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The Utility of Fisher's Geometric Model in Evolutionary Genetics

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Abstract

The accumulation of data on the genomic bases of adaptation has triggered renewed interest in theoretical models of adaptation. Among these models, Fisher's geometric model (FGM) has received a lot of attention over the past two decades. FGM is based on a continuous multidimensional phenotypic landscape, but it is mostly used for the emerging properties of individual mutation effects. Despite its apparent simplicity and limited number of parameters, FGM integrates a full model of mutation and epistatic interactions that allows the study of both beneficial and deleterious mutations and, subsequently, the fate of evolving populations. In this review, I present the different properties of FGM and the qualitative and quantitative support they have received from experimental evolution data. I then discuss how to estimate the different parameters of the model and outline some future directions to connect FGM and the molecular determinants of adaptation.

INTRODUCTION

Phenotypic complexity: the number of statistically independent phenotypic traits an organism exposes to natural selection in a given environment

With the rise of genomics and massive sequencing, the quantitative investigation of the genetic bases of adaptation seems finally at hand. Precise analysis of quantitative trait loci (QTL), genome-wide association studies, and experimental evolution coupled to whole-genome sequencing have provided new opportunities to identify individual mutations contributing to adaptation (Barrick & Lenski 2013) and to assess mutation fitness effects at large-scale (Acevedo et al. 2014, Hietpas et al. 2011, Robins et al. 2013). As a consequence, some fundamental questions in evolution can now be experimentally tackled. What is the type and effect of mutations that contribute most to adaptation? How do these mutations interact with one another? How do the rates and effects of beneficial and deleterious mutations vary among populations or among species? All these questions are linked to the properties of the genotype to phenotype to fitness map that defines a fitness landscape.

The forces that shape these fitness landscapes have biochemical, physiological, or ecological origins, and despite the progress being made to unravel them (Lewis et al. 2012), they may be difficult to apprehend other than empirically. Yet, this apparent complexity and diversity may not exclude the existence of some simplified models capturing their quantitative properties. Therefore, theoreticians have tried over the past two decades to define simplified abstract fitness landscapes that may have relevant mathematically tractable properties. The objective of such models is to define a minimal number of intelligible and measurable parameters that allow the model to capture the large body of experimental data being produced on both deleterious and beneficial mutations as well as the interactions among mutations and with the environment.

In *The Genetical Theory of Natural Selection*, R.A. Fisher (1930), one of the three founding fathers of population genetics, briefly outlined a geometrical model that synthetized his vision of adaptation. The description of the model took no more than one page, and though often cited, the model was infrequently used until the 1990s. Yet, during the past 20 years, it has been widely utilized to study the quantitative properties of adaptation and confront experimental data. The aim of this review is to present the specificity of the model, its emergent properties, and the benefits and challenges of using it to interpret data on adaptation.

FISHER'S GEOMETRIC MODEL

In Fisher's geometric model (FGM), an organism is characterized by a set of independent phenotypic traits, each corresponding to an axis in an n-dimensional Euclidian space. The axes correspond to idealized traits; they are a combination of traits that produce the orthogonal bases of a space. A genotype is assumed to generate a single phenotype and is therefore characterized by a point in this space. The dimensionality of this space is referred to as phenotypic complexity, defined as the number of independent and evolvable traits an organism exposes to the action of natural selection in a given environment.

All phenotypes are supposed to be under stabilizing selection, i.e., each has an optimal value. As a result, an optimal combination of phenotypic values exists. Fitness decays as the distance to this optimum increases. Fisher used an isotropic model, meaning that fitness was supposed to decay similarly along all axes. As a consequence, in the canonical FGM, fitness isoclines are hyperspheres centered on the optimum (**Figure 1*a***). Several functions can be used to describe how fitness declines with the distance to the optimum. For the sake of simplicity and similarity with multivariate quantitative genetics models (Lande & Arnold 1983), the following quadratic decay function has been used:

$$W(d) = e^{-\frac{d^2}{2}}, \qquad 1.$$

where d is the distance to the optimum (**Table 1**). This function has sometimes been extended (Tenaillon et al. 2007) to

$$W(d) = e^{-\alpha d^Q}. \quad 2.$$

Universal pleiotropy: the fact that all mutations affect all phenotypic traits; in standard FGM, it is equivalent to the isotropy of mutations

In FGM, mutations are represented by a vector that moves an ancestral phenotype to the mutant's phenotype. Mutations are classically supposed to be isotropic, which means they have no preferred direction and affect all phenotypic axes similarly. Termed universal pleiotropy (Paaby & Rockman 2013, Wagner & Zhang 2011), as most mutations affect all traits simultaneously, this hypothesis is not without consequences, but it has been widely used for the sake of simplicity.

To summarize, FGM can be fully described with only three metaparameters: phenotypic complexity, the fitness function, and the way mutations affect the phenotype of an organism. This limited number of parameters is the great strength of FGM: Once the parameters have been defined, all the interesting properties of the fitness landscape that constrain adaptation emerge. Indeed, despite its apparent simplicity, FGM integrates a very rich model of mutation or epistatic interactions.

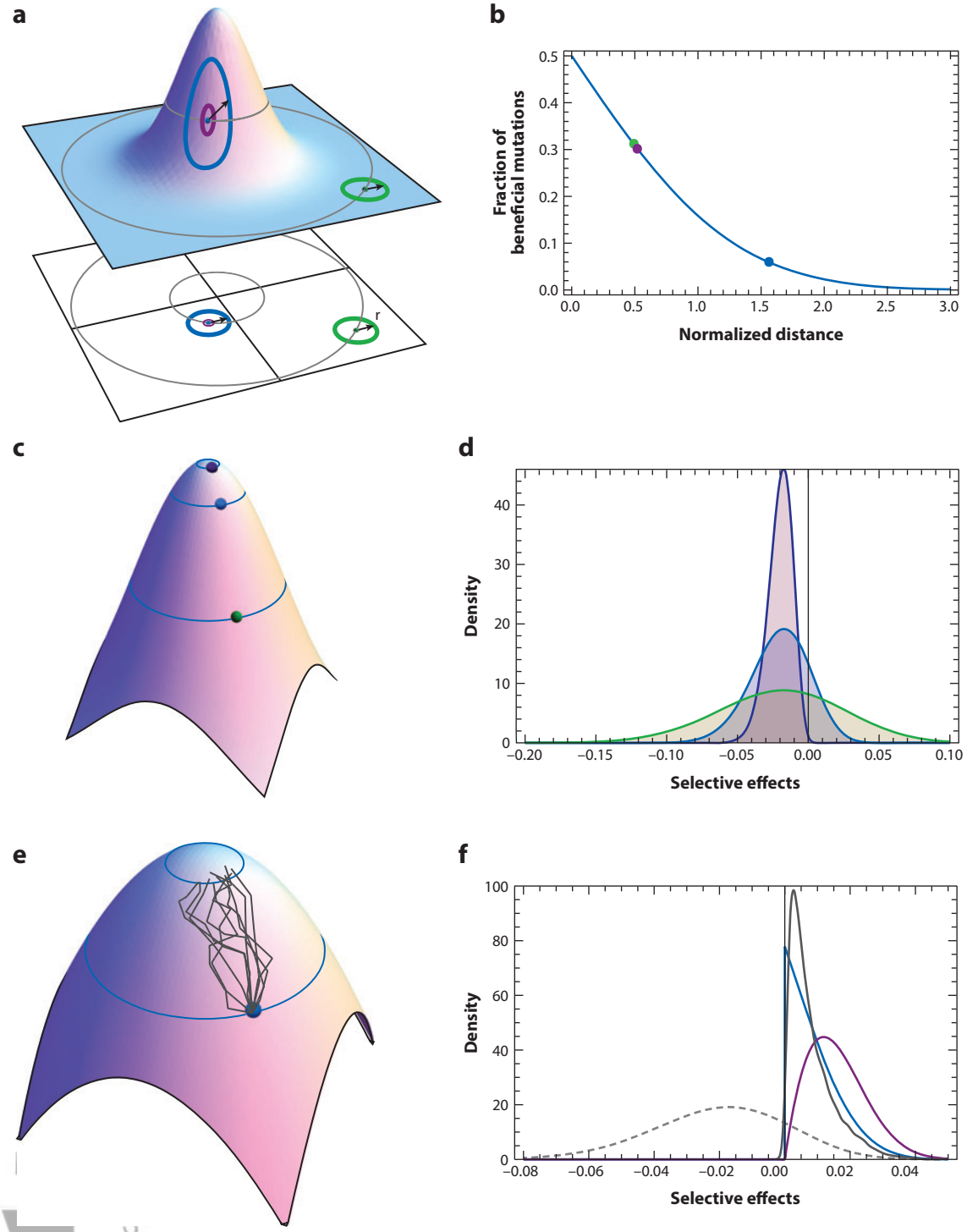
The first and only usage of FGM by Fisher took full benefit of the model's strength. Fisher supported the microevolutive vision of evolution stipulating that adaptations proceeded by mutations with very small effects. To support his vision, he showed that the fraction of beneficial mutation increases as the length of mutation vector decreases and that when phenotypic complexity is large beneficial mutations are almost exclusively of small effect (**Figure 1*a*,*b***). In other words, for complex organisms, adaptation would most likely proceed through small steps rather than a large one. Though Fisher missed the major contribution of drift to the selective process (Kimura 1983), the full power of the model is illustrated here: FGM has emerging properties concerning mutation effects and their interactions that depend on the parameters chosen but are not the direct choice of the modeler. Moreover, intuitive and visual interpretation of these properties is possible (**Figure 1**).

