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COMPLEXITY, PLEIOTROPY, AND THE FITNESS EFFECT OF MUTATIONS

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One of the assumptions underlying many theoretical predictions in evolutionary biology concerns the distribution of the fitness effect of mutations. Approximations to this distribution have been derived using various theoretical approaches, of which Fisher's geometrical model is among the most popular ones. Two key concepts in this model are complexity and pleiotropy. Recent studies have proposed different methods for estimating how complexity varies across species, but their results have been contradictory. Here, we show that contradictory results are to be expected when the assumption of universal pleiotropy is violated. We develop a model in which the two key parameters are the total number of traits and the mean number of traits affected by a single mutation. We derive approximations for the distribution of the fitness effect of mutations when populations are either well-adapted or away from the optimum. We also consider drift load in a well-adapted population and show that it is independent of the distribution of the fitness effect of mutations. We show that mutation accumulation experiments can only measure the effect of the mean number of traits affected by mutations, whereas drift load only provides information about the total number of traits. We discuss the plausibility of the model.

KEY WORDS: **Drift load, genetic drift, models, molecular evolution, natural selection.**

The proportion and strength of mutations that are deleterious, neutral, or advantageous, or in general the distribution of the fitness effect of mutations (DFEM) is a fundamental aspect in evolutionary genetics and molecular evolution. Major theories are based on assumptions regarding this distribution, affecting the theoretical predictions that can be made about evolutionary processes (Eyre-Walker and Keightley 2007). Although large amounts of data are being accumulated—mostly from mutation accumulation (MA) experiments and comparative analysis of molecular data (reviewed in Eyre-Walker and Keightley 2007)—and several mathematical distributions have been proposed (Keightley 1994; Shaw et al. 2002; Piganeau and Eyre-Walker 2003; Eyre-Walker et al. 2006), there is still no consensus about the functional form of the DFEM (Welch et al. 2008).

From a theoretical point of view, a substantial amount of work has focused on Fisher's geometrical model (FGM) (Fisher 1930). In FGM, phenotypes are represented as points in a multidimensional space, the axes of which correspond to phenotypic characters (traits); fitness is a decreasing function of the distance of the phenotype to a local optimum (a combination of trait values that confers the maximal possible fitness in current environmental conditions); and mutations are random vectors that create new phenotypes from pre-existing ones. Depending on the orientation of these vectors (Fig. 1), mutations can be advantageous (if the new phenotypes are closer to the optimum) or deleterious (if otherwise).

Although FGM is in essence a model of adaptive evolution (Orr 2005a,b), it has the desirable property of making the DFEM emerge based only on a small number of assumptions. Beginning with the work of Fisher, the FGM has been a fundamental theoretical tool in such diverse areas as the genetics of adaptation (Hartl and Taubes 1998; Orr 1998, 1999, 2000a,b, 2006; Burch and Chao 1999; Welch and Waxman 2003), drift load (Hartl and Taubes 1996; Peck et al. 1997; Poon and Otto 2000; Silander et al. 2007; Tenaillon et al. 2007), evolution of sex and recombination (Peck et al. 1997), hybridization (Barton 2001), and pleiotropy (Fisher 1930; Orr 2000a; Welch and Waxman 2003).

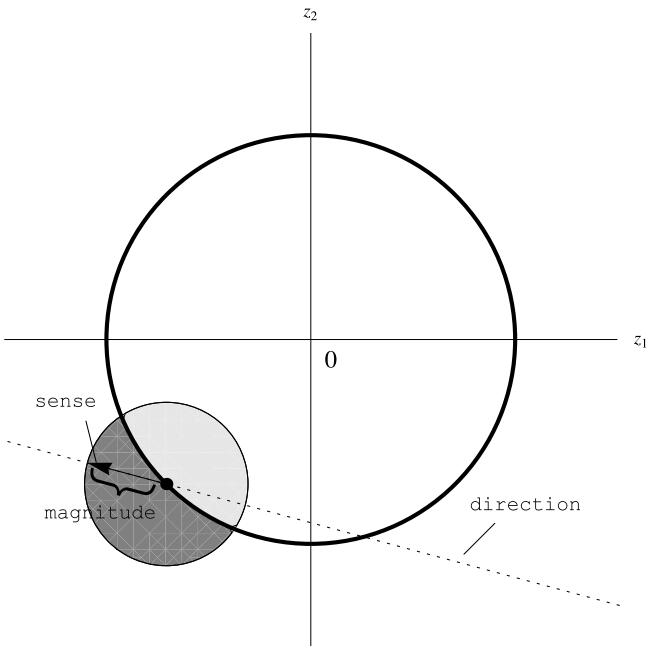

Figure 1. **Fisher's geometrical model in a two-dimensional phenotypic space. A phenotype is represented by a black point. The phenotype lies on a fitness isocline (thick circle) grouping all the phenotypes that are at the same distance from the origin. Mutations (vectors) of a given magnitude can displace the phenotype to new locations (thin circle). Mutations have a given magnitude (the length of the vector), a direction (the doted line), and a sense (indicated by the arrowhead; in one direction, there are exactly two possible senses, corresponding to the two possible orientations of a vector in the line). The orientation of a mutation is the combination of its direction and sense. The probability of a mutation being advantageous or deleterious equals the probability of the phenotype being displaced closer (light gray area enclosed by thin semi-circle) or further away (dark gray area enclosed by thin semi-circle) from the optimum. A higher number of traits decreases the probability of mutations being advantageous. This probability increases with the distance to the optimum.**

THE PARADOX OF COMPLEXITY

A central concept in FGM is complexity (i.e., the number of traits under selection). Fisher originally developed his model to calculate the probability of mutations being advantageous. Assuming that mutations can potentially affect all traits at the same time (universal pleiotropy, see Table 1), he concluded that this probability decreases as the number of traits under selection increases (Fisher 1930). Later, Orr used Fisher's approach to calculate the rate at which adapting populations increase in mean fitness, and found that more complex organisms have lower rates of adaptation, indicating a "cost of complexity" that is considerably higher than Fisher had previously suggested (Orr 2000a). These results have encouraged attempts to estimate complexity across species using methods directly based on Fisher's model. In the following, we briefly review two recent studies that have taken different roads toward this goal.

First, Martin and Lenormand (2006) made a significant contribution to the generalization of FGM (Waxman and Welch 2005; Waxman 2006) by developing a multivariate Gaussian model (Lande 1980; Welch and Waxman 2003; Zhang and Hill 2003; Martin and Lenormand 2006) that takes into account mutational and selective covariances between traits. They showed how the joint effect of these interactions result in a DFEM that would be predicted by a smaller number of independent traits, which they called effective complexity. They then estimated effective complexity by fitting their DFEM to empirical data from MA and direct measurements of single-mutation fitness effects. Surprisingly, effective complexity was very low and varied little from viruses to nematodes and fruit flies.

Second, Tenaillon et al. (2007) developed a method to estimate complexity based on the drift load—the decrease in mean population fitness in a well-adapted population that is due to the stochastic fixation of slightly deleterious mutations (Kimura et al. 1963; Hartl and Taubes 1998; Poon and Otto 2000). They showed that drift load is directly related to complexity and inversely related to effective population size. They used their method to estimate the complexity of two viruses, using data from evolution experiments with controlled population size. The results they obtained differed markedly from those of Martin and Lenormand: estimated levels of complexity were relatively high and differed significantly between the two species (45 for the bacteriophage $\phi X174$ and 10 for the vesicular stomatitis virus VSV, see Fig. 2).

