotypic landscape resembling that of Figure 1, which can be modeled by Equation 1 in FGM.

Using FGM and an exact solution in the limit of a small mutation rate, we have shown that contrary to what is commonly assumed, the selection for genetic robustness does not require high mutation rates. In fact, our results suggest that any mechanism that impedes the maintenance of a population at the maximal fitness through natural selection will tend to select for genetic robustness, even in asexual populations [contrary to what has been suggested before (GARDNER and KALINKA 2006)]. Our present work illustrates that genetic drift efficiently promotes selection for mutational robustness. More precisely, Equations 3 and 6 reveal how drift alone affects the position of a population in phenotypic space and its corresponding fitness, while Equation 9 reveals how drift affects the action of selection on a modifier of robustness. When the mutation rate is small, intense drift promotes the selection for genetic robustness regardless of the real value of the mutation rate. This result is in agreement with the KRAKAUER and PLOTKIN (2002) study: when drift is strong, the population is, on average, far from the optimum (Equations 3 and 6 and Figure 3), and mutation-loaded individuals benefit directly from increased robustness. When the drift intensity is low, then the population is concentrated closer to the optimum, and robustness is deleterious because of its cost. Contrary to what has often been assumed (WAGNER 2005; GARDNER and KALINKA 2006), this result is independent of the mutation rate: mutational robustness can be selected for even at a vanishing mutation rate in finite populations.

Mutational robustness is a property of mutant fitness. It can be selected for only in conditions in which the alleles present in the optimal genotype of the population experience the load associated with the presence of deleterious mutations. In other words, the genotypes that will contribute to the future of the population must feel the effect of deleterious mutations and how a modifier of robustness affects them. In an infinite asexual population with no back mutations, as used in classical models (GARDNER and KALINKA 2006), a copy of an allele that is associated with a deleterious mutation is doomed to disappear without any further contribution to the mutation-free class. Hence selection for a modifier of robustness is impossible as the mutation-free class, the only contributor to the evolutionary future of the population, never experiences the effect of the modifier: the load is independent of the effect of mutations (HALDANE 1937). In the presence of recombination, the situation is different. A copy of a beneficial allele can be at some time associated with a deleterious allele at another locus and still contribute to the future of the population since it may join the mutation-free class through a recombination event. Hence a fraction of the optimal genotypes comes from mutation-loaded individuals influenced by the modifier of robustness (GARDNER and KALINKA 2006). This is illustrated by the contribution of the effect of mutations to the load (DESAI *et al.* 2007), which allows selection for robustness (GARDNER and KALINKA 2006). Similarly in asexual infinite populations, the existence of back mutations would allow selection for robustness. A modifier reducing the effect of deleterious mutations will enrich to a larger extent its mutation-free class with the reversion of mutation-loaded genotypes, as its mutants that remain longer in the population will have more chance to generate the back mutation. This is indeed the property used in the selection for robustness in asexual populations evolving on neutral networks. In such networks, in which fitness is either maximal or null, mutations between maximal fitness genotypes cannot be