FGM IN LIGHT OF ALTERNATIVE MODELS OF EVOLUTION

FGM and Quantitative Genetics

As a continuous model based on phenotype traits, FGM is clearly linked to quantitative genetics models, which also define fitness from a set of phenotypic traits, often under stabilizing selection (some work on disruptive selection has also been performed). Selection and mutation may affect the traits differentially, and in most cases, Gaussian deviates are used. So, in essence, the multivariate landscaped defined in quantitative genetics and FGM are very similar.

FGM and quantitative genetics models differ in the focus they give to the mutation process. FGM primarily emphasizes the role of de novo mutation in the process of an asexual population with no standing variation. By contrast, quantitative genetics primarily focuses on the adaptive process of sexual populations with some standing genetic variation. In that process, adaptation is not a single genotype moving in the phenotypic space, but rather a population of recombining and mutating genotypes, which can be characterized in most conditions by a Gaussian cloud (Turelli & Barton 1994). A variance-covariance matrix, termed the G matrix, identifies the populations in that landscape (Lande 1979, Walsh & Blows 2009). Accordingly, mutations are not studied independently but collectively through their contribution to standard genetic variation. The underlying idea is that mutations have small effects and are distributed among many loci. Interestingly, Fisher's use of FGM convinced scientists that, because beneficial mutations have very small effects, there would be no experimental power to analyze them (Orr & Coyne 1992). As a consequence, the individual properties of mutations have rarely been the main focus of quantitative genetics, which concentrated on mean traits and fitness evolution, and few studies have



tried to connect quantitative genetics with the adaptive fate of a single mutation (but see Chevin & Hospital 2008). By contrast, per our current understanding of FGM and in line with Fisher's early work, de novo mutations are the center of attention. To unravel the properties of these mutations, most FGM studies use asexual populations with a low mutation rate such that the populations are monomorphic and can be described by a single point in the adaptive landscape (the classic strong-selection weak-mutation hypothesis).

Why has FGM's perspective on de novo mutation been so popular in recent years and not before? The recent success of FGM's perspective on mutation came from the discrepancies between the small-effect mutation hypothesis used in the models and the genetic information gathered in QTL analysis in the 1980s and 1990s (Orr & Coyne 1992): QTL analysis revealed the existence of large-effect loci. So, a new formalism was needed to uncover the genetic bases of adaptations.

Epistasis: the impact on fitness of mutation interactions, measured for pairs of mutations as the difference between the double-mutant log-fitness effect and the sum of single-mutant log-fitness effects

The Rise of FGM as a Genetic Model of Adaptation

Along with the observation of quantitative genetic variation, the rise of molecular evolution, the beginning of genomics, and the dawn of the age of experimental evolution, the genetic bases of adaptation started to trigger increasing interest. Microbial experimental evolution played a particularly important role. Most experiments in that domain use asexual organisms and initiate populations with a single individual. Therefore, adaptation must result from de novo mutations. The existence of mutations that provide resistance to phage and then to antibiotics suggested that massive selective advantages were possible. As early as 1951 (Atwood et al. 1951), researchers used a chemostat to show that a succession of selective sweeps was observed within a few hundreds of generations. In the 1980s and 1990s, the use of fitness assays through competitions enabled researchers to uncover the effect of fixed mutations. In particular, the long-term evolution of 12 replicate populations of *Escherichia coli* unraveled a succession of mutation fixations that had fitness effects of up to 10% (Lenski & Travisano 1994). Large-effect mutations appeared to be the drivers of adaptation. Consequently, the microbial experimental evolution community favored genetic models of adaptation with mutations of large effects explicitly modeled and not just integrated through their effects on standing variation.

Multilocus genetic models defining an adaptive landscape have been developed for a long time. For instance, Haigh (1978) developed the simplest genetic landscape as a set of biallelic loci, each having a similar contribution to fitness. This landscape has a single optimum, and the fitness of an individual carrying k mutations is simply $w(k) = (1 - s)^k$, where s is the selective effect of the nonoptimal allele. The model was further modified in the 1980s to consider epistasis: the impact of mutation interactions on selection (Charlesworth 1990, Kondrashov 1988). More elaborate

Figure 1

(*a*) Fitness landscape of Fisher's geometric model, with fitness on the vertical axis and below the projection on a two-dimensional plot. Fitness decays as the phenotype moves away from the optimal phenotype. Mutation of phenotypic size r moves the phenotype on a hypersphere. The same mutation (*arrow*) may have different effects depending on the initial phenotype. (*b*) Fraction of beneficial mutations as a function of the scaled effect of mutation $x = \frac{r\sqrt{n}}{2d}$. The points represent the situations illustrated in panel *a*, assuming 100 dimensions. A mutation with a large r (*blue*) is less likely to be beneficial than one with a small r from the same phenotype (*purple*) or a mutation of similar r affecting a less optimal phenotype (*green*). (*c*) The distribution of fitness effects of mutations depends on the initial position of the phenotype. (*d*) The distribution is presented for the points shown in panel *c* assuming $n = 20$; $\bar{s} = -0.01$; and initial log-fitness of −0.01 (*purple*), −0.1 (*blue*), and −0.5 (*green*). (*e*) Ten adaptive walks moving log-fitness from −0.1 to −0.01 are presented. (*f*) Different distributions of fitness effects of mutations produced from a phenotype of log-fitness −0.1 are presented (other parameters as in panel *d*): total mutations (*dashed gray*), beneficial mutations (*blue*), beneficial mutations surviving drift (*purple*), mutations fixed during adaptive walks starting from a given phenotype (*solid gray*).