These contrasting results present a paradox, as both methods are based on the same model (FGM) and, in one case, use data from the same species (the virus VSV). The paradox suggests that some assumption of the model is being violated, and we hypothesize it to be universal pleiotropy. Our hypothesis was inspired by empirical studies such as the one by Wagner et al. (2008b), in which the authors studied the effect of quantitative trait loci (QTL) on 70 skeletal traits in mouse, and found that a large portion of the QTL affect only a small fraction of the measured traits (50% of the QTL affect less than 10% of the traits), suggesting that pleiotropy in real life is probably far from universal.

To test our hypothesis, we developed a model of partial pleiotropy based on FGM, where the assumption of universal pleiotropy is relaxed by assuming that single mutations may affect only a subset of all traits under selection. We then used this model to analytically calculate the DFEM, the rate of adaptation, and the drift load, and to check whether partial pleiotropy, as a departure from the assumption of universal pleiotropy, would bias (or otherwise render irrelevant) inferences based on a model of universal pleiotropy.

Table 1. Table summarizing definitions of pleiotropy and complexity.

"Complexity" is usually associated with the number of different phenotypic components that characterize biological organization at different levels (from molecular to supra-organismal). However, even if complexity is discernible in nature, the concept remains elusive, as different attempts to measure it have sometimes lead to conflicting results. Due to these shortcomings, Tenaillon (2007) proposed a top-down objective biological metric that corresponds to the number of genetically "uncorrelated" traits contributing to an organism's fitness (= the number of "uncorrelated" traits under selection), and is well captured in Fisher's model by the number of axes in the coordinate system.
"Pleiotropy" is the genotype-to-phenotype phenomenon by which mutations can simultaneously affect multiple traits. As so, it can be used either as a quality (pleiotropic mutations are those that affect more than one trait) or as a metric (the number of different trait affected by mutations).
Gu specified that, from the point of view of a gene, pleiotropy corresponds to the number of distinct components in the fitness affected by all possible mutations in that gene (gene pleiotropy), as opposed to pleiotropy from the point of view of single mutations, meaning the number of traits under selection affected by single mutations (allelic pleiotropy). In this text, we use the somewhat more generalized term of "mutation pleiotropy" as the equivalent of Gu's "allelic pleiotropy" concept. Furthermore, Gu's "gene Pleiotropy" concept corresponds to the number of axes in Fisher's geometric model, and therefore, to phenotypic "complexity."
When mutation pleiotropy is inferior to complexity, pleiotropy is said to be "partial," as opposed to "universal pleiotropy," where mutations can potentially affect all of the traits under selection at the same time. In "modular pleiotropy," traits are organized into modules, and each mutation has effects limited to a single module.
Pleiotropy is said to be "isotropic" if the phenotypic effect of mutations on single traits are distributed the same way, or "anisotropic" (also called "variable pleiotropy"), if this distribution varies with trait.
Pleiotropy and complexity are two related concepts that describe the same reality but from different points of view. *Ceteris paribus*, if "loci" tend to be more pleiotropic in one organism, we intuitively expect this organism to be more complex. How are pleiotropy and complexity related in FGM? In Fishers original model, where traits are uncorrelated both for mutation and selection, both pleiotropy (as a metric) and complexity correspond to the dimensionality of the phenotypic space. However, when traits are not assumed to be uncorrelated, "effective Complexity" may be significantly lower than pleiotropy. In this case, it is still possible to replace the initial system by an equivalent system with a lower number of uncorrelated traits, the number of which correspond to effective complexity (Martin and Lenormand 2006).

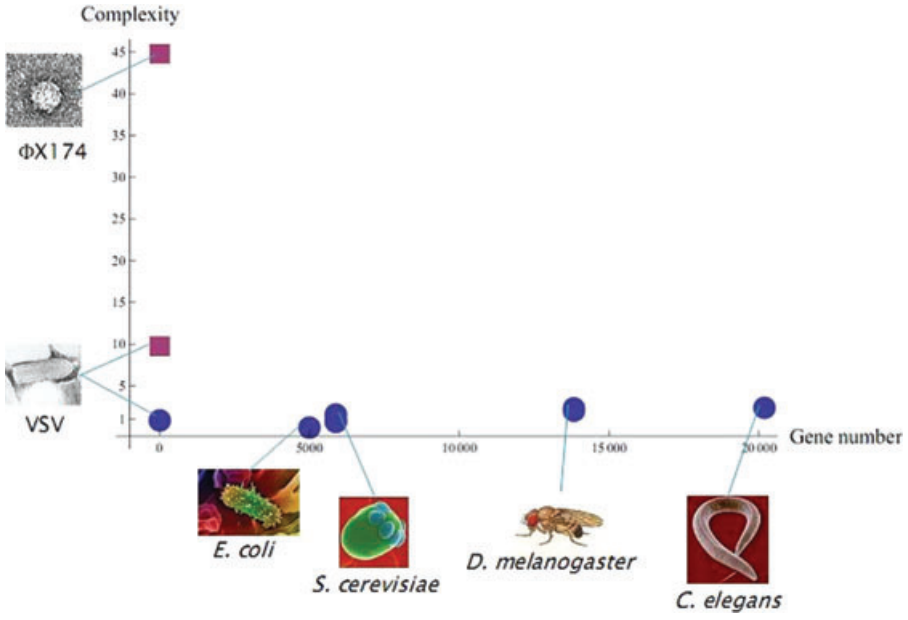

Figure 2. Estimated levels of complexity from Tenaillon et al. (2007) (squares) and Martin and Lenormand (2006) (circles) across several species, compared to the number of open reading frames (gene numbers) as surrogates for complexity. We used the gene numbers reported in Martin and Lenormand (2006) plus an estimate of 11 genes in the bacteriophage $\phi X174$ from the Kyoto Encyclopedia of Genes and Genomes (available at http://www.genome.jp).

The Model

Consider a haploid population of effective size N_e, undergoing selection, drift, and mutation. The population is described as a collection of phenotypes geometrically represented as points in an n-dimensional coordinate system, the orthogonal axes of which correspond to continuous quantitative traits. Traits are assumed to be under stabilizing selection of identical intensity around a local optimum, that is made to reside, by convenience, in the origin of the coordinate system.

Let $(z_1, z_2, \ldots, z_n)$ be the position of a phenotype. Fitness is determined by a Gaussian function of the Euclidean distance $z = \sqrt{z_1^2 + z_2^2 + \cdots + z_n^2}$ to the optimum,

$$w(z) = \exp(-z^2). \tag{1}$$

GEOMETRY OF MUTATIONS IN THE PHENOTYPIC SPACE

The geometry of mutations is described by two parameters—mutation pleiotropy m, the number of traits affected by single mutations; and total pleiotropy or complexity n, the total number of traits collectively affected by all mutations (Fig. 3). These parameters correspond to Gu's "allele pleiotropy" and "gene pleiotropy" (Gu 2007). The sets of traits affected by single mutations are uncorrelated, so that partial overlapping of trait sets between mutations is possible.

