The Genetics of Adaptation: The Roles of Pleiotropy, Stabilizing Selection and Drift in Shaping the Distribution of Bidirectional Fixed Mutational Effects

Cortland K. Griswold[1] and Michael C. Whitlock

Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada

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ABSTRACT

Pleiotropy allows for the deterministic fixation of bidirectional mutations: mutations with effects both in the direction of selection and opposite to selection for the same character. Mutations with deleterious effects on some characters can fix because of beneficial effects on other characters. This study analytically quantifies the expected frequency of mutations that fix with negative and positive effects on a character and the average size of a fixed effect on a character when a mutation pleiotropically affects from very few to many characters. The analysis allows for mutational distributions that vary in shape and provides a framework that would allow for varying the frequency at which mutations arise with deleterious and positive effects on characters. The results show that a large fraction of fixed mutations will have deleterious pleiotropic effects even when mutation affects as little as two characters and only directional selection is occurring, and, not surprisingly, as the degree of pleiotropy increases the frequency of fixed deleterious effects increases. As a point of comparison, we show how stabilizing selection and random genetic drift affect the bidirectional distribution of fixed mutational effects. The results are then applied to QTL studies that seek to find loci that contribute to phenotypic differences between populations or species. It is shown that QTL studies are biased against detecting chromosome regions that have deleterious pleiotropic effects on characters.

A major goal of modern biology is to identify the individual mutations that are the genetic basis of phenotypic differences among individuals, populations, and species. An important class of mutations that cause phenotypic differences between populations and species are fixed mutational differences, *i.e.*, mutations that are fixed in one population and absent in the other.

Often quantitative trait locus (QTL) studies seek the fixed mutational differences that are the genetic basis of phenotypic differences between two interfertile species. Many of these studies observe bidirectional QTL effects; *i.e.*, some QTL for a trait in a species have an effect in the direction of the difference between it and the other species and some QTL have effects in the opposite direction. For instance, consider the study by Bradshaw *et al.* (1998) that determined the QTL that distinguish floral characters between the sibling species *Mimulus lewisii* and *M. cardinalis* (Figure 1a). The effects are for those QTL present in *M. cardinalis* and are standardized such that negative effects are more *M. lewisii*-like and positive effects are more *M. cardinalis*-like. Overall, out of the 11 characters studied, 8 had all of the QTL with positive effects and 3 had some positive and some negative effects. Likewise, bidirectional effects were found in the QTL that cause species differences in male sexual characters between *Drosophila simulans* and *D. sechellia* (Figure 1b; MacDonald and Goldstein 1999). Overall, out of 6 characters studied, 2 had only positive effects, 3 had bidirectional effects, and 1 had all negative effects.

The presence of bidirectional QTL effects extends from domesticated organisms undergoing artificial selection to phenotypically divergent populations or species (Doebley and Stec 1993; Tanksley 1993; Orr 1998b; Rieseberg *et al.* 2003). For instance, in a review of 96 QTL studies, 22.1% of the 3252 QTL had negative effects (Rieseberg *et al.* 2003). The interpretation of the origin and maintenance of bidirectional effects has not been clearly presented. The pleiotropic nature of mutation and evolutionary forces such as stabilizing selection, hitchhiking, and random genetic drift have been invoked to explain QTL with bidirectional effects. Stabilizing selection may yield bidirectional effects because an average individual in a population may sometimes be above and sometimes below an optimum for a character. Hitchhiking may lead to bidirectional effects because a mutation with a negative effect for a character may be linked to a mutation with a larger beneficial effect for the same or another character. With random genetic drift, a mutation fixes independently of the magnitude of its effect and its direction. Yet no study has quantified how these processes and forces individually or collectively shape the expected distribution of fixed

[1]*Corresponding author:* Department of Zoology, 6270 University Blvd., University of British Columbia, Vancouver, BC V6T 1Z4, Canada. E-mail: griswold@zoology.ubc.ca