Table 1 Parameters used in Fisher's geometric model

Parameter	Definition
n	Phenotypic complexity, dimensionality of the phenotypic space
d	Phenotypic distance to the optimum of the reference genotype
α, Q	Robustness and epistasis parameter influencing the decay rate and the curvature of the fitness function $W(d) = e^{-\alpha d^Q}$
$s0$	Maladaptation of the reference genotype: $s0 = -\log[W(d)] = \alpha d^Q$
r	Length of the mutational vector
σ	Standard deviation of normal deviates of the mutational vector on each axis: $\Delta z_i = N(0, \sigma)$
x	Scaled distance to the optimum $x = \frac{r}{2d}\sqrt{n}$
e	Epistasis defined as $e = \log(\frac{w_{12}}{w_0}) - \log(\frac{w_1}{w_0}) - \log(\frac{w_2}{w_0})$
m	Pleiotropy of mutations: number of phenotypes affected by mutations
N	Effective population size
μ	Mutation rate

models were further developed, but in every case, the modeler fully defined all the mutational parameters of the model. Consequently, the focus was more on mean fitness improvement or decay (Gordo & Charlesworth 2000) rather than on mutations.

As experimental evolution unraveled some properties of adaptation, modelers turned to models with some emerging properties concerning adaptive mutations, e.g., the house of cards model (Gillespie 1983, Kingman 1978). In that model, mutations are sampled from a fixed distribution. Consequently a large population, through the action of natural selection, will move toward the high-fitness tail of that distribution. Extreme value theory, which describes the properties of the tails of distribution, provided a theoretical framework to analyze the patterns of adaptation of populations (Gillespie 1991). Per extreme value theory, for instance, over a large range of conditions (Joyce et al. 2008), the distribution of fitness effects of substitutions fixed during adaptation should be exponential (Orr 2003). However, because the model is equivalent to a single-locus model with an infinite number of alleles, interactions among mutations could not be studied. Concurrently, experimental data had started to produce results on epistasis (Visser et al. 2011).

In contrast, the NK model (Kauffman 1993) was built precisely on the notion of genetic interactions between mutations. In this model, N loci are each in epistatic interaction with K others, where K tunes the ruggedness of the landscape. A single peak exists with $K = 0$, whereas the landscape is random (very rugged) with $K = N$. Although the model has some rich properties, it suffers from several drawbacks. Only a limited number of sites can be modeled, and most importantly, the statistical properties of the model are difficult to capture intuitively. The interesting domain in which multiple peaks of different heights are present seems incompatible with the convergence in fitness observed in most experimental evolution settings (Wiser et al. 2013).

With NK or the house of cards models, the perspective on beneficial mutation became the complete opposite of the view provided by theoretical quantitative genetics. Adaptation was now envisioned as the result of a handful of large-effect mutations and suggested that the use of discrete adaptive landscape was relevant. FGM, with its continuous space, initially appeared to be at odds with this perspective.

Yet, two highly influential papers uncovered the relevance and power of FGM. First, in a pioneer paper, Allen Orr (1998) showed that the FGM approach was relevant to the study of the genetics of adaptation. Rather than look at the fraction of mutations that were beneficial or at the probability that these mutations would get fixed (Kimura 1983), he followed an adaptive path. He

showed that the distribution of effects fixed in an adaptive walk was almost exponential and included large-effect mutations. This observation matched some QTL analysis and showed that FGM could be used to study adaptation with a focus on individual beneficial mutations. Second, Burch & Chao (1999) used a bacteriophage hampered by a deleterious mutation to show that small populations could adapt through the selection of numerous small-effect beneficial mutations. As Fisher showed in his model, they concluded that beneficial mutations of small effect were more numerous. Their paper had two major impacts. It showed that FGM offered a reasonable framework to study microbial evolution, and in doing so, it also suggested that the mutations were numerous enough for the continuous landscape used in FGM to be relevant.

Both papers revealed the paradoxical strength of FGM: Although based on a continuous phenotypic landscape, it is relevant to the study of the discrete genetic properties of the adaptive process. Over the past ten years, several studies have shown that the rate of beneficial mutations was high enough for a continuous approach to be relevant to the study of adaptation (Hietpas et al. 2013, Perfeito et al. 2007, Sanjuan et al. 2004b, Silander et al. 2007, Trindade et al. 2012). Moreover, the recent coupling of experimental evolution with whole-genome sequencing has confirmed that a large spectrum of beneficial mutations existed (Achaz et al. 2014). For instance, Tenaillon et al. (2012) evolved 114 populations of *E. coli* to 42.2°C. The sequencing of the lineages revealed that a few functional units are the targets of selection but that many alternative mutations may affect these targets. Up to hundreds of mutations may be selected for in a given gene, even when the gene keeps its function. Moreover, these mutations have slightly different selective effects as well as different pleiotropic side effects (Ostrowski et al. 2005, Rodríguez-Verdugo et al. 2013). The presence of hundreds or thousands of alternative beneficial mutations is enough to support a shift from a discrete to a continuous adaptive landscape.

MUTATIONAL PROPERTIES OF FGM

The above establishes the spirit of FGM. In the following, I describe in more detail some of its emerging properties and their experimental support. All parameters are defined in **Table 1**.

Fraction of Beneficial Mutations

Let us start by describing the bases of Fisher's first use of the model (**Figure 1*a*,*b***). What is the fraction of beneficial mutation, P_{ben}, for an individual at a distance d from the optimum and a mutation vector of size r with phenotypic complexity n? Initially derived by Fisher in 1930, the derivation was made much more explicit by Hartl & Taubes (1996). For $n > 10$ and $x = \frac{r}{2d}\sqrt{n}$,

$$P_{\text{ben}}(x) \approx \sqrt{\frac{1}{2\pi}} \int_x^{\infty} e^{-\frac{u^2}{2}} \mathrm{d}u = \frac{1}{2}\mathrm{erfc}\left(\frac{x}{\sqrt{2}}\right).$$

Several observations can be derived from this formula. First, $\mathrm{erfc}(\frac{x}{\sqrt{2}}) < 1$, so $P_{\text{ben}}(x) < 1/2$; there is always an excess of deleterious mutations. This is in agreement with common sense: Mutations are more likely to disrupt things than to improve them. It also agrees with the mutation accumulation experiments done in microbes (Chao 1990, Kibota & Lynch 1996).

Second, when mutations have a small effect compared with the distance to the optimum ($r/d \ll 1$), P_{ben} can be close to 50%. Thus, beneficial mutations can be extremely frequent when their effect is small. Geometrically, in these conditions, the curvature of the fitness isoclines at a distance d and is negligible compared with the curvature of the mutation radius. This was Fisher's initial point, which has been recovered in several experimental systems (Chao 1990, Maisnier-Patin et al. 2002).

Similarly, if r/d is small because d is large (in other words, if the initial individual is extremely maladapted), then the fraction of beneficial mutations will be high. The high rate of beneficial mutation in maladapted genotypes has been observed in many settings (Hietpas et al. 2013, Perfeito et al. 2007, Sanjuan et al. 2004b, Silander et al. 2007). Microbiologists commonly find that extremely debilitating genotypes can be compensated for within a single colony growth. Using a more quantitative approach based on the evolution of bacteriophage, a rate of beneficial mutations as high as 20% may be found in extremely low-fitness clones (Silander et al. 2007). By contrast, no beneficial mutations have been detected in high-fitness clones.

Finally, as the number of dimensions increases, the fraction of beneficial mutations decreases. This complies with the common sense intuition that mutation is more likely to disrupt a complex system than a simple one. In terms of mathematics, the fraction of vectors that points toward the origin is vanishingly small in high dimensions.