Assume that the effects (r_j) of a mutation on the traits follow a zero mean Gaussian distribution with standard deviation σ,

$$p(r_j) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{r_j^2}{2\sigma^2}\right). \tag{2}$$

Here, σ works as a scaling parameter for the phenotypic effect of mutations. In the remainder of the text, we will not focus our attention on σ, as our main interests are the effects of complexity and pleiotropy. The choice of a Gaussian distribution, while mathematically convenient, is consistent with the finding that mutations of small effect are more common than mutations of large one (Davies et al. 1999; Wloch et al. 2001; Wingreen et al. 2003; Eyre-Walker and Keightley 2007). It is also convenient for comparisons, as it is commonly used in FGM.

Assuming the effects of mutations across traits are independent (according to the so-called Euclidean superposition model—see the discussion below), then the total phenotypic effect of a mutation is given by

$$r = \sqrt{\sum_{j=1}^{m} r_j^2}, \tag{3}$$

which itself is random and expected to follow a generalization of the Chi distribution with m degrees of freedom,

$$\phi(r) = \frac{2^{1-\frac{m}{2}} \exp\left(-\frac{r^2}{2\sigma 2}\right)\left(\frac{r}{\sigma}\right)^{m-1}}{\sigma\Gamma\left(\frac{m}{2}\right)}. \tag{4}$$

Three illustrative examples of this distribution are given in Figure 4. For $m > 1$, most mutations have intermediate effect on phenotype.

From the geometric point of view, mutations are vectors randomly oriented within a subspace of the phenotypic space, which means that they are not randomly oriented in the n-dimensional phenotypic space. However, if we further assume that the orientation of the optimum relative to mutant phenotypes is random and independent of the orientation of mutations, then the probability of a mutation being deleterious or advantageous is just a function

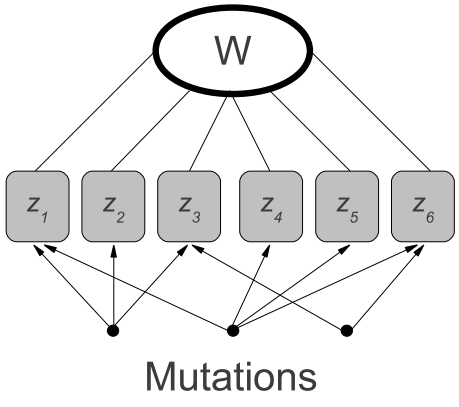

Figure 3. An illustration of how mutations affect traits in a model of partial pleiotropy. Each mutation affect a subset of traits that is uncorrelated to the sets of traits affected by other mutations. Adapted from Welch and Waxman (2003).

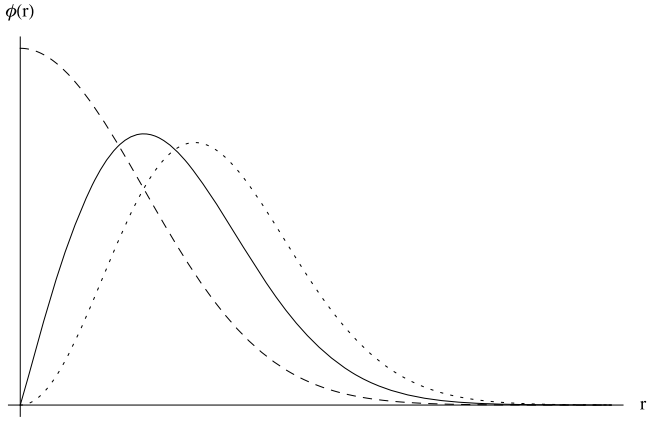

Figure 4. Distribution of the magnitude of mutational effects under the model of partial pleiotropy. The figure compares the generalization of the Chi distribution (eq. 4) when $m = 1$ (dashed curve), $m = 2$ (solid curve), and $m = 3$ (dotted curve). For $m > 1$, most mutations have intermediate effect on phenotype. The case of $\sigma^2 = \frac{\pi}{8}$ is shown.

of the distance to the optimum of the mutant phenotype, the magnitude of the mutation, and the dimensionality of the phenotypic space, as in Fisher's original analysis.

This decouples the magnitude from the orientation of mutations, as we can make the model "forget" the axial components r_j of mutations, and retain only r. Thus, to simulate a mutation vector, one just needs to randomly draw a direction and a sense in an n-dimensional space, and a magnitude from the generalization of the Chi distribution with m degrees of freedom (eq. 4).

For simplicity, we assumed selection and mutation are spherically symmetric. However, as demonstrated by Martin and Lenormand (2006), distortions of the spherical symmetry effectively reduce the number of independent traits. In that sense, the parameters of the present model are comparable to Martin and Lenormand's "effective" parameters.

THE FITNESS EFFECT OF MUTATIONS

Let δz denote the change in the distance to the optimum due to a mutation. The fitness effect of the mutation is

$$s = \frac{w(z + \delta z)}{w(z)} - 1. \tag{5}$$

Assuming that s is small enough that $s \approx \log(1 + s)$, and using equation (1), we can approximate the above expression to

$$s \approx z^2 - (z + \delta z)^2. \tag{6}$$

Results

We start by deriving an approximation for the DFEM at any distance from the optimum, for example, in a population adapting to an environmental change, and we use this approximation to determine the rate of adaptation. Next, we derive the DFEM for well-adapted populations, that is, populations in a state of dynamic equilibrium between selection, mutation, and drift in a constant environment. For this purpose, we first derive an approximation of the drift load and the mean phenotypic distance of the population to the optimum.

DFEM IN A POPULATION AWAY FROM THE OPTIMUM

Let $\psi(s; r, z)$ be the probability density distribution of s when mutations of a given magnitude r occur at random in all directions in the phenotypic space, and phenotypes are at distance z from the optimum. It has been demonstrated that, for the fitness function in equation (1), when $n \gg 1$, s is approximately Gaussian distributed (Waxman and Peck 1998)

$$\psi(s; r, z) = N\left(-r^2, \frac{4r^2z^2}{n}\right), \tag{7}$$

where $-r^2$ is the mean and $\frac{4r^2z^2}{n}$ is the variance of the distribution. Two simple comments regarding the role of n in the proportion of deleterious mutations: first, because the distribution has a negative mean, there is always a higher proportion of deleterious mutations; second, although the mean is independent of n, the variance decreases with increasing n, so the proportion of mutations that are deleterious is expected to increase when n increases—the cost of complexity.

Integrating $\psi(s; r, z)$ over r according to $\phi(r)$ (eq. 4) gives the full distribution for s at a given distance z from the optimum

$$\begin{aligned}\psi(s; z) &= \int_0^\infty \psi(s; r, z)\,\phi(r; \sigma, m)\,dr = \\ &= \frac{2^{-\frac{m+1}{2}} \exp\left(-\frac{ns}{4z^2}\right) \sqrt{n} \left(\frac{1 + \frac{4z^2}{n\sigma^2}}{s^2}\right)^{\frac{1-m}{4}} \sigma^{-m}}{\sqrt{\pi} z \Gamma\left(\frac{m}{2}\right)} \\ &\quad \times K_{\frac{m-1}{2}}\left(\frac{1}{4\sqrt{\frac{z^2}{ns^2\left(\frac{n}{z^2} + \frac{4}{\sigma^2}\right)}}}\right),\end{aligned} \tag{8}$$

where $K_a(\cdot)$ denotes the modified Bessel function of the second kind (Abramowitz and Stegun 1964). We tested this expression by comparing it to exact computer simulations of the model, which were run using the software MATHEMATICA (see Appendix 1). As shown in Figure 5, equation (8) provides a good approximation to the DFEM for various values of m, n, and z.