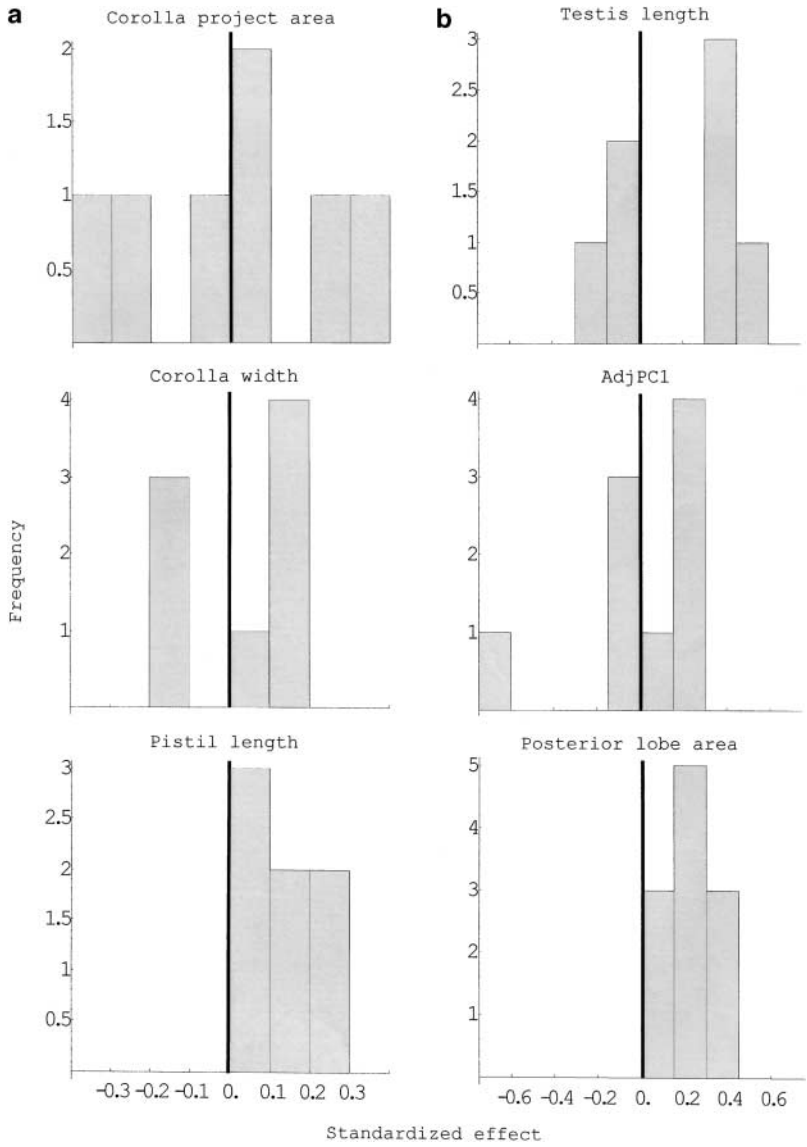

FIGURE 1.—QTL effects from two recent studies: (a) BRADSHAW *et al.* (1998) on floral differences distinguishing two species of Mimulus and (b) MACDONALD and GOLDSTEIN (1999) on male sexual characteristics distinguishing two Drosophila species. In the Mimulus study, the effect was standardized such that it is the difference between the homozygous effects of a QTL of *M. cardinalis* and *M. lewisii* divided by the homozygous effect of *M. lewisii*. A negative effect makes a *M. cardinalis* plant more like *M. lewisii* and a positive effect is in the direction of *M. cardinalis*. Corolla projected area is a measure of the surface area of the corolla that would be visible to a pollinator. AdjPC1 is a principal-components measure of the shape of the posterior lobe. The magnitude of effects for the Drosophila study is the percentage of the difference between the two species explained by substituting an introgressed *D. simulans* allele for a *D. sechellia* allele.

mutational effects on phenotypic characters. In this article, we quantify analytically the contribution pleiotropy and directional selection make in generating a bidirectional distribution of fixed mutational effects. Through simulation, we quantify contributions made during stabilizing selection and random genetic drift.

KIMURA (1983) derived the distribution of the magnitude of the overall effect of the next fixed mutation on a phenotype by employing FISHER's (1930) geometrical model and found that mutations of intermediate effect fix most frequently. Recently, ORR (1998a), who also used Fisher's model, derived the distribution of fixed mutational magnitudes during a bout of adaptation in which more than one mutation fixes and found the overall distribution to be approximately exponentially distributed. Later ORR (1999) showed that the distribution of the magnitude of fixed effects on specific dimensions is also approximately exponentially distributed.

With the exception of ORR (1999), none of the results are directly relevant to empirical studies that seek to understand the evolution of single characters because the effect of a mutation is the length of the vector it makes in an *n*-dimensional hypersphere—not its effect on a single character. ORR's (1999) result is useful in that it predicts the magnitude of fixed effects on single characters.

Furthermore, all of the previous results have focused on the magnitude of fixed effects, that is, their unsigned absolute value. Biologically, of course, whether a fixed effect increases or decreases the value of a trait is a crucial bit of information. In this article we distinguish between the size and magnitude of mutational effects. With "size" we refer to the signed value of the effects, while with "magnitude" we refer to its absolute value. To understand the evolution of bidirectional effects, we of course focus on size rather than magnitude.