Distribution of Fitness Effects of Mutations

The evolutionary fate of populations is not only conditioned by the rate of beneficial mutations, but also based on the whole distribution of fitness effects of mutations (DFEM) that includes both beneficial and deleterious effects. To infer the DFEM, the distribution of mutation sizes and the fitness decay function have to be further defined. In the canonical version of FGM, with isotropic mutations and circular fitness isoclines and using the fitness function of Equation 2,

$$p(s) = \frac{e^{-\frac{\left(\frac{-s+s0}{\alpha}\right)^{2/Q}+\left(\frac{s0}{\alpha}\right)^{2/Q}}{\sigma^2}}}{\alpha Q\sigma^2}\left(\frac{-s+s0}{\alpha}\right)^{\frac{\frac{n}{2}+1-Q}{Q}}\left(\frac{s0}{\alpha}\right)^{(1-\frac{n}{2})/Q} I_{\frac{n}{2}-1}\left[\frac{1}{\sigma^2}\left(\frac{-s+s0}{\alpha}\right)^{1/Q}\left(\frac{s0}{\alpha}\right)^{1/Q}\right],$$

in which $s0$ is the maladaptation of the ancestor $s0 = -\log(W_0)$, σ^2 is the variance of the normal deviates used for mutations along each axes, $I_k(y)$ is the modified Bessel function of the first kind, and Q controls the curvature of the fitness function (**Table 1**). The moments are

$$\bar{s} = -\alpha\sigma^Q 2^{\frac{Q}{2}}\frac{\Gamma\left(\frac{n+Q}{2}\right)}{\Gamma\left(\frac{n}{2}\right)}{}_1F_1\left[-\frac{Q}{2},\frac{n}{2},-\frac{1}{2\sigma^2}\left(\frac{s0}{\alpha}\right)^{\frac{2}{Q}}\right]-s0$$

and

$$\mathrm{var}(s) = \alpha^2\sigma^{2Q}2^Q\frac{\Gamma\left(\frac{n}{2}+Q\right)}{\Gamma\left(\frac{n}{2}\right)}{}_1F_1\left[-Q,\frac{n}{2},-\frac{1}{2\sigma^2}\left(\frac{s0}{\alpha}\right)^{\frac{2}{Q}}\right]-(\bar{s}+s0)^2,$$

where ${}_1F_1$ is the Kummer confluent hypergeometric function. This simplifies in the case $Q = 2$ to

$$\bar{s} = -\alpha n\sigma^2$$

and

$$\mathrm{ver}(s) = 2\alpha\sigma^2(-2s0-\bar{s}) = -\bar{s}2\alpha\sigma^2(1+2\varepsilon), \text{ with } \varepsilon = -\frac{s0}{\bar{s}} = \frac{s0}{\alpha n\sigma^2} > 0 \text{ as } s0 > 0.$$

Using a moment-matching approach, Martin & Lenormand (2006a) showed that, with $\alpha = \frac{1}{2}$ and $Q = 2$, the distribution of effects was well approximated by a shifted negative gamma distribution:

$$p(s) = \frac{e^{-\left(\frac{s0-s}{a}\right)}}{\Gamma(b)}(s0-s)^{b-1}a^{-b};$$

$$a = \sigma^2\frac{(1+2\varepsilon)}{(1+\varepsilon)}, \quad b = \frac{n}{2}\frac{(1+\varepsilon)^2}{(1+2\varepsilon)}.$$

This distribution converges toward a normal distribution when n is large:

$$p(s) \sim \mathrm{N}\left[-\frac{\sigma^2 n}{2}, \sqrt{2\alpha\sigma^2\left(2s0 + \frac{\sigma^2 n}{2}\right)}\right],$$

which is quite similar to the distribution of effects found when the effect size of mutations is kept constant to r, as derived by Waxman & Peck (1998) (with $r^2 = n\sigma^2$):

$$p(s, r) \sim \mathrm{N}\left(-\frac{r^2}{2}, \sqrt{2\frac{r^2}{n}s0}\right).$$

Several observations can be made from these formulas. First, as long as selection is stabilizing as modeled by Equation 2, the mean effect of mutations is always deleterious. Second, when $Q = 2$, the average mutation effect is constant but the variance increases with the distance to the optimum (Martin & Lenormand 2006b). In other words, in stressful environments, the variance in effects, not the mean effect, should increase (**Figure 1*c,d***) (for some experimental support, see Martin & Lenormand 2006b). Third, the negative gamma distribution for the proposed DFEM has also received strong experimental support (Hietpas et al. 2013, Jacquier et al. 2013, Martin & Lenormand 2006b, Sanjuan et al. 2004b, Trindade et al. 2012). Experiments in which single mutations were introduced into a gene (Hietpas et al. 2013, Jacquier et al. 2013), a virus (Sanjuan et al. 2004b), or a bacterial genome (Elena et al. 1998) have all produced this type of distribution when mutant fitness or proxies have been measured. Fourth and most importantly, these mathematical derivations suggest a highly dynamic DFEM in the adaptive landscape. As mentioned above, the fraction of beneficial mutations as well as the overall shape of the distribution (**Figure 1*c,d***) change. Using antibiotic resistance mutations (Trindade et al. 2012) or mutations centered in the active site of the chaperon HSP90 (Hietpas et al. 2013), experiments have shown that changes in the environment affect the variance of the distribution. Moreover, using two clones of beta-lactamase TEM-1 separated by a single stabilizing mutation, Jacquier et al. (2013) found a very different DFEM on the enzyme efficiency. Finally, Barrick et al. (2010) demonstrated that the distribution of beneficial mutations changes among clones that differ in fitness as a result of a single-point mutation. Clones with lower initial fitness have access to beneficial mutations of larger effects.

Distribution of Epistasis

The change in the DFEM with the position in phenotypic space suggests that FGM has a built-in model of epistasis. Graphically, this can be pictured by looking at the effect of a given mutation vector in different places within this space. The fitness effect of a mutation may drastically change depending on the mutation's starting point (**Figure 1*a***). For instance, a mutation that moves an individual phenotype closer to the optimum is beneficial, but the same change of phenotype will be deleterious at the optimum. Epistasis can be defined as the difference in log-fitness between the effects of the double mutant (w_{12}/w_0) and those of the single mutant (w_1/w_0 and w_2/w_0):

$$\varepsilon = \log\left(\frac{w_{12}}{w_0}\right) - \log\left(\frac{w_1}{w_0}\right) - \log\left(\frac{w_2}{w_0}\right).$$

Martin et al. (2007) were the first to investigate the distribution of epistasis among pairs of mutations. If fitness is defined as in Equation 1, then the average epistasis is null in the whole phenotypic space $\bar{\varepsilon} = 0$ and is distributed approximately normally with a variance twice as large as the variance of the DFEM at the optimum, V_m^*. $\mathrm{var}(\varepsilon) = 2V_m^*$. Interestingly, these results hold in the case of deviations from the canonical version of FGM with ellipsoid fitness isoclines and have found

quantitative support from two data sets. Under fitness Equation 2, the average epistasis at the optimum can be computed as $\bar{e} = (2 - 2Q/2)\bar{s}$ (Gros et al. 2009).

Genetic robustness: the mean selective effect of mutations

The fact that epistasis is distributed with both positive and negative values in FGM contrasts drastically with genetic models that have studied the role of epistasis and allowed only positive or negative epistasis (Kondrashov 1988). Yet, experimental data on viruses (Sanjuan et al. 2004a), bacteria (Elena & Lenski 1997), and yeast (Costanzo et al. 2010) have all supported these broad distributions of epistasis as well as the predictions of FGM. When Q differs from 2, the mean epistasis can be shifted toward positive ($Q < 2$) or negative ($Q > 2$) values. Accordingly, the intensity of epistasis is directly connected to the intensity of the mutation effects, showing that epistasis and genetic robustness are intrinsically connected as suggested by computational models (Wilke & Adami 2001). When $Q = 2$, the model also predicts that epistasis among beneficial mutations will be negative on average (Blanquart et al. 2014, Martin et al. 2007). Because beneficial mutations tend to point toward the optimum, they tend to follow the curvature of the fitness function, which has a diminishing return. Once again, this prediction fits quite well with experimental data (Chou et al. 2011, Khan et al. 2011).