Figure 5 also illustrates how the DFEM depends on pleiotropy and complexity. As expected, the proportion of mutations that are advantageous increases with increasing distance from the optimum (large z) and strongly decreases with overall complexity (large n). Pleiotropy (m) mainly influences the shape of the distribution (Figs. 5 and 6). For small m, the distribution has a sharp peak at $s = 0$, such that many mutations are close to neutral. As m increases, the distribution becomes less peaked and the mode is shifted toward negative s. In consequence, more mutations are either clearly deleterious or clearly advantageous. Pleiotropy also affects the proportion of mutations that are advantageous: as m increases, the probability of a mutation being advantageous decreases.

It is worth pointing out that this transition is gradual and that the case $m = 1$ does not represent a special case for the DFEM, even though it does so for the distribution of phenotypic effect sizes (the generalization of the Chi distribution, equation (4), is monotonically decreasing for $m = 1$ but unimodal for $m > 1$, see Fig. 4).

The simulations also show that, as expected, close to the optimum, the vast majority of mutations are deleterious, whereas away from the optimum there is a significant proportion of mutations with advantageous effects. Simulations with different values of n confirm that, when $z \gg 0$, complexity greatly influences the proportion of mutations that are advantageous or deleterious.

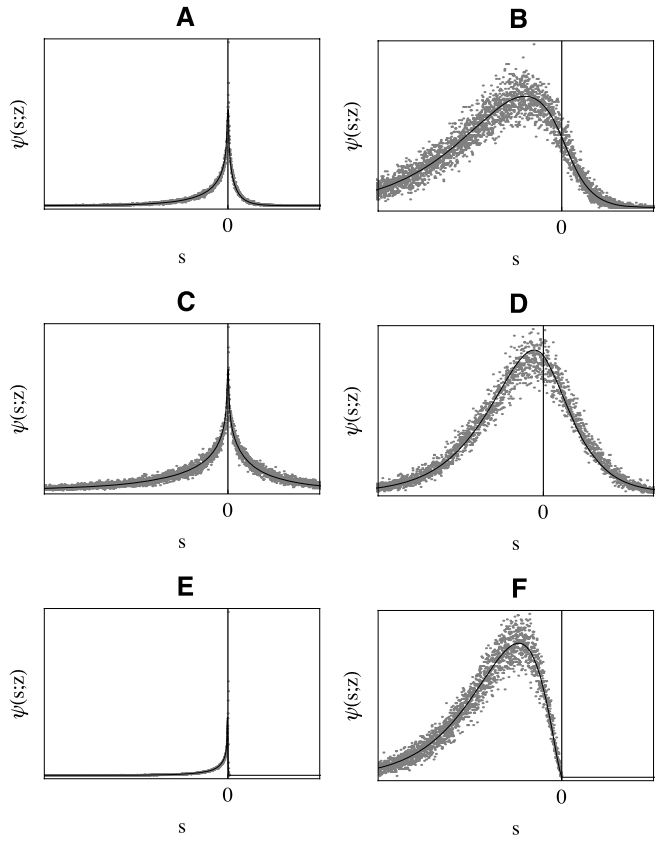

Figure 5. Distribution of the fitness effect of mutations under the model of partial pleiotropy. The figure compares the results of six exact computer simulations (described in Appendix 1) (gray dots) to the approximation in equation (8) (thin curves) using different combinations of values of *m* and *z*. The case of $\sigma^2 = \frac{\pi}{200}$ is shown. (A) $n = 120$, $m = 1$, $z = 1$ distance unit from the optimum; (B) $n = 120$, $m = 5$, $z = 1$ distance unit from the optimum; (C) $n = 10$, $m = 1$, $z = 1$ distance unit from the optimum; (D) $n = 10$, $m = 5$, $z = 1$ distance unit from the optimum; (E) $n = 120$, $m = 1$, $z = 0.1$ distance unit from the optimum; (F) $n = 120$, $m = 5$, $z = 0.1$ distance unit from the optimum.

RATES OF ADAPTATION

Assuming that in a large population away from the optimum only advantageous mutations have reasonable probabilities of fixation, we can use equation (8) to derive the rate of adaptation of a population to a new environment when the mean phenotypic distance to the optimum is z (Orr 2000a; Poon and Otto 2000; Welch and Waxman 2003; Martin and Lenormand 2006)

$$\frac{d\bar{w}}{dt} \approx N_e \mu \int_0^\infty s\psi(s;z)\, F(s)\, ds, \qquad (9)$$

where μ is the per generation mutation rate, and $F(s) \approx 2s$ is the fixation probability of a mutation with selective advantage s. $\frac{d\bar{w}}{dt}$ has a complicated expression but can be computed simply by using equation (8) and the approximation for $F(s)$ mentioned above. An example of a plot of $\frac{d\bar{w}}{dt}$ as a function of n and m is shown in Figure 7. It indicates that $\frac{d\bar{w}}{dt}$ is a decreasing (approximately exponential) function of complexity n, which is consistent with the previous studies on the cost of complexity (Orr 2000a; Welch and Waxman 2003), but it depends on m in a more complicated way: $\frac{d\bar{w}}{dt}$ is a concave function of mutation pleiotropy m with a maximum for intermediate values. The most obvious reason is that m acts on the rate of adaptation through its effect

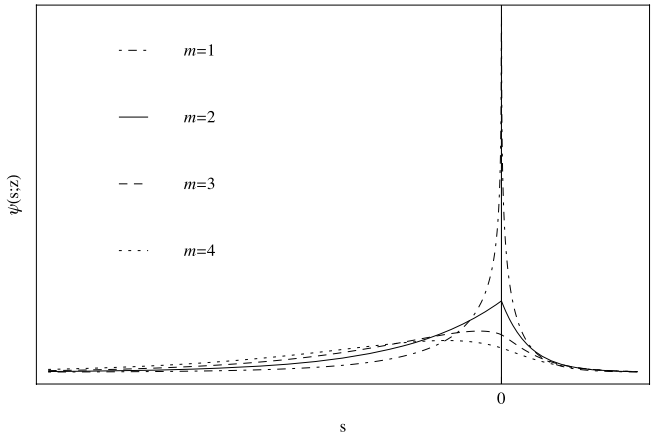

Figure 6. The effect of mutation pleiotropy on the distribution of the fitness effects of mutations. The figure compares the DFEM (eq. 8) for different levels of *m*. $\sigma^2 = \frac{\pi}{200}$, $n = 120$, and $z = 1$ units distance from the optimum.

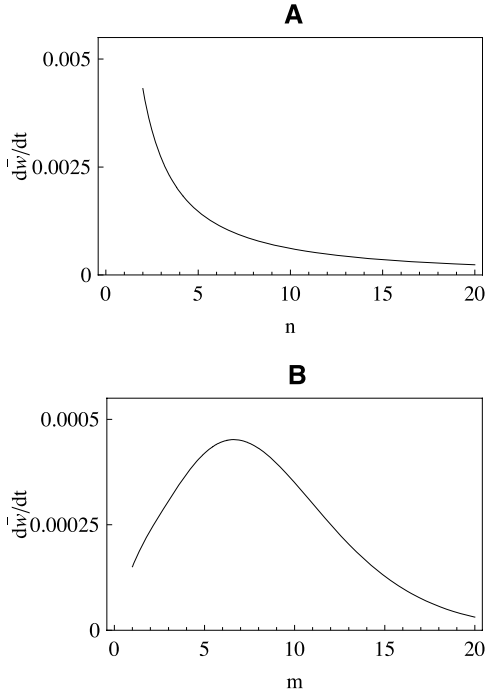

Figure 7. Rate of per generation increase of mean fitness as a function of *n* (A) and as a function of *m* (B). The case of $z = 1$, $N_e = 1000$, $\sigma^2 = \frac{\pi}{200}$, and $\mu = 9 \times 10^{-5}$ was considered. In the top plot, $m = 2$ and in the bottom plot $n = 20$.

on the magnitude of mutations. The optimal mutation size, the one that is expected to yield the greatest gain in fitness, corresponds to a mutation of intermediate phenotypic size that is in accordance with previous analyses (Orr 2000b). Similarly, the rate of increase in mean fitness peaks for intermediate values of m.