Here we employ a model that is conceptually simpler than Fisher's, yet allows for the retention of information about the direction fixed mutations have on characters. The comparable results to Fisher (1930), Kimura (1983), and Orr (1998a, 1999) are duplicated despite the simpler approach. In our model, mutations are assumed to independently affect some number of characters. The results from this analysis are more relevant to empirical studies that measure characters. Analysis shows that information about both the direction and the magnitude of fixed mutations can potentially be used to estimate how characters are associated pleiotropically through mutation and to infer the role selection has taken in shaping the evolution of a character. At the end of the analysis we scale up from individual mutations to QTL.

In this study, three factors affecting the distribution of fixed mutational effects are evaluated: pleiotropy, drift, and stabilizing selection. By varying these factors separately and together we evaluate how they contribute to the existence of fixed bidirectional mutational effects and change the shape of the distribution of fixed mutational effects.

We assume that mutation acts independently on characters and that a mutation has an effect on a character that is a random draw from a bilaterally symmetric gamma distribution (in the appendix we allow for the possibility of asymmetric mutation). Empirical evidence supports that the effects of mutation may be bilaterally symmetric; Mackay *et al.* (1992) and Lyman *et al.* (1996) found that random *P*-element inserts in Drosophila affected bristle number in a bilaterally symmetric manner with the frequency of effects with larger magnitude decreasing in an exponential-like way or possibly with even higher kurtosis. Fitness is modeled as a linearly decreasing function of the phenotype's distance from the optimum in simulations and as a linear function of a mutation's individual effects on characters in the analytical analyses. Following previous work, we assume that beneficial mutations are rare, so that there is no selective interference among mutations (Hill and Robertson 1966).

Figure 2 illustrates how the processes of random genetic drift, stabilizing selection, and directional selection with pleiotropy by themselves or collectively lead to the fixation of bidirectional mutations. In Figure 2, a–d, the axes are characters, the optimum character state is at the origin for both characters, and the current phenotype, *i.e.*, the combination of two character states, is represented by a point. Mutations that bring the phenotype to be within the circle are beneficial overall because the new phenotype would be closer to the optimum. Random genetic drift leads to the fixation of bidirectional effects because chance plays a role in which mutations fix, allowing for the fixation of mutations in all directions (Figure 2a). Stabilizing selection becomes important when the probability that a mutation overshoots the optimum becomes nonnegligible, and then the sequential overshooting of the optimum leads to bidirectional fixed effects (Figure 2b). Pleiotropy allows for the fixation of bidirectional effects, in the absence of drift and stabilizing selection, because even though one or a few characters may have effects in the opposite direction to their optima, other characters may have stronger effects in the direction of their optima (Figure 2c). Unidirectional effects are expected under pure directional selection and when mutations that fix have effects only in the direction of the optima for both characters (Figure 2d).

Analytical expressions are derived for varying levels of pleiotropy in the absence of drift and stabilizing selection for exponentially distributed mutations. Some useful approximations cannot be made when mutation is gamma distributed with shape parameters <1.0, and expressions giving the distribution of fixed mutational effects are presented in the appendix under these conditions that require numerical integration. Simulations are used to determine distributions under drift and stabilizing selection and mixtures of varying levels of drift, stabilizing selection, and pleiotropy. We characterize the effects of drift, stabilizing selection, and pleiotropy on fixed mutational effects with three measures: (1) the expected size of a fixed mutational effect on a character, (2) the distribution of fixed mutational effects, and (3) the relative frequencies of bidirectional fixed mutational effects, *i.e.*, the frequency of positive *vs.* negative fixed effects.

MODEL

We assume that organisms are haploid. The current state of n characters pleiotropically affected by mutation is represented by a vector $\boldsymbol{z} = \{z_1, z_2, \ldots, z_n\}$. The effect of a random mutation on a character (δ_i) is a random draw from a bilaterally symmetric gamma distribution, $f(\delta; \alpha, \beta)$, with shape parameter α and scale parameter β. The result is a mutation vector $\boldsymbol{\delta}$ that adds to the vector $\mathbf{z}$. The selection coefficient of a new mutant phenotype is determined by the relationship $w_{\text{wild type}}(1 + s) = w_{\text{mutant}}$, where $w_{\text{wild type}}$ is the fitness of the phenotype without the mutation and w_{mutant} is the fitness with the mutation.

Simulations: $w_{\text{wild type}}$ is scaled to equal one and w_{mutant} is equal to $1 + (|\boldsymbol{z}| - |\boldsymbol{z} + \boldsymbol{\delta}|)\sigma$, where σ is the magnitude of the slope of the fitness function and $|\boldsymbol{x}|$ denotes the length of a vector $\boldsymbol{x}$. The selection coefficient of a mutation is then $s = (|\boldsymbol{z}| - |\boldsymbol{z} + \boldsymbol{\delta}|)\sigma$. A mutation that causes the phenotype to be greater than $|\boldsymbol{z}| + 1/\sigma$ is assumed to have zero fitness. A mutation with selection coefficient s fixes with probability $(1 - e^{-2N_e s/N})/(1 - e^{-2N_e s})$ (Kimura 1957), where N_e and N are the effective and census population sizes. When a mutation fixes, the phenotype of all individuals in the population takes on the value $\boldsymbol{z} + \boldsymbol{\delta}$.