The study of epistasis among beneficial mutations can be extended to focus not only on pairs of mutations, but also on all possible combinations of mutations found in an adaptive walk. In several experimental systems, all possible combinations based on four to five beneficial mutations were generated and an adaptive landscape was built from their fitness effects (Chou et al. 2011, Khan et al. 2011, Weinreich et al. 2006). Despite the original continuity of this landscape, similar landscapes can be obtained in FGM when focusing on a panel of mutations, for instance, a set of mutations fixed in an adaptive walk (Blanquart et al. 2014). Interestingly, landscapes built from different realizations of stochastic adaptive walks in the same exact conditions were highly variable according to epistasis and accessible paths. Hence, FGM suggests that, despite their appealing simplicity, these small experimental landscapes contain limited information about the underlying architecture of the global adaptive landscape.

Distribution of Dominance

FGM can also be used to study evolution in diploid populations (Manna et al. 2011). In this case, mutations will affect only one of the two copies of each gene. Assuming additivity within the phenotypic space between alleles at each locus, mutations generate heterozygotes whose phenotype is intermediate between both homozygotes. Dominance is then akin to the study of epistasis between two identical mutations in the haploid model.

Subsequent derivations that suggest an average dominance of 0.25 have found global support from experimental data but need more accurate testing (Manna et al. 2011). Contrary to other qualitative discussions on the emergence of dominance, FGM has the benefit of proposing a quantitative model that can be tested. Moreover, every kind of dominance is present in FGM: For example, overdominance emerges when the heterozygous state is closer than both homozygous states to the optimum, as a mutation overshoots the optimum (Sellis et al. 2011).

ADAPTIVE PROPERTIES OF FGM

Distribution of Substitutions

Once the DFEM has been characterized, the mutations that will be fixed through adaptation from a given position in the landscape may be studied further. To contribute to population adaptation, beneficial mutations must not only appear, but also survive genetic drift, a process that is directly coupled to the mutation's impact on fitness. Kimura (1983) was the first to study this problem and

to moderate Fisher's micromutationist conclusions. He showed that most of the frequent small-effect mutations are lost by drift and that mutations of intermediate size contribute primarily to adaptation.

Numerical solutions of the exact distribution and the gamma distribution or analytical solutions for the normal approximation can be used to show that fixed mutations have larger effects than do beneficial mutations (**Figure 1*e,f***). When $s0 \ll 1$, n is large, and $Q = 2$, the distribution of a beneficial mutation, s_b, and of fixed mutations, s_f, can be described by beta distributions $\frac{s_b}{|s0|} \sim \text{Beta}(1, n/2)$ and $\frac{s_f}{|s0|} \sim \text{Beta}(2, n/2)$, respectively (Martin & Lenormand 2008). If $x = \frac{r}{2d}\sqrt{n} = \sqrt{\frac{-n\bar{s}}{4s0}} = \frac{n\sigma}{2\sqrt{2s0}} \gg 1$ and n $\gg 1$, then $\frac{s_b}{s0} \sim \text{Exponential}\left(\frac{n}{2s0}\right)$, $\frac{s_f}{s0} \sim \text{Exponential}\left(\frac{n}{4s0}\right)$, and $\overline{s_f} = \frac{4s0}{n} = 2\overline{s_b}$. However, for this approximation to be valid, beneficial mutations must be extremely rare, such that extreme value theory can be applied to the tail of the distribution. As such, the scaling by the overall mean mutation effects disappears (Martin & Lenormand 2008, Orr 1998, 2006).

Adaptive Walks

Adaptation is the process of a single beneficial substitution, but it also results from several substitutions. To study adaptive walks, one must remember that the DFEM changes with position in the phenotypic space. Yet, Orr (1998) observed self-similarity in the process. For instance, when $x \gg 1$, after a mutation is fixed with an average effect of $\frac{4s0}{n}$, the same problem found with a new $s0$ of $s0_1 = s0\left(1 - \frac{4s0}{n}\right)$. The distribution of mutations fixed over an adaptive walk can then be studied and appears to be roughly exponential, as suggested by QTL analyses (**Figure 1*e,f***).

The rate of adaptation can also be computed (Orr 2000) when mutations are rare enough not to interfere. Following the log-fitness, the rate of improvement of log-fitness is the production of a beneficial mutation per generation, $N\mu\int_0^{+\infty} p(s)\mathrm{d}s$, times the probability the beneficial mutation will survive drift, $\frac{\int_0^{+\infty} 2sp(s)\mathrm{d}s}{\int_0^{+\infty} p(s)\mathrm{d}s}$, times the selective effects of mutations surviving drift, $\frac{\int_0^{+\infty} 2s^2 p(s)\mathrm{d}s}{\int_0^{+\infty} 2sp(s)\mathrm{d}s}$. Hence, $\frac{\mathrm{d}\ln(W)}{\mathrm{d}t} = N\mu\int_0^{+\infty} 2s^2 p(s)\mathrm{d}s$, which can be numerically evaluated with exact or approximate solutions.

From this equation, a triple cost of complexity emerges (Orr 2000). As complexity increases, (*a*) there are fewer beneficial mutations. These mutations have a smaller fitness effect, which diminishes (*b*) their chances of surviving drift and (*c*) their influence on the rate of adaptation. At a given distance from the optimum, the rate of adaptation is maximal for an intermediate size of mutations (r or σ). However, this optimum effect size r changes with distance to the optimum, so no mutational size is optimal in the whole landscape: When far (close) from the optimum, large (small) mutations maximize the rate of adaptation.

Here, I focus only on models of asexual populations with a fixed optimum; however, the model has also been used to analyze a moving optimum (Gordo & Campos 2013, Kopp & Hermisson 2009) or, in some cases, the impact of recombination (Peck et al. 1997). Moreover, recent developments have been based on studies of diploids rather than haploids. If mutations of large effects are common, then large-effect mutations that overshoot the optimum will regularly be selected for. As a result, the adaptive walk will recruit mutations with overdominance effects (Sellis et al. 2011).

EQUILIBRIUM PROPERTIES

If adaptation proceeds for long enough, what will the fate of finite populations be in FGM? The evolutionary fate of a finite population depends on the balance between the probability of

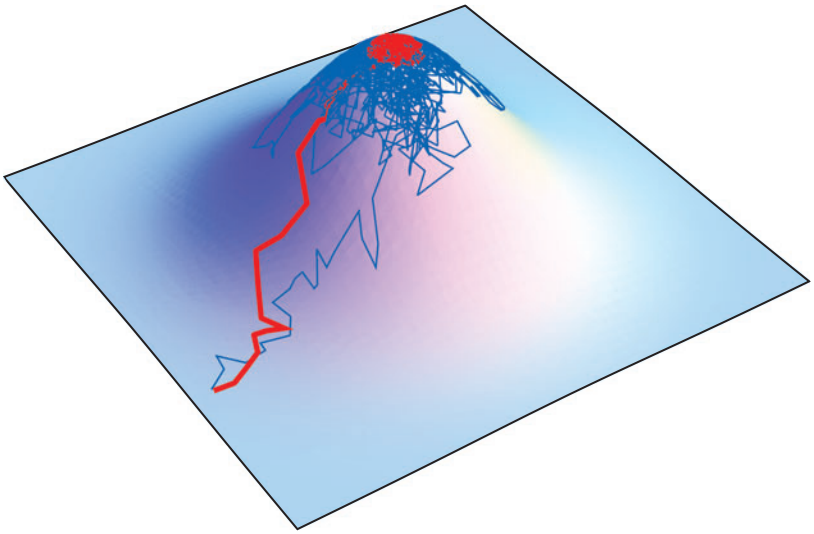


Figure 2

Populations evolve toward some mutation-selection-drift equilibrium depending on their population size. Here, the adaptive walks are presented starting from two different nonoptimal phenotypes: (*blue*) population size of 5; (*red*) population size of 100.