In summary, both mutation pleiotropy and (total) complexity are expected to affect the rate of adaptation of a population to a new environment, with n being more implicated in the cost of complexity than m.

DRIFT LOAD AND THE DFEM IN WELL-ADAPTED POPULATIONS

Even in a long-term stable environment, populations are never perfectly adapted. Instead, because of the stochastic fixation of slightly deleterious mutations, phenotypes are expected to be distributed around the optimum, at a mean phenotypic distance that results from a dynamic equilibrium between mutation, selection, and drift. The resulting decrease in mean population fitness is commonly known as the drift load, and is inversely related to the effective population size (Kimura et al. 1963; Hartl and Taubes 1998; Poon and Otto 2000). It has also been suggested that drift load is directly proportional to complexity: because fitness is a measure of how successfully organisms interact with the environment, a higher number of these interactions can make simultaneous optimization more difficult (Tenaillon et al. 2007).

Consider that z_{eq} is the equilibrium mean phenotypic distance to the optimum. In the low mutation rate limit, we can neglect the effect of segregating mutations. Thus, by definition of z_{eq}, when $z = z_{eq}$ the expected decrease in mean fitness induced by the fixation of deleterious mutations is perfectly matched by the expected increase in mean fitness induced by the fixation of advantageous mutations. Using equation (9), this translates to the equilibrium condition

$$\int_{-\infty}^{0} s\psi(s < 0; z_{eq})F(s)\,ds = -\int_{0}^{\infty} s\psi(s > 0; z_{eq})F(s)ds, \tag{10}$$

where

$$F(s) \backsimeq \frac{2s}{1 - \exp(-2n_e s)}$$

(Hartl and Taubes 1998; Piganeau and Eyre-Walker 2003). To solve equation (10) for z_{eq}, we follow the same strategy applied in Hartl and Taubes (1998): the symmetry in the expressions allows us to write

$$\int_{0}^{\infty} s[\psi(s; z_{eq})F(s) - \psi(-s; z_{eq})F(-s)]ds = 0, \tag{11}$$

and if we substitute the expressions for $\psi(\cdot)$ (eq. 8) and $F(\cdot)$ in equation (11), we get

$$\int_0^\infty \frac{2^{\frac{1-m}{2}}\sqrt{n}\pi s^2\left(\frac{1+\frac{4z_{eq}^2}{n\sigma^2}}{s^2}\right)^{\frac{1-m}{4}}}{\sigma^m z_{eq}\Gamma\left(\frac{m}{2}\right)} \times K_{\frac{m-1}{2}}\left(\frac{s}{4\sqrt{\frac{z^2}{n\left(\frac{n}{z^2}+\frac{4}{\sigma^2}\right)}}}\right) csch(N_e s)\, sinh\left(N_e s - \frac{ns}{4z_{eq}^2}\right) ds = 0, \tag{12}$$

where $csch(\cdot)$ is the hyperbolic cosecant function and $sinh(\cdot)$ is the hyperbolic sine function (Abramowitz and Stegun 1964). Note that the integrand in equation (12) is either strictly positive, when $N_e s - \frac{ns}{4z_{eq}^2} > 0$, or else strictly negative, when $N_e s - \frac{ns}{4z_{eq}^2} < 0$. Therefore, the equality is expected to hold if and only if $N_e s - \frac{ns}{4z_{eq}^2} = 0$, which leads to

$$z_{eq} = \sqrt{\frac{n}{4N_e}}. \tag{13}$$

This result is equivalent to the expression for the equilibrium value of phenotypes given in Hartl and Taubes (1998), and an approximation of the expression for the mean equilibrium fitness in Tenaillon et al. (2007). From equation (13), we can calculate the mean fitness of the population at equilibrium

$$\bar{w}_{eq} = \exp\left(-\frac{n}{4N_e}\right). \tag{14}$$

The most surprising consequence is that drift load, while proportional to complexity and inversely proportional to effective population size, is not expected to depend on m (or σ). When the complexity of organisms increases, the proportion of mutations that are deleterious increases also, and so the mean distance to the optimum tends to increase until the balance between the effects of fixed deleterious and advantageous mutations is reestablished. When effective population size increases, there are fewer effectively neutral deleterious mutations (the ones with a fair chance of being fixed), and so the distance to the optimum at equilibrium tends to decrease until the balance is reestablished. When populations are so small that drift overwhelms selection, there is a higher frequency of effectively neutral deleterious mutations that fix, causing fitness to decline, until the increase in the rate of fixation of advantageous mutations reestablishes the equilibrium—an effect termed compensatory epistasis (Silander et al. 2007). Very large populations maintain nearly optimal mean population fitness regardless of phenotypic complexity, whereas small populations can maintain high mean fitness only when there are a small number of traits. The fact that drift load is largely independent of the DFEM in FGM, is valid under the assumption of alleles having continuous effects. Under alternative models (such as a model

Table 2. Examples of simulations (described in Appendix 2) in which the effect of the parameters of the model on the mean population fitness at mutation–selection–drift equilibrium were studied. In each simulation, the simulated mean distance to the optimum of the population ($\bar{z}_{eq}$) is compared to the expected values ($\hat{\bar{z}}_{eq}$) calculated following equation (13). The case where $\mu = 0.000012$ is shown. (a) n=10, m=1 and $\sigma^2 = \frac{\pi}{200}$; (b) N_e=1000, m=1 and $\sigma^2 = \frac{\pi}{200}$; (c) N_e=1000, n=40 and $\sigma^2 = \frac{\pi}{200}$; (d) N_e=1000, n=40 and m=1. Simulations show that, although N_e and n have a significant impact on $\bar{z}_{eq}$, m and σ have an insignificant impact on $\bar{z}_{eq}$ as expected from equation (14).

a			b			c			d		
N_e	$\bar{z}_{eq}$	$\hat{\bar{z}}_{eq}$	n	$\bar{z}_{eq}$	$\hat{\bar{z}}_{eq}$	m	$\bar{z}_{eq}$	$\hat{\bar{z}}_{eq}$	σ^2	$\bar{z}_{eq}$	$\hat{\bar{z}}_{eq}$
100,000	0.011	0.005	5	0.037	0.035	1	0.103	0.100	$\frac{\pi}{8}$	0.097	0.100
20,000	0.014	0.011	10	0.052	0.050	2	0.101	0.100	$\frac{\pi}{200}$	0.103	0.100
5,000	0.023	0.022	40	0.103	0.100	5	0.101	0.100	$\frac{\pi}{800}$	0.101	0.100
1,000	0.052	0.050	100	0.161	0.158	10	0.166	0.100	$\frac{\pi}{20{,}000}$	0.101	0.100

where alleles have discrete effects), the drift load may depend on the fitness effect of mutations (see discussion in Rosas et al. (2010) and Poon and Otto (2000)).