Each character begins adaptation the same distance

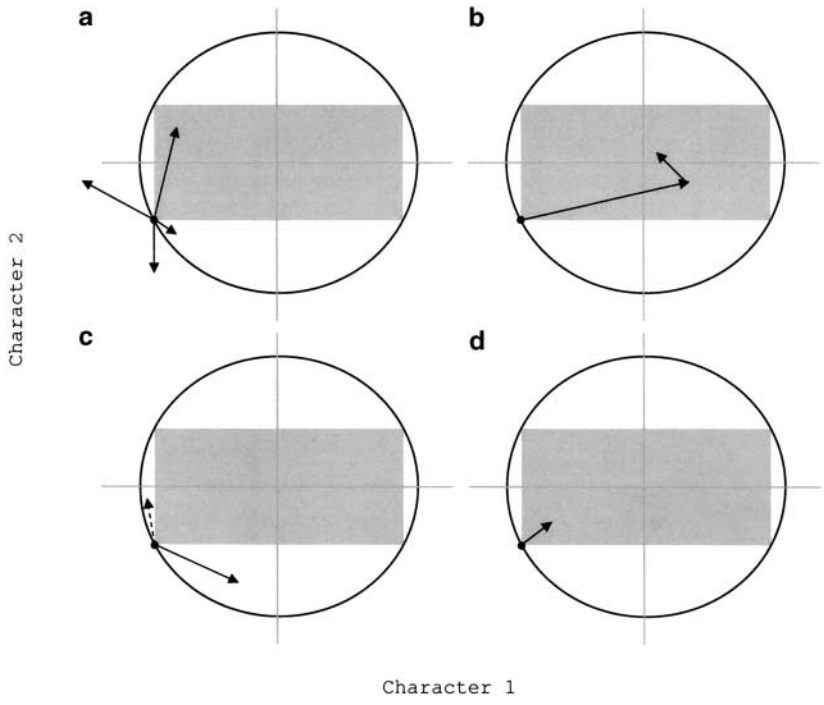

FIGURE 2.—How (a) random genetic drift, (b) stabilizing selection, and (c) directional selection with pleiotropy may lead to the fixation of bidirectional mutational effects, for mutations that pleiotropically affect two characters. The optimum state for each character is at the origin. The point on the circle gives the current state of both characters. A mutation, represented by a vector, may cause a character to take on a new state. Mutations bringing the phenotype to be within the circle are beneficial overall because the new phenotype is closer to the optimum. (a) Under drift, mutations fix in all directions, leading to bidirectional fixed effects. (b) A character experiencing stabilizing selection undergoes sequential overshooting of the optimum, leading to the fixation of bidirectional mutations. (c) Pleiotropy and directional selection lead to the fixation of bidirectional effects because although a particular character may have an effect opposite in direction to its optimum, another character may compensate by having a larger effect in the direction of its optimum. The mutation is beneficial overall because it is within the circle; *i.e.*, it brings the phenotype to be closer to the optimum, but is outside the rectangle because it has a deleterious effect on one of the characters. (d) When both characters have effects in the direction of the optimum, for mutations pleiotropically affecting two characters, the mutation is always beneficial overall.

from its optimum. The beginning distances and stopping points vary among simulations to model the effects of the role of stabilizing selection in shaping the distribution of fixed mutational effects. Effective and census population sizes are varied to model the effects of random genetic drift.

Mathematical analysis: Here we assume that there is no random genetic drift or stabilizing selection and investigate how pleiotropy alone causes the fixation of bidirectional effects. Because each character experiences directional selection only, we employ the convention that mutations with positive effects are in the direction of the optimum and mutations with negative effects are in the opposite direction of the optimum. For both the one-character case and the multicharacter cases $w_{\text{wild type}}$ is scaled to equal one and w_{mutant} is equal to $1 + \sigma\Sigma_{i=1}^{n}\delta_i$. Thus, unlike the simulations, the fitness of a mutant is a linear function of the effect of a mutation on each character. It is possible to measure fitness this way because, for the mathematical analyses, we assume that there is no stabilizing selection; *i.e.*, there is no overshooting of the optimum and the average magnitude of an effect of a random mutation on a character is small such that terms involving δ_i^2 or higher order are negligibly small. Accordingly, the selection coefficient of a mutation is $s = \sigma\Sigma_{i=1}^{n}\delta_i$. In the no pleiotropy case, we analyze the probability of initiating stabilizing selection. To do this we make adjustments in the integral functions. Furthermore, in all cases we assume infinite population size. Therefore, it is possible to approximate the probability of fixation of a mutant with selection coefficient, s, to be $2s$ (CROW and KIMURA 1970). This approximation assumes that no mutations that fix have selection coefficients $<1/N_e$ and selection is weak, *i.e.*, $s \ll 1/2$.