producing and fixing beneficial mutations and the probability of producing and fixing deleterious mutations. As shown for FGM, the DFEM and the fraction of beneficial mutations are highly variable (**Figure 2**). The fact that fitness improvements are more likely as fitness decreases suggests the existence of fitness equilibriums (Hartl & Taubes 1998, Poon & Otto 2000, Tenaillon et al. 2007, Wagner & Gabriel 1990). Within the limit of a small mutation rate, if fitness is defined by Equation 2 and the effective population size is N, an exact solution for the distribution of log-fitness may be derived (Gros & Tenaillon 2009, McCandlish et al. 2014, Tenaillon et al. 2007):

$$p(-\log(w)) = \Gamma\left(-\log(w), \frac{n}{Q}, 2N-2\right) = \frac{(2N-2)^{n/Q}}{\Gamma(n/Q)}\left[-\log(w)\right]^{\frac{n}{Q}-1} e^{(2N-2)\log(w)}$$

$$\overline{log(w)} = -\frac{n}{Q(2N-2)} \approx -\frac{n}{2QN}.$$

Drift load: the loss of fitness associated with the fixation of deleterious mutations caused by the inefficiency of selection in small populations

Pleiotropy of a mutation: the number independent phenotypic traits a mutation affects

The equilibrium represents the loss of fitness due to drift: the drift load. The contribution to drift load of the ratio between effective population size and phenotypic complexity reveals that natural selection requires very large population sizes to be efficient enough to optimize many traits simultaneously. The epistasis parameter Q also contributes to the load. The higher the value of Q, the lower is the mean epistasis and the lower the load. Increasing Q leads to increasingly drastic costs of deleterious mutations as they accumulate; therefore, natural selection can more easily filter them out. Finally, as long as the mutational process is symmetrical (mutating from phenotype A to phenotype B is as likely as going from B to A), it does not contribute to the drift load. The pleiotropy and size of the mutations (see below), the existence of modules, and the mutation rate have no impact, and each of these traits contributes an equal share of $1/4N$ to the load (Tenaillon et al. 2007). Despite some experimental evidence, the existence of these equilibria is still debated. Using bacteriophages, it was shown that the fitness of populations with initial low and high fitness converged toward a similar value when populations of similar sizes

evolved: High-fitness populations decayed, whereas low-fitness populations adapted, suggesting convergence in fitness (Silander et al. 2007, Tenaillon et al. 2007).

Indeed, if the mutation rate is such that $N\mu \ll 1$, the contribution of recurrent mutations to the load, or mutation load, is $-\mu$. Thus, the mutation load can be added to the drift load. When $N\mu$ is greater than 1, the mutation load can be computed as $-\sqrt{\frac{n}{2}\mu\bar{s}}$, which is no longer independent of the mutation process (Lande 1980, Martin & Gandon 2010, Roze & Blanckaert 2014).

Mutation load: the loss of fitness due to the recurrent production of deleterious mutations

MOLECULAR EVOLUTION

FGM can also be used to study molecular evolution. Interestingly, at mutation selection drift equilibrium (MSDE), evolution is ongoing, and an equal share of beneficial or deleterious mutations are fixed (Hartl & Taubes 1996; Razeto-Barry et al. 2011, 2012; Sella & Hirsh 2005; but for an interesting perspective on mutations at equilibrium, see McCandlish et al. 2014). MSDE can therefore be used to study molecular evolution and the accumulation of incompatibilities between populations evolving independently (Chevin et al. 2014). Under simplifying assumptions, Gu (2007) suggested that the mean rate of evolution as well as its heterogeneity among sites could be linked to the parameters of FGM. Moreover, using recurrent shifts of the optimum, Lourenco et al. (2013) found that the rate of molecular evolution was linked more to phenotypic complexity than to population size.

SELECTIVE PRESSURE ON FGM PARAMETERS

As we have seen, the various parameters of the FGM influence the adaptive process. So, if these parameters show heritable variation, they could evolve by natural selection. In FGM formalism, these parameters are kept constant and their impact on the processes can be compared. Although the selective pressure controlling the evolution of the parameters cannot be analyzed in FGM, it can be explored with the use of modifiers: An additional locus controlling the value of the parameters is added to FGM, and its quantitative evolution is then followed.

This approach has been performed on the genetic robustness parameter α (Gros & Tenaillon 2009) and on the epistasis parameter Q (Gros et al. 2009) at equilibrium with a low mutation rate. For instance, with some similarity to a previous genetic model (Krakauer & Plotkin 2002), if reducing the fitness consequences of mutation effects on traits (by reducing α) comes at a cost, then two selective regimes exist. If the ratio of population size to phenotypic complexity is large, $N/n \gg 1$, selection removes efficiently deleterious mutations and the cost of robustness is too much of a burden; minimal robustness evolves. However, in the opposite case, $N/n \ll 1$, the populations are loaded with deleterious mutations. Thus, there is a short-term benefit to decreasing the fitness consequences of mutations, and costly robustness is selected for. However, once the new robustness is selected for, the population moves to the new equilibrium fitness and its fitness decreases again. Hence, in this regime, the population is captured in a vicious situation, termed the paradox of robustness (Frank 2007), in which ever-increasing robustness is selected for and fitness keeps declining in the long term (Gros & Tenaillon 2009).

These observations are relevant in the context of protein repair by chaperones. The overexpression of chaperones decreases the effect of deleterious mutations (Fares et al. 2002), as chaperones enforce the folding of destabilized mutant proteins. Yet, this repair comes at a cost: the production of chaperones and use of ATP. Interestingly, the paradox of robustness seems to be at work in obligate endosymbionts that have a low effective population size, very high production of chaperones, and low-stability proteins (Gros & Tenaillon 2009). Finally, though the commonly accepted hypothesis stipulates that an elevated mutation rate is required to select for genetic robustness

(de Visser et al. 2003), these models further show that drift alone can also select for robustness. Although these modifier approaches have been studied only at MSDE, they may also be studied in other regimes, for instance, during adaptation or when the mutation rate is high.

PARAMETERS OF FGM

FGM is relevant for understanding qualitative and quantitative facets of evolutionary genetics. It is now becoming a standard reference model on which any quantitative observation on mutations and adaptation may be projected. Thus, it is becoming similar to the idealized Wright-Fisher model in population genetics. The constant population size of hermaphrodites in panmixia within the Wright-Fisher model has no biological reality. However, a population with a different demography and reproductive regime can be projected on that model and can be attributed an effective population size, which is the only parameter of the model. As a result, populations of farm animals and of *Drosophila*, for instance, may be compared. FGM is more complex than the Wright-Fisher model, but as shown above, it can be characterized by three metaparameters defining the landscape and one defining the initial fitness of the population. How can such parameters be estimated or chosen?

Fitness Function

The fitness decay of the form $W(d) = e^{-\alpha d^Q}$ has been most commonly used. The parameter α is a scaling factor that can be compensated for by a change of scale in the phenotypic axis. However, if some phenotypic measures are performed and linked to fitness, then it can be fitted to the data. What should the value of Q be? Measures of epistasis strongly support an almost centered distribution of epistasis and, therefore, also support a value of $Q = \sim 2$. Moreover, when close to an optimum, an FGM-like model with $Q = 2$ seems to emerge in a very large set of conditions (Martin 2014). A value of Q different from 2 should nevertheless be used only if the mean epistasis significantly deviates from 0, or if the mean effect of mutations consistently changes with the level of adaptation, which may be the case at the gene level (Jacquier et al. 2013).