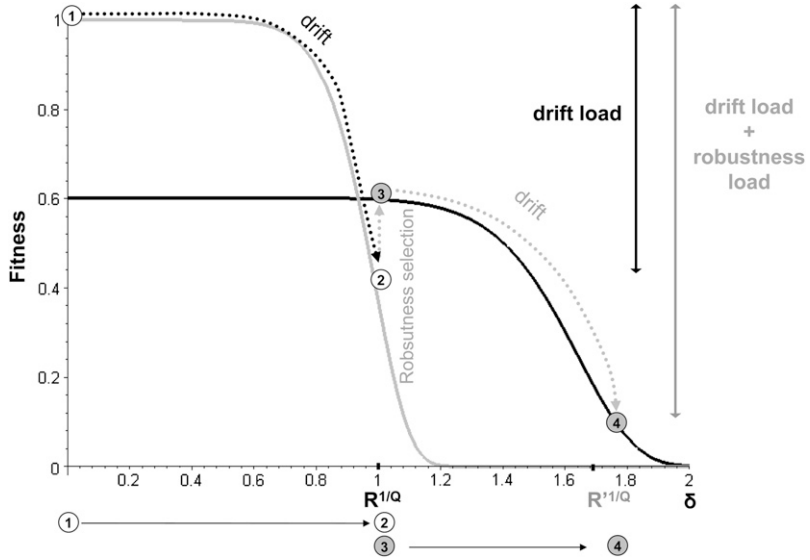

Figure 6.—Schematic view of the action of drift and selection on robustness and fitness evolution. When drift is strong, the population, initially fit (1), is drifting toward its equilibrium value (2). A mutation increasing robustness is then beneficial and can be selected for (3). The new equilibrium value has increased in proportion (4). In the new equilibrium value (4) reverting mutations that decrease robustness are strongly deleterious: "the ratchet has clicked." The circles (open and shaded) denote the robustness alleles.

neglected, as all such genotypes are as stable in the face of natural selection and can contribute to the future of the population. However, genotypes that have more neutral neighbors will get more back mutants from these neighbors and therefore be selected for (Wilke and Adami 2003). In all these models it is the mutational pressure that generates the load that promotes the selection for robustness, the mutation-free class being always present. We show here that under the same prerequisite, drift generates another form of load that is sufficient to promote the selection for robustness: it allows the population to fix deleterious mutations and therefore the optimal genotypes in the population to experience directly the effect of a modifier of robustness. The effect being direct, selection for robustness can be intense if drift is strong.

Importantly, however, the extent to which the drift can influence the selection for robustness is not solely determined by the population size. In this study, the intensity of drift ($1/N_e$) is determined by three parameters. $1/N_e = 2n/NQ$: proportional to the phenotypic complexity (n) of the organism and inversely proportional to the population size (N) and the epistasis parameter (Q). Thus decreasing the population size increases drift intensity, as has commonly been observed experimentally (Silander *et al.* 2007). High phenotypic complexity also increases drift intensity (Tenaillon *et al.* 2007). This effect of phenotypic complexity is related to the "cost of complexity in adaptation" observed by Orr (2000) in a similar model studying the dynamics of adaptation. Here, the increased load associated with increased phenotypic complexity is due to the increased deleteriousness of mutations in complex organisms as noted by Orr (1998, p. 938): changes "are more likely to disrupt a complex, tightly integrated organism than a simple one." This results in mutations that are on average more deleterious in complex organisms and in a higher drift load. Finally, epistasis (Q) can also influence drift intensity. Larger values of Q (>2) imply more negative epistasis between pairs of mutations (Gros *et al.* 2009) and thus increase selection intensity: deleterious mutations have smaller probabilities of becoming fixed when epistasis is more negative. It is hence not a surprise to find that drift intensity is inversely related to Q.

Most importantly, our model suggests that complex organisms with small effective population sizes should select robustness preferentially. This prediction is in good agreement with the observation that complex organisms [that generally have small effective population size (Lynch and Conery 2003)] seem to have evolved more mechanisms promoting robustness than simpler organisms. This gives also some consistency to the debated role of direct selection for mutational robustness in the evolution of miRNA in several eukaryote species (Borenstein and Ruppin 2006), which first appeared in contradiction with previous large population–high mutation rate theory (Szollosi and Derenyi 2009).

We have modeled the action of selection on a global modifier of robustness, *i.e.*, one acting on all phenotypic traits. This model can be extended to study the selective pressure acting on modifiers that affect only a subset of traits. We can define sets of traits n_i under Q_i epistasis and affected by modifiers R_i such that fitness is defined as $f(\boldsymbol{\delta}, \mathbf{R}) = C(R_1, \ldots, R_m)^{-1}\exp(-\sum_{i=0}^{m} R_i^{-1}\delta_i^{Q_i})$. Drift intensity is then just the sum of the drift intensity for all the set of traits, $1/N_e(n, Q, N) = \sum_i (1/N_e(n_i, Q_i, N))$, and the selection for each robustness modifier will depend only on the set of traits it affects (File S1, Appendix F). As a consequence, global modifiers, such as GroESL [this chaperone affects the folding of at least 250 proteins in *E. coli* and is required for the proper folding of 84 proteins, including 13 essential ones (Kerner *et al.* 2005)], are more likely to be selected for by drift only. Conversely, local modifiers of robustness, such as mutations involved in the folding dynamics of a single protein, are less likely to be selected for by drift

only. Presumably, high mutation rates would be required to promote their selection, as has been observed experimentally (Bershtein *et al.* 2008).