No pleiotropy: Prior work, under the assumption of directional selection and that the fitness effects of random mutation are gamma distributed with shape parameter α and scale parameter β, showed that the distribution of fixed mutational effects is gamma distributed with shape parameter $\alpha + 1$ and scale parameter β (OTTO and JONES 2000). It can be shown that under our assumptions, the same distribution is obtained. No bidirectional fixed mutations are expected.

Effects of stabilizing selection: The previous analysis (OTTO and JONES 2000) assumes only directional selection is happening. Another way of viewing this is that the population is sufficiently far from the optimum that overshooting of the optimum and, therefore, stabilizing selection is not happening. It is of interest to determine how far a population needs to be to safely assume stabi-

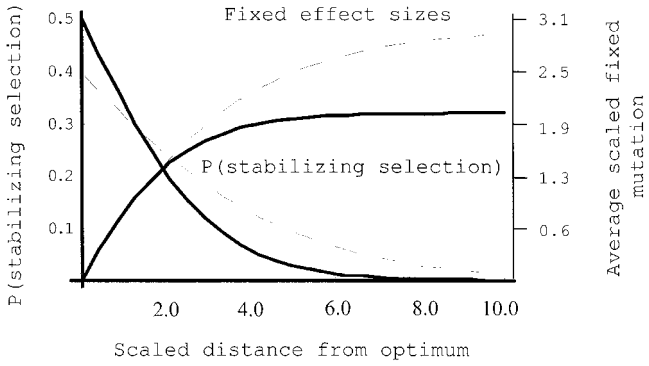

FIGURE 3.—The probability that the next fixed mutation initializes stabilizing selection (left axis) and the average scaled size of the next fixed mutation (right axis) as a function of the distance that the population is from the optimum. Solid lines are for random mutations that are exponentially distributed and dashed lines are for mutations that are gamma distributed with a shape parameter equal to 0.5. The average magnitude of a random mutation is the same for both mutational distributions. The fixed mutation size and distance from the optimum are scaled relative to the average magnitude that a random mutation has.

lizing selection is unimportant. The probability that stabilizing selection is initiated, *i.e.*, the probability of overshooting the optimum with the next fixed mutation, is

$$a(z) = \begin{cases} \dfrac{\int_{|z|}^{|2z|} 2(2|z| - \delta)\sigma f(\delta;\, \alpha,\, \beta)\, d\delta}{k_-} & \text{for } z < 0 \\ \dfrac{\int_{-2z}^{-z} 2(2z + \delta)\sigma f(\delta;\, \alpha,\, \beta)\, d\delta}{k_+} & \text{for } z > 0, \end{cases}$$

evaluating to

$$a(z) = \frac{\exp[|z|/\beta](|z| - \beta) + \beta}{(\exp[|z|/\beta] - 1)^2\beta}$$

when $\alpha = 1$; *i.e.*, mutation is exponentially distributed, for both positive and negative z, where z is the current character state, k_- is a normalization constant when $z < 0$ equal to $\int_0^{|z|} 2\delta\sigma f(\delta;\, \alpha,\, \beta)\, d\delta + \int_{|z|}^{|2z|} 2(2|z| - \delta)\sigma f(\delta;\, \alpha, \beta)\, d\delta$, and k_+ is a normalization constant for $z > 0$ equal to $\int_{-z}^{0} -2\delta\sigma f(\delta;\, \alpha,\, \beta)\, d\delta + \int_{-2z}^{-z} 2(2z + \delta)\sigma f(\delta;\, \alpha,\, \beta)\, d\delta$. As the population approaches the optimum, for exponentially distributed mutation, the probability of initializing stabilizing selection grows from being vanishingly small to being 0.5 (Figure 3, solid curve). When mutation is gamma distributed and the shape parameter is such that the distribution is more leptokurtic than an exponential distribution is, there is a higher probability of initiating stabilizing selection farther from the optimum despite the same average magnitude of a random mutation (Figure 3, dashed curve). As the population approaches the optimum, the potential effects of stabilizing selection grow, and the size of the next fixed mutation becomes increasingly dependent on z, such that the expected size of the next fixed mutation is