Within FGM, fitness isoclines are assumed to be circular, yet the model can be extended to include anisotropy in the selection and mutation processes, leading to ellipsoidal fitness isoclines and a mutational cloud (Martin & Lenormand 2006a, Waxman & Welch 2005). This extension can be performed assuming a mutation matrix M and the fitness function $W(z) = e^{-\frac{z^T S z}{2}}$, where z is the position vector and S is a selection matrix. In both M and S, nondiagonal terms represent covariances among the different phenotypes. Nevertheless, the model can be reduced to an isotropic FGM that has similar mutational properties and an effective number of dimensions equal to

$$n_e = \frac{n}{1 + CV(\sigma_i^2)^2} = \frac{n\,\mathrm{E}(\sigma_i^2)^2}{\mathrm{E}(\sigma_i^4)} \quad \text{and} \quad \sigma_e^2 = \mathrm{E}(\sigma_i^2)(1 + CV(\sigma_i^2)^2) = \frac{\mathrm{E}(\sigma_i^4)}{\mathrm{E}(\sigma_i^2)},$$

where σ_i^2 is the eigenvalues of the SM matrix, CV is the coefficient of variation, and E indicates Esperance (Martin & Lenormand 2006a). This is a moment-matching transformation. It computes n_e as the number of dimensions, each affected similarly (whereas the $n - n_e$ other dimensions are not affected), such that the mean and variance of σ_i^2 over all n traits are identical to the one observed. For instance, if there are large differences in the selective pressure acting on the different axes [$CV(\sigma_i^2)^2 \gg 1$], then the mutational properties of the system will be equivalent to FGM with a very limited number of dimensions.

Effect Size of Mutations

Two theoretical approaches have been primarily used to obtain the effect size of the mutation vector: either a single value r (Fisher 1930, Orr 1998) or a (chi-squared) distribution resulting from a multivariate Gaussian deviation of standard deviation $\boldsymbol{\sigma}$ along each phenotypic axis (Gros et al. 2009, Martin & Lenormand 2006a). In both cases, and especially when comparing with data, most authors maintain constant the effect size of the vector across phenotypic complexity. Hence, in the constant r model, r is fixed across dimensions; when r is drawn in a distribution, $\sigma^2 = -\frac{\bar{s}}{\alpha n}$ such that the mean effect of mutations is kept constant and can be fitted to an experimental measurement of $\bar{s}$. Under this scaling, both models converge when n is large. Indeed, in the Gaussian multivariate model, the square of the length of the vector r^2 has a chi-squared distribution with n degrees of freedom and with mean $-\frac{\bar{s}}{\alpha}$ and variance $\frac{2\bar{s}^2}{\alpha^2 n}$, which is roughly equivalent to a constant r when n is large.

When at the optimum, this scaling can give some nonbiological distribution of effects for large dimensions (**Figure 1*d***). Thus, the model implies that all mutations have approximately the same deleterious effects and the absence of both large- and small-effect mutations. Consequently, either dimensionality is small, or the hypothesis of universal pleiotropy is wrong.

Pleiotropy

The concept of pleiotropy was defined more than a century ago (Stearns 2010) and refers to the idea that a mutation affects several phenotypic traits simultaneously. Yet, in the different fields of biology, pleiotropy has different meanings that only partially overlap (Paaby & Rockman 2013). With recent high-throughput methods (Nichols et al. 2011, Wang et al. 2010) and quantitative genomics approaches (Wagner et al. 2008), large-scale analyses of pleiotropy have been performed. They suggest (mostly using gene knockdown) that only a few mutations affect many traits and seem to reject the concept of universal pleiotropy (Nichols et al. 2011, Wagner & Zhang 2011, Wagner et al. 2008, Wang et al. 2010). Moreover, they suggest that more pleiotropic mutations have larger effects than do less pleiotropic mutations. However, technical issues on the contribution of measurement error, the definition of the phenotypic traits that are sometimes measured in different environments, and the estimation of pleiotropy for lethal mutations (Paaby & Rockman 2013) all contribute to the continued debate on the extent of pleiotropy. The point here is to see how departure from universal pleiotropy affects the results discussed above and how pleiotropy may be estimated from the data (for a detailed discussion of pleiotropy, see Wagner & Zhang 2011).

Restricted pleiotropy does not affect the drift load results (Tenaillon et al. 2007), but the distribution of mutations is fully dependent on it (Chevin et al. 2010, Lourenco et al. 2011). If all mutations have a pleiotropy of m, then at the optimum the mean effect of mutation scales with m rather than with n (Lourenco et al. 2011) and could be used to infer pleiotropy. However, away from the optimum and especially if there is a distribution of pleiotropy, all derived formula cannot be computed, though some quantitative predictions may still hold (Chevin et al. 2010).

The cost of complexity may be affected by pleiotropy, but it may also depend on the scaling that is used between mutation effects and pleiotropy. In FGM, pleiotropy may be defined as the dimension of the hyperspace that mutations in a locus may reach (Chevin et al. 2010) and, therefore, may be independent of size effects. Alternatively, it can be a directly linked to the length of the mutation vector (Lourenco et al. 2011): A more pleiotropic mutation affects a greater number of traits and has an overall increased length. In the latter case, intermediate pleiotropy results in a higher adaptation rate (though, as discussed above, the optimal pleiotropy changes with adaptation) (Wang et al. 2010). Yet, there always seems to be a cost of complexity, as the fraction

of mutations (Welch & Waxman 2003) pointing toward the optimum is always limited when the number of dimensions is high.

Phenotypic Complexity

The final parameter of FGM is the dimensionality of the phenotypic space. This parameter is quite important for its mathematical contribution to previous results and for its biological interpretation. Just like pleiotropy, complexity is a widely mentioned concept in biology that extends further than its mathematical definition in FGM. Nevertheless, it has myriad definitions, most of which are imprecise and subject to anthropocentrism. Within FGM, phenotypic complexity is a quantitative measure that reflects the number of variationally quasi-independent traits an organism is exposing to the action of natural selection in a given environment. The definition of complexity provided by FGM is powerful because it is centered on natural selection as the unifying concept of biology and can therefore be applied to all evolving organisms. It also offers a full theoretical background for its analysis (Le Nagard & Tenaillon 2013, Le Nagard et al. 2011).

On the basis of mathematical derivations, FGM offers various ways to estimate effective phenotypic complexity. Unfortunately, these derivations reveal different facets of the model. Thus, they echo limitations of the concept of effective population size whose definition differs depending on whether the focus is on neutral, deleterious, or beneficial mutations.