We tested the accuracy of equation (13) with simulations using representative combinations of parameter values (see Appendix 2). The results are presented in Table 2, and confirm that z_{eq} does not depend significantly on m.

In contrast, plugging equation (13) into equation (8) shows that the DFEM at mutation–selection–drift balance (i.e., of a population with $z = z_{eq}$) depends only on m, and not on n

$$\psi\left(s; z_{eq}\right) \approx \frac{2^{\frac{1-m}{2}}\sqrt{N_e}\left(|s|\right)^{\frac{m-1}{2}}\left(1+\frac{1}{N_e\sigma^2}\right)\exp\left(-N_e s\right)}{\sqrt{\pi}\sigma^m\Gamma\left(\frac{m}{2}\right)} \times K_{\frac{m-1}{2}}\left(N_e\,|s|\sqrt{1+\frac{1}{N_e\sigma^2}}\right). \quad (15)$$

In algebraic terms, the reason for the DFEM being independent of n is that equation (8) depends only on the ratio $\frac{n}{z^2}$, which equals $4N_e$ when $z = z_{eq}$. In the resulting expression for the DFEM at equilibrium, the parameter n cancels out. Geometrically, at a given distance to the optimum, increasing n decreases the proportion of advantageous mutations (due to the cost of complexity), but at the same time, increasing n moves the equilibrium away from the optimum, which increases the potential for advantageous mutations.

It could be argued that the "true" expression for the DFEM of well-adapted populations should take into account the entire distribution of phenotypic distances to the optimum, and not only the average. However, although the computation of such expression would require an integration that is mathematically intractable, simulations (results not shown) indicate that it would not be significantly different from the DFEM in equation (15).

As the results in Appendix 3 show, when $n \to \infty$ and $z_{eq} \to 0$ (i.e., the population is perfectly adapted), the DFEM becomes a negative gamma distribution, equivalent to that in Martin and Lenormand (2006), which is still a function of m but not of n. This is obvious, taking into account that for $z_{eq} = 0$, all mutations are deleterious and the DFEM depends only on the distribution of the total effect of mutations.

In summary, drift load depends only on complexity (n), whereas the DFEM at mutation–selection–drift balance depends only on mutational pleiotropy (m).

Discussion

In this article, we focus on two key concepts in FGM, complexity, and pleiotropy, and their effects on the DFEM, the drift load, and the cost of complexity. We introduce a new model assuming partial pleiotropy, in which each new mutation affects a subset of traits of arbitrary size. This reconciles two opposed models for the distribution of the total effect of mutations, and has important implications for the influence of complexity on the DFEM and for the estimation of the variation of complexity across species. We discuss these various aspects, and compare the new formalism to alternative attempts that have been made to relax the universal pleiotropy assumption, namely modular models.

A PARTIAL PLEIOTROPY MODEL TO RECONCILE ESM AND ITEM

Two models are usually considered in FGM for the distribution of the total effect of mutations: the Euclidean superposition model (ESM) and the invariant total effect model (ITEM) (Hermisson and McGregor 2008; Wagner et al. 2008b).

The ESM assumes that the expected squared effect of a mutation on a trait is the same regardless of how many other traits are also affected by the mutation. This model was used by Turelli (1985), Wagner (1988), Wagner (1989), and Waxman and Peck (1998) among others. Under the ESM, as complexity increases, the distribution of the total phenotypic effect of mutations is displaced toward higher values, and the proportion of mutations of

small effect rapidly vanishes (Fig. 4). Because empirical data strongly suggest that mutations of small effect are the most frequent, the assumptions of the ESM with universal pleiotropy lead to a bias toward small values of complexity when estimated from empirical data, a point that has already been made elsewhere (e.g., Orr 2000a; Wingreen et al. 2003).

In turn, the ITEM model, proposed by Orr (2000a) and Wingreen et al. (2003) among others, assumes that the distribution of the total effect of mutations is independent of the degree of mutation pleiotropy. The advantage of such a model is that slightly deleterious mutations are not suppressed when the dimensionality of the phenotypic space increases. However, as pointed out by Wagner et al. (2008b), the ITEM model would predict a decrease of the phenotypic effect of mutations on individual traits as the level of pleiotropy increases, which is not supported by QTL data in mouse (Wagner et al. 2008b).

An ESM model with partial pleiotropy reconciles these two approaches by uncoupling the effects of complexity and pleiotropy on two aspects of the geometry of mutations: orientation (dependent on complexity) and magnitude (dependent on mutation pleiotropy). However complex an organism may be, if every single mutation affects a small subset of traits, then the distribution of phenotypic effect of mutations (displacements in the phenotypic space) is identical to that of a low-complexity organism. The magnitude of mutations is not affected by the total number of traits under selection, and the phenotypic effect of mutations on individual traits is not changed by mutation pleiotropy.

Is the ESM model a reasonable assumption about the pleiotropic scaling of mutation effects? Wagner et al. (2008b) present evidence of an increase in the total effect of QTL with increasing degree of pleiotropy, that is even stronger than predicted by the ESM model. This evidence lead them to reject the ESM model. However, as pointed out in Hermisson and McGregor (2008), the authors did not consider the possibility of some of the analyzed QTL regions containing multiple mutations, which could lead to an overestimation of the effects of mutations with higher pleiotropy. In a reply, the authors acknowledged that these overestimations can reconcile the observed data with the ESM model, so this model remains a reasonable assumption (Wagner et al. 2008a).

ESTIMATING COMPLEXITY

A number of recent studies attempted to estimate complexity in various species using FGM-based approaches, yielding contradictory results. Fitting their model to DFEM data obtained from MA lines and single-mutation experiments, Martin and Lenormand (2006) reported low levels of effective complexity (0.21–2.58) in the five species they analyzed. Using experimental measurements of drift load, Tenaillon et al. (2007) found much higher levels of complexity in two viruses, including an effective complexity of 10 for the VSV virus, whose effective complexity was estimated to be 1.07 by Martin and Lenormand (2006) (Fig. 2).

Our analytical results provide a plausible explanation for these differences. Equation (15) indicates that, under partial pleiotropy, the DFEM at mutation–selection–drift equilibrium does not depend on complexity (our parameter n). What determines the deleterious effects of a specific mutation in a well-adapted population is m, the mutation pleiotropy. MA data, as well as direct measurement of fitness effects in well-adapted populations, can only reflect the effects of m in the fitness effects of mutations. Drift load, on the other hand, is largely determined by complexity n (eq. 14), and not by m. We suggest that the distinction between mutation pleiotropy and total pleiotropy (or complexity), could explain much of the discrepancy between the studies done by Martin and Lenormand (2006) and Tenaillon et al. (2007)—the former approach essentially estimating m, and the latter n.

Overall, estimates of complexity from approximations of the DFEM of well-adapted populations tend to give relatively low values, which do not vary much across species, whereas estimations of complexity from drift load data tend to give relatively high, variable values. As argued above, we speculate that this disparity is simply due to complexity being generally much higher and much more variable than mutation pleiotropy. If our model has any biological relevance, the DFEM of well-adapted populations should not be used on its own to approximate the DFEM of populations away from the optimum, or to make inferences about the rates of adaptation.