$$b(z) = \begin{cases} \dfrac{\int_0^{|z|} \delta(2\delta\sigma) f(\delta;\, \alpha,\, \beta)\, d\delta + \int_{|z|}^{2|z|} \delta(2(2|z| - \delta)\sigma) f(\delta;\, \alpha,\, \beta)\, d\delta}{k_-} \\ \quad = \dfrac{2(z + \beta - \exp[|z|/\beta]\beta)}{(1 - \exp[|z|/\beta])} \quad \text{for } z < 0 \text{ and } \alpha = 1 \\ \dfrac{\int_{-z}^{0} \delta(-2\delta\sigma) f(\delta;\, \alpha,\, \beta)\, d\delta + \int_{-2z}^{-z} \delta(2(2z + \delta)\sigma) f(\delta;\, \alpha,\, \beta)\, d\delta}{k_+} \\ \quad = -\dfrac{2(z + \beta - \exp[|z|/\beta]\beta)}{(1 - \exp[|z|/\beta])} \quad \text{for } z > 0 \text{ and } \alpha = 1\,. \end{cases}$$

The average size of the next fixed mutation is asymptotically constant when the population is sufficiently far from the optimum and only directional selection is occurring, as inferred by OTTO and JONES (2000). But as the population approaches the optimum, the average size of the next fixed mutation decreases to zero (Figure 3). Note that the average fixed effect is larger when the random mutational distribution is more leptokurtic, as expected from OTTO and JONES's (2000) work.

With pleiotropy: *Two-character case:* In the absence of random genetic drift, mutations with nonzero probabilities of fixation satisfy the condition $\delta_1 + \delta_2 > 0$. Thus, if a mutation fixes and its fitness effect on one character is negative, its effect on the other character must be positive with a greater magnitude.

Given that a mutation arises and is bilateral and exponentially distributed, the probability that it has an effect of x on a character and fixes is $g(x;\, 1,\, \beta) = f(x;\, 1,\, \beta)\int_{-x}^{\infty} 2(x + y)\sigma f(y;\, 1,\, \beta)\, dy$, where y is its effect on the other character. The overall distribution of fixed effects for mutations affecting two characters is $c_2(\delta_i;\, 1,\, \beta) = g(\delta_i;\, 1,\, \beta)/\int_{-\infty}^{\infty} g(x;\, 1,\, \beta)\, dx$. Upon simplification, this distribution is

$$c_2(\delta_i;\, 1,\, \beta) = \begin{cases} \dfrac{\exp[2\delta_i/\beta]}{3\beta} & \text{for } -\infty < \delta_i < 0 \\ \dfrac{\exp[-2\delta_i/\beta](2\delta_i \exp[\delta_i/\beta] + \beta)}{3\beta^2} & \text{for } 0 < \delta_i < \infty. \end{cases}$$

Also, for exponentially distributed mutations, the expected size of a fixed mutation's effect on a character averaged over both negative and positive effects is $4\beta/3$. Given that an effect is negative its expected size is $-\beta/2$, and given that it is positive it is $17\beta/10$. The proportion of negative effects is $1/6$ and the proportion positive is $5/6$. See the APPENDIX for equations when random mutation is more leptokurtically distributed.

More than two characters: For n characters, the selection coefficient of a new mutation is $s = \sigma\Sigma_{i=1}^{n}\delta_i$, which provides a convenient approach for deriving the distribution of fixed effects for a single character. Given a mutation with an effect of x for a particular character, the

selection coefficient is $s = \sigma(x + \sum_{i=1}^{n-1}\delta_i)$, where $\sum_{i=1}^{n-1}\delta_i$ is the sum of the effects over the other characters.

When each effect, δ_i, of the other $n - 1$ characters is a bilaterally symmetric exponentially distributed random variable, then by the central limit theorem, the sum of their effects is approximately normally distributed with mean zero and variance $\theta = 2\beta^2(n - 1)$. Using this distribution of effects for the other component characters, analysis proceeds in a similar manner as in the two-character case. For a gamma-distributed mutation with shape parameters <1 the normal approximation is poor, and the distribution of fixed effects is given in the APPENDIX. Let $h(y; \theta)$ be the probability that the sum of the effects on the $n - 1$ other characters is y. $h(y; \theta)$ will be approximately normally distributed with mean zero and variance θ. Given a mutation, the probability that it has effect x on a component character and fixes is $l(x; \beta, \theta) = f(x; 1, \beta)\int_{-x}^{\infty} 2(x + y)\sigma h(y; \theta)\,dy$, and the overall distribution of fixed mutational effects is $c_n(\delta_i; \beta, \theta) = l(\delta_i; \beta, \theta)/\int_{-\infty}^{\infty} l(x; \beta, \theta)\,dx$, or

$$c_n(\delta_i; \beta, \theta) = \frac{\exp[-|\delta_i|/\beta](\delta_i + 2\beta\exp[-\delta_i^2/(2\theta)]\sqrt{(n-1)/\pi} + \delta_i \mathrm{erf}[\delta_i/(\sqrt{2\theta})])}{2\beta^2\Omega}, \quad \text{for } -\infty < \delta_i < \infty$$

where erf[ ] is the error function and $\Omega = \exp[n - 1](1 - \mathrm{erf}[\sqrt{n - 1}]) + 2\sqrt{(n - 1)/\pi}$. The expected size of a fixed effect on a character is $2\beta/\Omega$. The probability that a mutation has a negative effect on a character is $1/2 - 1/(2\Omega)$, and the probability a mutation has a positive effect is $1/2 + 1/(2\Omega)$.