If selection for genetic robustness is favorable in the short term, our analysis reveals that it can be costly in the long term as populations experience a fitness reduction over long timescales (Figures 4 and 5). This decline results from the fact that robustness reduces the selective effect of deleterious mutations and so facilitates their fixation. Note that the distance of the population to the fitness maximum (Equation 3), which is linked to the fixation of deleterious mutations, does not depend on the cost function and that δ^Q/R is constant at mutation–selection–drift equilibrium, such that increasing robustness results in a proportional increase of the distance to the optimum. Hence when drift intensity is strong, higher robustness will be selected for. The population will then reach a new equilibrium distance distribution with an increased fitness load associated with the cost of the increased robustness (Figure 6). Once this process is initiated, selection for decreased robustness is not possible, as the population is now loaded with many more deleterious mutations that require the presence of the modifier of robustness to be tolerated; hence selection will lead to maximal robustness and its maximal cost. During this process, increased robustness becomes essential, as now many traits are maladapted and require high robustness to be functional. This prediction of the model is in agreement with the observation that some chaperones such as GroESL are essential proteins required for the folding of at least 13 essential proteins (Kerner *et al.* 2005) and that GroESL is overproduced in endosymbionts (Moran 1996).

This process has recently been called "the paradox of robustness in evolution" by Frank who explains how at a short timescale robustness can increase fitness but leads to a reduction of "performance" at longer timescales (Frank 2007). Frank does not apply the theory directly to fitness (in his study fitness always increases) but to what he calls a "maladapted" character: robustness leads to a "decrease in the performance of a character relative to what could be achieved by natural selection in the absence of robustness" (Frank 2007, p. 3). In his view the character would correspond in our model to the set of traits that are perturbed by an increased number of deleterious mutations in their underlying genes or quantitative trait loci (represented by an increased distance to the optimum). Here we provide evidence that fitness can in fact decrease because of the selection for robustness, an even more paradoxical situation. We predict that fitness will decrease until either robustness reaches a maximal value (R_{Max}) or the population goes extinct. This mechanism is qualitatively similar to the well-studied action of Muller's ratchet (Muller 1932) in small populations, in which drift impedes the maintenance of the fittest genotype in the population and leads to a ratchet-like decrease in fitness. In the situation we have modeled here, populations reach a stable fitness equilibrium in the absence of a modifier of robustness, while an increase in robustness leads to a ratchet-like decrease in fitness. This mode of evolution can also be compared to the advantages brought by mutator alleles in the case of adaptation to a new environment (Tenaillon *et al.* 1999, 2000). Mutator alleles benefit from the short time-scale advantage of the beneficial mutations they overproduce, before they pay the price of the concomitant overproduction of deleterious mutations. This phenomenon also results in a selection for an ever-increasing mutation rate that may result in population extinction (Andre and Godelle 2006; Gerrish *et al.* 2007).

The case of the obligate intracellular Buchnera offers some good illustration of the predictions of our model. Population size reduction through intense bottlenecks has increased drift intensity and is presumably the primary cause of the mutation accumulation observed in this species. Concomitantly the GroESL protein is overproduced in endosymbionts and constitutes 10% of the total proteins (Moran 1996; Fares *et al.* 2004). Moreover the *groESL* gene is less affected by drift than the other genes and seems to have fixed adaptive mutations (Fares *et al.* 2004). Since this species evolves in a stable environment, genetic robustness (as opposed to environmental robustness) has presumably been the direct target of selection in this nonrecombining species [contradicting other theoretical studies (Gardner and Kalinka 2006)]. Furthermore, the demonstrated thermodynamic instability of most of Buchnera's proteins [with the notable exception of the other chaperone DnaK (van Ham *et al.* 2003)] may illustrate the paradox of robustness: most proteins must have already acquired stronger dependency to chaperones and the ratchet-like mechanism is probably still going on.