Our model can also explain why the estimates of complexity in Gu (2007), based on substitution patterns during gene divergence across vertebrate species, tend to be intermediate between the two approaches (ranging from 2.3 to 20.4). During the course of species divergence, changes in population sizes or environmental changes can move populations away from the equilibrium, making the DFEM (and substitution patterns) depend on both m and n (eq. 8).

Our results may also have specific consequences for molecular evolution. First, from a technical point of view, the DFEM at equilibrium given by equation (15) has very flexible shapes and reconciles displaced and reflected gamma solutions. Equation (15) can thus be directly used in methods based on polymorphism data (e.g., Eyre-Walker et al. 2006; Eyre-Walker and Keightley 2009) to estimate DFEM and the degree of mutation pleiotropy. Second, because mutation pleiotropy varies little across species, it validates the classical assumption that, for genes without significant adaptive episodes (i.e., in a long-lasting state of mutation–selection–drift equilibrium), variation in dN/dS ratios across species (which depend on the DFEM) should be largely determined by differences in effective population size. Differences in effective population size have also been invoked to explain the strong variation of the proportion of adaptive

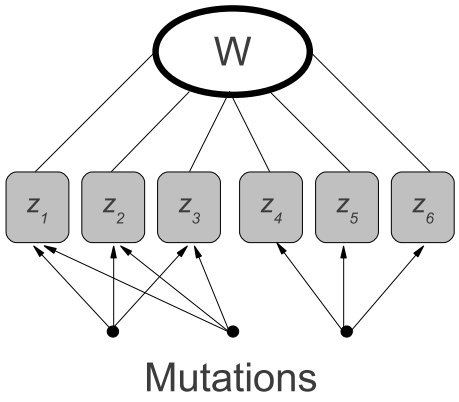

Figure 8. An illustration of how mutations affect traits in a model of modular pleiotropy. Traits z_1, z_2, and z_3 form a module, and every mutation that affects one of this traits can potentially affect all the other traits in the module at the same time, but none of the traits in the other modules. Adapted from Welch and Waxman (2003).

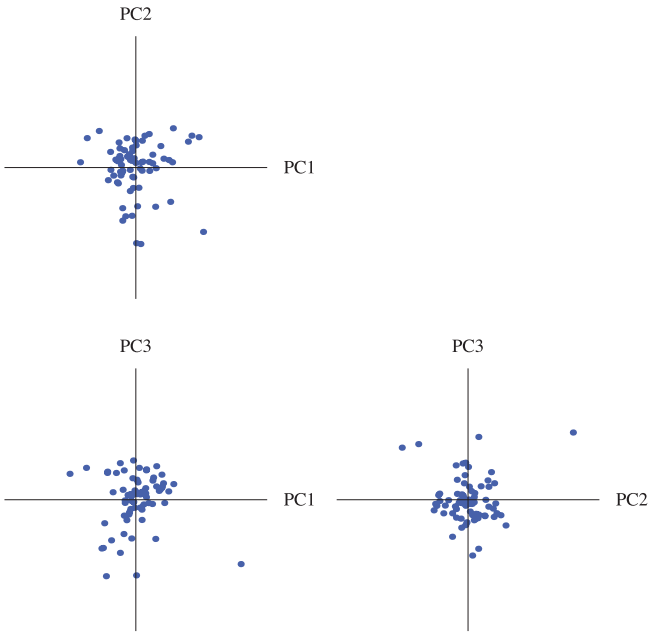

Figure 9. Skeletal traits plotted against three principal components from an analysis on mutation-trait scores. Each graph plots the 70 skeletal traits against a combination of two of the first three principal components of an analysis involving the scores of 105 pleiotropic loci.

substitutions between species—between *Drosophila* and humans for instance (Eyre-Walker 2006). However, the proportion of adaptive substitutions does not seem to be related to population size in several plant species (Gossmann et al. 2010). For this much debated issue in molecular evolution, our results suggest complexity could also matter.

PARTIAL VERSUS MODULAR PLEIOTROPY

One biologically plausible way to relax the universal pleiotropy assumption that has been proposed in the literature is modular pleiotropy (Fig. 8). In a modular pleiotropy model, sets of traits are grouped into more or less fixed modules, and mutations have their effects restricted to single modules (Welch and Waxman 2003). This is different from the newly introduced partial pleiotropy model, where the effect of each mutation is restricted to a randomly chosen subset of traits (Fig. 3).

In modular pleiotropy models, mutation pleiotropy is equivalent to the average number of independent traits within modules (our *m* parameter), and complexity is equivalent to the total number of traits (our *n* parameter). Similarly to the partial pleiotropy model, complexity and pleiotropy operate at two levels: the orientation of mutations is mostly influenced by the number of modules, whereas the total effect of mutations depends on the number of effectively independent traits within modules.

Is the DFEM predicted by our model equivalent to the DFEM expected under modular pleiotropy? Simulations (not shown) demonstrate that this is indeed the case, at least for well-adapted populations close to the optimum. This is because, at equilibrium, phenotypes are expected to be randomly distributed around the optimum due to the fixation of mutations with slightly (effectively neutral) deleterious effects in all modules, and the position of the optimum relative to mutated phenotypes can be considered random. Therefore, the probability of mutations being deleterious or advantageous is not affected by the modular nature of mutations, and identical in the two models. This equivalence, however, does not generalize to the case of adapting populations away from the optimum. In this case, phenotypes are not randomly distributed around the optimum, and some degree of correlation is expected between the orientation of mutations, and the orientation of the optimum relative to the mutated phenotypes.

Is a modular pleiotropy model more plausible than a partial pleiotropy model? The partial pleiotropy and modular pleiotropy models differ in that the former assumes independent direction of all mutations (each new mutation affects a random subset of traits), whereas the latter involves correlations between mutations of a common module. We reanalyzed the dataset of Kenney-Hunt et al. (2008), who measured the phenotypic effect of 105 loci (here taken as mutations) on 70 skeletal traits in mouse. No significant correlation between mutations was detected from this dataset (see Fig. 9 and Appendix 4), which is in agreement with the partial pleiotropy model—each mutation affecting only a small subset of traits, which are distinct and uncorrelated between mutations.

Conclusion

Phenotypic complexity is itself a complex concept, the relationship between dimensionality in FGM and measurable traits in real organisms being anything but trivial (Martin and Lenormand

2006). Following Welch and Waxman (2003) and Gu (2007), we show that besides correlations between traits, the difference between mutation pleiotropy and total pleiotropy can confuse further inferences of organismal complexity. Our results show that mutation pleiotropy and complexity have separate effects on the predictions of FGM about the DFEM and drift load. For well-adapted populations, average mutation pleiotropy (m) has an effect on the DFEM, whereas complexity (n) influences drift load, of well-adapted populations. Therefore, we conclude that FGM based methods using the predictions on the DFEM, in contrast to the ones based on drift load, can underestimate true complexity. The newly introduced partial pleiotropy model, which captures this distinction and reconciles alternative viewpoints regarding the relationship between complexity and magnitude of mutations, might serve as a useful basis for future developments on these topics. Finally, because our results are based on FGM and the concepts of complexity and pleiotropy, our findings can potentially be applied to a vast array of biologically relevant questions, besides estimating complexity.

NOTE ADDED IN PROOF

While this manuscript was in press, we were aware of the publication of the article of Chevin, Martin, and Lenormand on a related topic (Chevin, L. M., G. Martin, and T. Lenormand. 2010. Fisher's model and the genomics of adaptation: restricted pleiotropy, heterogenous mutation, and parallel evolution. Evolution 64:3213–3231.).