RESULTS

No pleiotropy: During directional selection and in the absence of drift and pleiotropy, no bidirectional effects fix (Figure 4a). For exponentially distributed mutation, the analytical expectation of OTTO and JONES (2000) closely follows the simulated distribution, and the theoretical expectation of the scaled mean fixed effect (2.0) is close to the simulated average (1.98). When adaptation begins closer to the optimum (Figure 4, b and c) and proceeds until the population travels 90% of its original distance to the optimum, bidirectional mutations fix because of stabilizing selection: lowering the scaled average fixed-effect size to 1.43 for Figure 4b and 0.78 for Figure 4c. The distribution of the magnitudes of fixed mutations becomes approximately exponentially distributed when adaptation begins the average magnitude of two or less random mutations from the optimum. When mutation is more leptokurtic, such that the shape parameter of gamma-distributed random mutation is 0.5, the scaled average fixed mutation in simulations (2.98) agrees closely with the prediction of 3.0 by OTTO and JONES (2000). Note that OTTO and JONES (2000) predict that the average fixed effect is $(1 + \alpha)\beta$, and when this is scaled by the average magnitude of a random mutation ($\alpha\beta$), the expected scaled effect is $(1 + \alpha)\beta/\alpha\beta = 1/\alpha + 1$, where α is the shape parameter and β is the scale parameter of random mutation that is bilaterally symmetric and gamma distributed.

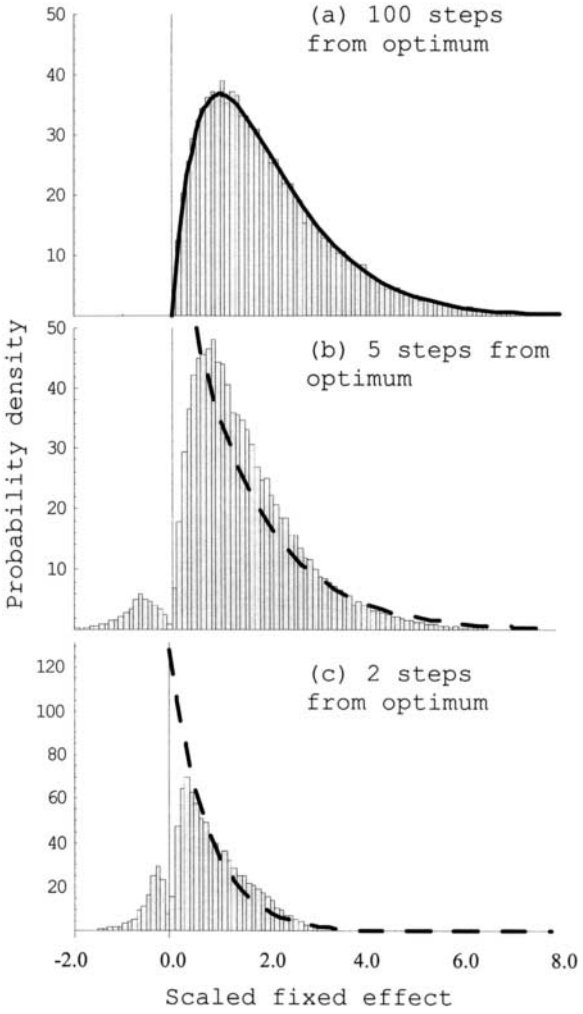

FIGURE 4.—The simulated (histograms) and analytical distributions (solid lines) of fixed mutational effects for mutations affecting a single character. Random mutations are bilaterally symmetric and exponentially distributed with scale parameter equal to 0.01. The magnitude of the slope of the fitness function is 1.0. The effective and census population sizes are both 200,000. Adaptation began the average magnitude of (a) 100, (b) 5, and (c) 2 random mutations below the optimum. Each replicate was stopped when the population traveled 90% of the distance to the optimum. The solid curves are the distribution of fixed effects predicted by the work of OTTO and JONES (2000). The dashed curve is an exponential distribution with a mean equal to the average effect of a fixed mutation in the simulation. The scale for the x-axis is the average magnitude of a random mutational effect on the character.