Additionally, our model agrees with the general view of Lynch (Lynch and Conery 2003), who proposed that the apparent increase of genome complexity (measured by the genome length and gene content) observed through evolution could be explained by a congruent increase of drift (through a reduction of the population sizes) that allows costly duplications (even whole-genome duplications) to propagate in populations as they are not efficiently counterselected. Duplication can increase genetic robustness through redundancy and later on increase complexity with functional divergence of the duplicated copies. Our model suggests that decreased population size will favor the selection of costly duplications that will in return lead to a long-term fitness decline, potentially associated with a population size decline and increased complexity, both of which contribute to an increased selection for gene duplications or other robustness mechanisms and so on. This ratchet mechanism could, however, be modulated by the evolution of epistasis, which could become more negative as robustness (Wilke and Adami 2001) and complexity (Sanjuan and Elena 2006) increase.

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Selection for Chaperone-Like Mediated Genetic Robustness at Low Mutation Rate: Impact of Drift, Epistasis and Complexity

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FILE S1

Appendix

A) Fitness definition

Fitness is defined as:

$$f(\delta,\alpha) = A(\alpha).e^{-\alpha.\delta^Q}$$

Note that KRAKAUER and PLOTKIN (2002) defined fitness as:

$$f(k,s) = \frac{(1-s)^k}{\sum_{k=0}^{L}(1-s)^k} = \frac{(1-s)^k}{C(s)} \approx \frac{e^{-s.k}}{C(s)}$$

where s is the effect of a single mutation on fitness (thus inversely related to robustness), k is the number of mutations, L the length of the genome, and $C(s)$ is the cost of being robust ($C(s)$ increases when s decreases *i.e.* when robustness $R=1/s$ increases). Epistasis could be introduced through an exponent Q acting on k:

$$f(k,s) = \frac{e^{-s.k^Q}}{C(s)}$$ (DESAI *et al.* 2007; WILKE and ADAMI 2001)

Therefore our model can be seen as an extension of their model in a continuous space instead of a discrete space and adding epistasis. To insist on the robustness parameter we finally choose to define fitness as:

$$f(\delta,R) = \frac{1}{C(R)}e^{-\frac{\delta^Q}{R}}$$

Note that we could replaced the constant e by any other constant for it does not influence the conclusions since most of the results are not modified by the real value if this exponential factor (TENAILLON *et al.* 2007). Moreover, it is worth noting that our results at equilibrium are not affected by the mutation process (as long as the distribution of the phenotypic changes is centered and symmetrical around its mean), nor by the isoclines of fitness: if we were to choose them as ellipsoidal instead of circular, our results would be similar (TENAILLON *et al.* 2007).

B) Genetic load

When μ increases ($\mu \sim 1/N$), the population is no longer monomorphic and several mutants appear each generation. Thus the population suffers from a mutation load due to the segregation of some deleterious mutants between two fixation events. If μ does not increase too much, the population is dominated in proportion by the fittest genotype from which mutants appears. This genotype is the most recent common ancestor of all individuals in the population *i.e.* the last genotype that fixed. The mean population fitness between two fixation events is then approximately given by:

$< f(\delta,R) > \approx f(\delta,R).e^{-\mu}$ (HALDANE 1937):

$f(\delta,R)$ is the fitness of the most recent common ancestor and $e^{-\mu}$ is the fraction of individuals that do not mutate.

Now taking into account the density probability distribution of genotypes fixations $p(\delta|R)$ the mean fitness becomes:

$$\overline{f(R)} \approx \frac{1}{C(R)}\left(\frac{v}{v+1}\right)^{\frac{n}{Q}} e^{-\mu}$$

and the total load:

$$L \approx \frac{\frac{1}{C(R)} - \frac{1}{C(R)} e^{-K-\mu}}{\frac{1}{C(R)}} = 1 - e^{-K-\mu}$$

(This formula was confirmed by simulations, data not shown)

C) Selective effect of robustness

The selective effect of a mutation from *R* to *R'* given that the phenotypic distance of the genotype is δ is by definition:

$$s_{R\to R'} = \frac{f(\delta,R') - f(\delta,R)}{f(\delta,R)}$$

Thus the mean selective effect of a mutation changing *R* to *R'* at MSDE is:

$$\bar{s}_{R\to R'} = \int_0^\infty \frac{f(\delta,R') - f(\delta,R)}{f(\delta,R)} . p(\delta|R) d\delta$$