- The first derivation is ideal and corresponds to a statistical vision of FGM (Le Nagard et al. 2011). If all traits could be measured for thousands of genotypes, then the dimensionality of the space could be derived through a principal component analysis. Though difficult to perform on real organisms, this analysis can be applied to artificial systems. Nevertheless, high-throughput data will make this approach more realistic in the near future.
- The second derivation comes from fitting the DFEM, including all mutations or only beneficial mutations. Martin & Lenormand (2006a) showed that the moments of the DFEM could be used to infer phenotypic complexity: Assuming a multivariate normal DFEM on traits, $n = \frac{\mathrm{var}(s)}{\bar{s}^2}$ at the optimum. Applying this formula to existing data revealed a surprisingly low phenotypic complexity ranging from 0.5 for bacteria to 1 for yeast (Hietpas et al. 2013) and 3 for *Caenorhabditis elegans*. Indeed, as discussed above, these estimates heavily rely on the hypotheses of universal pleiotropy and may be better interpreted as a proxy of pleiotropy (m) rather than the dimensionality of the space (n). Other methods have been derived on the basis of beneficial mutations. Bataillon et al. (2011) and Sousa et al. (2012) studied the distribution of beneficial mutations in various environments and genetic backgrounds (loaded with a deleterious mutation linked to antibiotic resistance) to compute a complexity ranging from ~3 for *Pseudomonas fluorescens* to 26 for *E. coli*. Later, Perfeito et al. (2014) used mutation accumulation and compensation to derive an estimate of 9 for *E. coli*. Finally, the precise distribution of epistasis between mutations may provide a proxy for complexity (Weinreich & Knies 2013), but experimental noise combined with the assumption that the initial genotype is at the fitness peak may limit the power of this method.
- MSDE derivations provide alternative estimates of complexity. Because they are independent of the mutation process, they are not affected by pleiotropy or any scaling of the mutation processes (Tenaillon et al. 2007). However, these results hold only if the mutation rate is low. Moreover, they require an evolution of populations that is long enough to reach equilibrium, an approach that is amenable to experiments only in microbes or artificial systems. When applied on two viruses, these derivations yield a complexity of 40 or 10.

Which complexity should a modeler use? Within the hypothesis of universal pleiotropy, a low effective complexity should be utilized. FGM will then be useful primarily in terms of the DFEM and the accumulation of random mutations or short-term adaptation. If interested in long-term adaptation, a modeler should likely use a higher phenotypic complexity associated with some restricted pleiotropy, but more theoretical developments are needed. Indeed, the adaptive process as well as the drift load are affected by the total number of dimensions of the phenotypic space, which defines the fraction of mutations that points toward the optimum.

PERSPECTIVES ON FGM AND GENETICS

FGM is a powerful framework to study evolution, yet as shown for dominance, pleiotropy, or an estimation of dimensionality, there is still some progress to be made. What is now needed is a deeper biological understanding of FGM. In other words, we need to understand how the molecular determinants of an organism in an environment generate an evolutionary process that is largely compatible with FGM.

Taking Account of the Nature of Mutations

Several aspects of the mutation process remain poorly studied in FGM. As here discussed, much progress could be made to uncover the effects of restricted pleiotropy on the distributions of effects as well as on long-term adaptation. The impact of large mutation rates on equilibrium properties has been studied (Martin & Gandon 2010, Roze & Blanckaert 2014, Wagner & Gabriel 1990), but extending these results to analysis of the adaptive process may provide another interesting perspective, especially if additive variance and a Gaussian cloud are used to describe the population (Hallatschek 2011, Wagner & Gabriel 1990).

For a comparison of FGM with real data, the integration of gene knockouts is also required. If gene expression is assumed to be a trait under selection, then the landscape of FGM does not appropriately incorporate the properties of gene knockouts. These mutations bring the trait's value to a minimum. As a consequence, the context dependency of gene knockout mutations may differ drastically from FGM predictions and may lead to the existence of some boundaries in the space of accessible trait values. Incorporating the statistical properties of these knockouts may make FGM more realistic.

The Meaning of Complexity

Much progress could be made if we gained a more biological understanding on phenotypic complexity and could infer it from the molecular constituents of an organism. First, the use of FGM to depict and eventually to predict organismal evolution could be extended to nonmodel organisms that are not amenable to evolutionary experiments in the laboratory. Second, different organisms, or their systems biology representation, could be compared within an evolutionary perspective. Take a metabolic network for instance. From genome sequences, the topology of these networks can be built and subsequently quantitatively analyzed using flux balance analysis (Lewis et al. 2012). Yet, it remains difficult to compare the evolutionary properties of different networks; networks could be ranked and compared within an FGM framework if their complexity could be computed. Third, comparative approaches could be used to study the selective pressures acting on phenotypic complexity, and the forces shaping the evolution of complexity could also be studied. The emergence and evolution of complexity are important issues in biology, and FGM offers an

interesting framework to study them, even though complexity has been associated with only a cost in FGM.

To proceed in this direction, we can use the phenotypic complexity estimates described above, but rather than apply them to real organisms whose complexity remains unclear, we can use them on artificial systems with calibrated complexity. Le Nagard et al. (2011) used this methodology to compare various estimates of phenotypic complexity on artificial neural networks that evolved in a Darwinian way. The networks had different sizes and were asked to perform tasks constituting a gradient of complexity: Simple and complex tasks were to fit polynomials of order 2 and 8, respectively. Yet, the authors found that the phenotypic complexity correlated with the complexity of the task performed more than with the size of the evolving network. Moreover, this abstract computational model revealed both a mechanistic and a selective process that resulted in higher complexity. Mutations that decoupled the impact of further mutations from the various traits under selection resulted in higher complexity. They could be selected for, as they allowed fine-tuning of the different phenotypic values. Hence, complexity resulted mechanistically from the decoupling of mutation effects from the different phenotypes and was selected for as it allowed fitness improvement that could not be reached if mutations affected all traits similarly. An intermediate complexity was selected for in each task, reflecting the balance between the aforementioned benefits of complexity and the costs that FGM predicts. Though based on a fully artificial system, these results provide some interesting insights on phenotypic complexity. When extrapolated to more real systems, they support the notion of complexity based on gene duplications proposed by Ohno (1970). After duplication, mutations can affect the expression or function of only one of the two copies, which may then perform a new task or may be expressed in different tissues. This initial work paves the way for rich perspectives, but more biological models must be tested to confront the power of this approach and to generate further insights into the molecular determinants of phenotypic complexity.

The Molecular Bases of Epistasis

Along the same lines, thanks to phenotyping methods, the molecular bases of epistasis could now be compared with the sources of epistasis in FGM. For instance, negative epistasis between beneficial mutations in FGM may come from optimum overshooting. When two highly beneficial mutations in the ancestral background are combined, they may overshoot the optimum, resulting in a decline in fitness. Recent work suggests that this pattern is sometimes found. Alternative mutations downregulating the expression of an exogenous gene carried on a plasmid presented a strong pattern of negative epistasis (Chou et al. 2014). Tight control of the expression of the gene revealed that, although individual mutations improved fitness through a decreased cost of expression, the combination of mutations excessively downregulated expression. Further experiments connecting the mutations involved in adaptation to phenotypes in light of FGM will help us better understand the relevance of this phenotypic model.

CONCLUSION

FGM is an integrated model of adaptation in which many quantitative predictions emerge. These predictions concern several facets of evolutionary genetics: distribution of fitness effects, epistasis, dominance, distribution of fixed mutations, adaptive trajectories, and equilibrium fitness. These quantitative predictions can be tested experimentally, and as here discussed, many have found some experimental support and, in some cases, allowed parameters of the model or effective parameters to be inferred. Therefore, FGM can readily be used as a reference model of adaptation. Rather

than develop new specific models, evolutionary biologists should use FGM in the set of models to be tested to confront evolutionary genetics data.

Despite its apparent simplicity, FGM is extremely rich. Some of its properties, such as the distribution of epistasis or the existence of non-null fitness equilibrium, challenge certain classic models of population genetics. They suggest revisiting the role of epistasis within the evolution of recombination or the process of Muller's ratchet (Wagner & Gabriel 1990). The pervasive role of epistasis in FGM, in agreement with observations from microbial experiments (Jacquier et al. 2013, Moore et al. 2000, Perfeito et al. 2014, Poon & Chao 2005, Silander et al. 2007), could now be used beyond the scope of microbes, and its consequences may be investigated in other fields of biology such as conservation biology or medical human genetics in which the conceptual framework that FGM offers could be very beneficial.

DISCLOSURE STATEMENT

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