Generalizing the Martin and Lenormand framework, Chevin et al. (2010) introduced what they called "restricted pleiotropy," which is basically equivalent to our "partial pleiotropy," although restricted pleiotropy is a property of loci in Chevin et al., but of mutations in our model. They also found that, at the optimum, the DFEM depends on the effective number of dimensions at the locus scale (not the total genome scale).

This is similar to our finding that the DFEM depends on m not n.

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Appendix 1

In this appendix, we describe the stochastic computer simulations used to obtain the DFEM presented in Figure 5. In each simulation, 100,000 random mutations were drawn, and their selection coefficients were calculated and recorded. For each mutation, a random initial phenotype at z units distance from the optimum was first computed by drawing a random vector from an n-dimensional multivariate Gaussian distribution, and then normalizing it to a magnitude z. Then, a mutation vector with a random orientation and magnitude was drawn according to the model described above, by drawing a random vector in an n-dimensional space using a multivariate Gaussian distribution, and then normalizing it to a magnitude r drawn from the generalization of the Chi distribution (eq. 4). The mutation vector was then added to the initial phenotype to establish the phenotypic position of the mutant phenotype, and the corresponding selection coefficient s was computed using equations (1) and (5).

Appendix 2

In this appendix, we describe the stochastic computer simulations used to study the effect of the parameters of the model on the mean population fitness at mutation–selection–drift equilibrium (Table 2).

The first component of the simulations was an "allelic genealogy", represented by a rooted tree, used to keep track of the ancestry of alleles (Gillespie 1993). The nodes of this tree correspond to data structures concerning the alleles and the mutations that created them. These data structures include a pointer to the parent allele, the coordinates of the alleles in the phenotypic space, the fitness $w(z)$ of the allele calculated according to equation (1), the selection coefficient s calculated according to equation (5), and the current frequency of the allele in the population $f(z)$. Should a mutation become fixed in the population (a substitution event, that is, when the sum of the frequency of the allele created by the mutation plus the frequency of all its descendant alleles attained one) the corresponding allele node became the new root node for all alleles in the population. When a "branch" of the allelic tree becomes extinct in the population, it was pruned to free computer memory (Gillespie 1993).

The second component of the simulation was responsible for the dynamics. In each generation, the entire population was first subjected to selection, which deterministically changes the frequencies of alleles using the standard formula for haploid selection $\Delta f(z) = \frac{f(z)[w(z)-\bar{w}]}{\bar{w}}$ (Gillespie 1993), where $\bar{w} = \sum f(z)w(z)$ is the mean population fitness. Then drift, which stochastically changes the frequencies of alleles, was simulated by drawing a multinomial random variate of size N_e, the number of independent trials, each trial having as outcome one of the extant alleles with probability $f(z)$. The frequencies of the alleles after drift correspond to the number of times each allele was observed over the N_e trials.

Mutation vectors of random magnitude and direction were produced according to the model described above, by first drawing a random vector in an n-dimensional space using a multivariate Gaussian distribution, and then normalizing it to a magnitude r drawn from the generalization of the Chi distribution (eq. 4). The mutation vector was then added to the parent allele to create a new allele that was added as a new "leaf" to the allelic tree, the frequencies of the alleles being readjusted accordingly.

The stochastic simulations started with the population being perfectly adapted, that is, with an initial unique allele ($f(z) = 1$) the coordinates of which correspond to the origin of the coordinate space (distance $z = 0$ from the optimum), and lasted until 1000 substitution events occurred after an initial burnin period empirically chosen so that the population had enough time to converge to a state of dynamic equilibrium.

In each generation, the mean population distance to the optimum $\bar{z}$ was recorded, and in the end of the simulations, an average over all generations was computed to obtain the observed mean distance to the optimum of the population at mutation-selection-drift equilibrium ($\bar{z}_{eq}$).

The results of Table 2 show that the expression of equation (13) is less accurate for large N_e (e.g., $N_e = 100{,}000$), but this comes from the fact that, in larger populations, segregating deleterious mutations are no more negligible, which bias mean population fitness downwards. Also, the expression seems to be less accurate for larger m (i.e., $m = 10$), but this results from the number of effectively neutral mutations available for fixation being drastically reduced, and adapting populations "freezing" in phenotypic space when they get close enough to the optimum, hardly ever reaching an effective state of dynamic equilibrium.

Appendix 3

In this appendix, we derive the probability distribution of s when $z = 0$, that is, when the population is perfectly adapted.

Let $\psi(s; 0)$ be the DFEM at the optimum, and $\psi(s; 0)$ be the corresponding cumulative distribution of s, that is, $\psi(s; 0) = \Psi'(s; 0)$. The cumulative distribution can be easily computed by noting that, when $z = 0$ and $\delta z = r$, $s = -r^2$ (from eq. 6), and given $r \geq 0$, we have

$$\begin{aligned}\Psi(s;0) &= Prob(-r^2 \leq s) = 1 - Prob(r < \sqrt{-s}) \\ &= 1 - \int_0^{\sqrt{-s}} \phi(r;\sigma,m)\,dr.\end{aligned} \tag{A1}$$

Then, it is straightforward that

$$\Psi(s;0) = \frac{\Gamma\left(\frac{m}{2}, -\frac{s}{2\sigma^2}\right)}{\Gamma\left(\frac{m}{2}\right)}, \tag{A2}$$

and by deriving $\Psi(s; 0)$ we get

$$\psi(s;0) = \frac{exp\left(\frac{s}{2\sigma^2}\right) 2^{-\frac{m}{2}} (-s)^{\frac{m-2}{2}} \sigma^{-m}}{\Gamma\left(\frac{m}{2}\right)}, \tag{A3}$$

which is a negative gamma distribution with a shape parameter $\alpha = \frac{m}{2}$ and a scale parameter $\beta = 2\sigma^2$, and is exactly the same distribution derived in Martin and Lenormand (2006), with $m = n_e$ and $\sigma = \sqrt{\lambda_e/2}$, where n_e is the effective number of traits and λ_e is the scale parameter in Martin and Lenormand (2006).

Appendix 4

In this appendix, we show how we used the dataset of Kenney-Hunt et al. (2008), showing the pleiotropic patterns of 105 QTL for 70 Murine skeletal traits, to test the nonmodularity of these traits.

We first built a table from table 3 in Kenney-Hunt et al. (2008) in which columns correspond to loci and rows correspond to traits. Each cell scores 1 or 0, depending on the corresponding trait being affected or not (respectively) by the corresponding locus. Using this table, we performed a principal components analysis, and plotted each trait against the first three principal components. If the traits were grouped into modules, we would expect the traits to be grouped into clusters. Results (Fig. 9) show that traits are not clustered as it would be expectable in the case of modular pleiotropy.

We performed a second test of modularity, by using the same dataset and computing the correlation between every pair of loci. In a model of modular pleiotropy, we would expect loci to be correlated whenever they affect traits in the same module. To test if the observed correlations between loci are significant at the 5% level, we performed a permutation test by drawing 10,000 random permutations of scores for each pair of loci, computing the corresponding correlations, and obtaining a reference distribution. Initially, we found 86 significant correlations of 5460, but after performing a Bonferroni correction, no significant correlations were found.

These results clearly show that the dataset of Kenney-Hunt et al. (2008) does not support a model of modular pleiotropy.