If we assume that the change of robustness is small such that *R'*~*R*, then a series expansion gives:

$$s_{R\to R'} = (R'-R)\frac{1}{f(\delta,R')}\left.\frac{\partial f(\delta,R)}{\partial R}\right|_R + o(R'-R)$$

$$s_{R\to R'} = (R'-R)\left.\frac{\partial \log(f(\delta,R))}{\partial R}\right|_R + o(R'-R)$$

$$\bar{s}_{R\to R'} = (R'-R)\int_0^\infty \left[\frac{\delta^Q}{R^2} - \left.\frac{d\log(C(R))}{dR}\right|_R\right] . p(\delta|R) d\delta + o(R'-R)$$

$$\bar{s}_{R\to R'} = (R'-R).\left[\frac{1}{R^2}\int_0^\infty \delta^Q p(\delta|R) d\delta - \left.\frac{d\log(C(R))}{dR}\right|_R\right] + o(R'-R)$$

$$\bar{s}_{R\to R'} = \left(\frac{1}{4Ne} - \frac{R}{C(R)}\frac{dC(R)}{dR}\right).\left(\frac{R'}{R} - 1\right) + o(R'-R)$$

with $\frac{1}{4Ne} = \frac{n}{v.Q} \approx \frac{n}{2N.Q}$

Remarks:

(i) If *C(R)=1* (no cost) then

$$\bar{s}_{R\to R'} \approx \frac{1}{4Ne}\left(\frac{R'}{R} - 1\right)$$

such that robustness is always advantageous ($\overline{s}_{R\to R'} > 0 \Leftrightarrow R' > R$) regardless of drift intensity. Nonetheless the strength of selection is directly proportional to the drift intensity.

(ii) Since

$$\delta^{Q} = -R[Log(C(R).f(\delta,R))] = -R.Log(f(\delta,R)/f_{Max})$$

and (for N not too small)

$$\overline{Log(f(R)/f_{Max})} \approx -Log(\overline{f(R)}/f_{Max}) = -Log(1-L)$$

we can approximate the mean selective effect by:

$$\overline{s}_{R\to R'} \approx (-Log(1-L) - \frac{R}{C(R)}\frac{dC(R)}{dR}).(\frac{R'}{R}-1)$$

or for a small load L:

$$\overline{s}_{R\to R'} \approx (L - \frac{R}{C(R)}\frac{dC(R)}{dR}).(\frac{R'}{R}-1)$$

emphasizing the role of the genetic load in the selection for genetic robustness.

D) Mean long-term robustness

When classical traits and the modifier trait are at MSDE, then their joint probability density can be computed as:

$$p(\delta,R) = \frac{f^{v}(\delta,R)\rho(\delta)\rho(R)}{B(Q,v,n,R)}$$

with the integration constant

$$B(Q,v,n,R) = \int\int f^{v}(\delta,R)\rho(\delta)\rho(R)d\delta.dR$$

as R is a 1-dimension variable, $\rho(R) = 1$ and

$$B(Q,v,n,R) = \int \frac{A(Q,v,n,R)}{C(R)^{v}} dR$$

Finally the mean robustness is:

$$\overline{R} = \frac{\int_{R_{\min}}^{R_{Max}} R.\frac{A(Q,v,n,R)}{C(R)^{v}} dR}{\int_{R_{\min}}^{R_{Max}} \frac{A(Q,v,n,R)}{C(R)^{v}} dR}$$

$$\overline{R} = \frac{\int_{R_{\min}}^{R_{Max}} \frac{R^{\frac{n}{Q}+1}}{C(R)^{v}} dR}{\int_{R_{\min}}^{R_{Max}} \frac{R^{\frac{n}{Q}}}{C(R)^{v}} dR}$$

If $C(R) = R^p$ then

$$\overline{R} = \frac{1+v[K(n,Q,v)-p]}{2+v[K(n,Q,v)-p]} \frac{R_{Max}^{\,2+v[K(n,Q,v)-p]} - R_{\min}^{\,2+v[K(n,Q,v)-p]}}{R_{Max}^{\,1+v[K(n,Q,v)-p]} - R_{\min}^{\,1+v[K(n,Q,v)-p]}}$$

and if drift intensity is strong ($K >> p$),

$$\overline{R}_{K>>p} \approx \frac{n+2Q}{n+Q} R_{Max} \approx R_{Max}$$

whereas if selection is strong ($K << p$),

$$\overline{R}_{K<<p} \approx \frac{1-v.p}{2-v.p} R_{\min} \approx R_{\min}$$

E) Robustness loads

The robustness load is given by:

$$L_{Rob} = 1 - \frac{C(R_{\min})}{C(R)}$$

for instance, with $C(R) = R^p$

$$L_{Rob} = 1 - (\frac{R_{\min}}{R})^p$$

And the total long time scale load:

$$L_{long} = 1 - C(R_{\min})\bar{f}.e^{-\mu} = 1 - (\overline{\frac{C(R_{\min})}{C(R)}})e^{-K(n,Q,v)-\mu}$$

with

$$\overline{(\frac{C(R_{\min})}{C(R)})} = C(R_{\min}).\frac{\int_{R_{\min}}^{R_{Max}} .\frac{A(Q,v,n,R)}{C(R)^{v+1}} dR}{\int_{R_{\min}}^{R_{Max}} \frac{A(Q,v,n,R)}{C(R)^{v}} dR}$$

$$\overline{(\frac{C(R_{\min})}{C(R)})} = C(R_{\min}).\frac{\int_{R_{\min}}^{R_{Max}} \frac{R^{n/Q}}{C(R)^{v+1}} dR}{\int_{R_{\min}}^{R_{Max}} \frac{R^{n/Q}}{C(R)^{v}} dR}$$

$$\overline{(\frac{C(R_{\min})}{C(R)})} = C(R_{\min})\frac{1+v[K(n,Q,v)-p]}{1+(v+1)[K(n,Q,v+1)-p]} \frac{R_{Max}^{\,1+(v+1)[K(n,Q,v+1)-p]} - R_{\min}^{\,1+(v+1)[K(n,Q,v+1)-p]}}{R_{Max}^{\,1+v[K(n,Q,v)-p]} - R_{\min}^{\,1+v[K(n,Q,v)-p]}}$$

And finally,

$$L_{long} \underset{K>>p}{=} 1-(\frac{R_{\min}}{R_{Max}})^{p} e^{-K(n,Q,v)-\mu} \underset{R_{Max} or K\to+\infty}{\to} 1$$

$$L_{long} \underset{K<<p}{=} 1-e^{-K(n,Q,v)-\mu}$$

F) Formula for subsets of traits:

(i) Drift intensity:

If fitness depends on *m* independent modules:

$$f(\boldsymbol{\delta},\mathbf{R}) = \frac{e^{-\sum_{i=0}^{m}\frac{\delta_i^{Q_i}}{R_i}}}{C(R_1,..,R_m)} = \frac{e^{-\sum_{i=0}^{m}\frac{\delta_i^{Q_i}}{R_i}}}{C(\mathbf{R})}$$

where $\mathbf{R}=(R_1,...,R_m)$, then

$$L_D = 1-e^{-\frac{1}{4Ne}}$$

with *1/4Ne* the total drift intensity:

$$\frac{1}{Ne} = \sum_{i=1}^{m}\frac{1}{Ne_i} \text{ with } Ne_i = N\frac{Q_i}{2n_i}$$

(ii) The selective effect of a mutations changing R_i to R_i' is:

$$\bar{s}_{R_i\to R_i'} = (\frac{1}{4Ne_i} - \frac{R_i}{C(\mathbf{R})}\frac{\partial C(\mathbf{R})}{\partial R_i}).(\frac{R_i'}{R_i}-1) + o(R_i'-R_i)$$

such that for each module *i* the strength of selection for or against robustness depends on its marginal drift intensity $1/Ne_i$.

More generally the selective effect of a multidimensional mutation changing **R** to **R'** is:

$$\bar{s}_{\mathbf{R}\to\mathbf{R}'} = \sum_{i=1}^{m}(\frac{1}{4Ne_i} - \frac{R_i}{C(\mathbf{R})}\frac{\partial C(\mathbf{R})}{\partial R_i}).(\frac{R_i'}{R_i}-1) + o(\|\mathbf{R}'-\mathbf{R}\|)$$

or more compactly

$$\bar{s}_{\mathbf{R}\to\mathbf{R}'} = (\mathbf{k}-\nabla Log(C(\mathbf{R})))\Delta\mathbf{R} + o(\|\mathbf{R}'-\mathbf{R}\|)$$

with

$$\mathbf{k} = (\frac{1}{4Ne_1R_1},...,\frac{1}{4Ne_mR_m})$$

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