Under conditions allowing random genetic drift, but in the absence of stabilizing selection, bidirectional effects also can fix (Figure 5a; Table 1). When $N_e = 200$, 7.0% of the mutations have negative effects and the average effect size is 1.74, or 13% less than that in the absence of drift (Table 1). Note that the average magnitude of a fixed effect decreases with a decrease in N_e

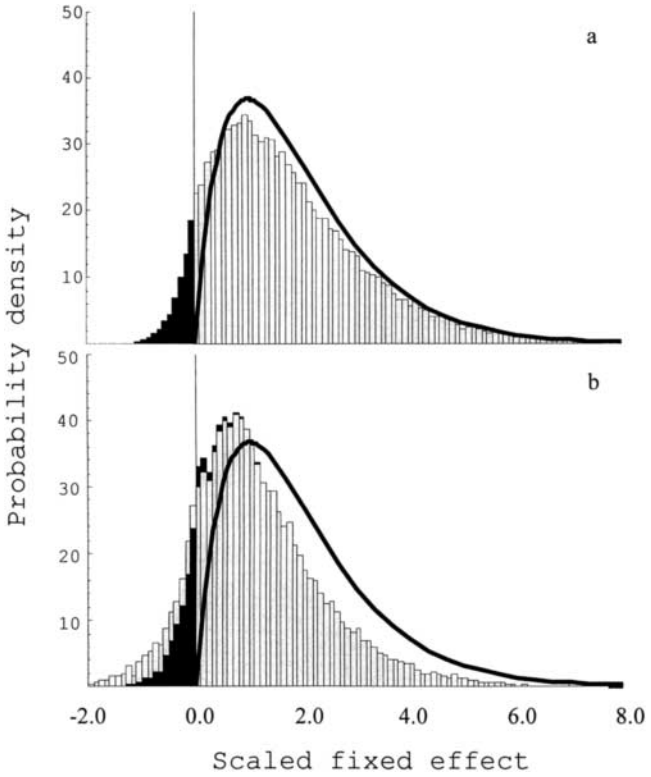

FIGURE 5.—The distribution of fixed effects for mutations affecting single characters under random genetic drift. Here, the effective size of the population is 200, and the census size is 2000. Mutations with effects opposite to the direction of the optimum are represented with solid bars and mutations in the direction of the optimum have shaded bars. Solid and shaded bars appear on both sides of the origin because stabilizing selection about the optimum was sometimes initiated in b. Together the shaded and solid bars add to the overall distribution of fixed mutational effect sizes. (a) The simulated distribution of fixed mutational effect sizes when the population began adaptation the average magnitude of 100 random mutational steps below the optimum. The stopping point for adaptation was when the population traveled 90% of the distance to the optimum. (b) The same as in a except that the population began adaptation a distance equivalent to the average magnitude of 5 mutational steps below the optimum. One thousand replicates of the adaptive process were performed to generate the histograms. The scale for the *x*-axis is the average magnitude of a random mutational effect on a particular character.

TABLE 1

The effects of lowering the effective size of a population on the distribution of the fixed mutational effects

		No. of characters			
N_e		1	2	5	25
50	Sc. effect	0.878	0.34	0.09	0.01
	Sc. magn.	1.23	1.04	1.01	1.00
	% neg.	28	42	48	51
100	Sc. effect	1.36	0.60	0.17	0.01
	Sc. magn.	1.55	1.11	1.01	1.00
	% neg.	16	35	46	49
200	Sc. effect	1.74	0.96	0.33	0.15
	Sc. magn.	1.78	1.28	1.04	1.01
	% neg.	7	26	42	46
2000	Sc. effect	1.96	1.33	0.79	0.34
	Sc. magn.	1.96	1.48	1.18	1.04
	% neg.	0.09	17	30	41
∞	Sc. effect	2.0	1.34	0.80	0.35
	Sc. magn.	2.0	1.50	1.19	1.03
	% neg.	0	17	30	40

Sc. effect, the scaled average effect of a fixed mutation; sc. magn., the scaled average magnitude of a mutation; % neg., percentage of fixed effects that were negative. All values are based on simulations in which at least 10,000 fixed mutations occurred and mutation was bilaterally symmetric and exponentially distributed.

(Table 1). Under conditions of genetic drift and stabilizing selection in Figure 5b, the frequency of negative effects increased to 13.7% and the average effect size decreased to 1.15.

Mutations pleiotropically affecting two characters: When mutation is bilateral and exponentially distributed, bidirectional mutational effects fix in the presence of pleiotropy and in the absence of drift and stabilizing selection (Figure 6a). The average scaled fixed effect in the simulations (1.32) agrees with our prediction (1.34). The frequency of negative effects in the simulations (16.1%) is accurately predicted by the theory presented here (16.67%). The average scaled fixed effect given that it is positive in the simulations (1.69) agrees with our prediction (1.70). When mutation is more leptokurtic, the average scaled fixed mutational effect increases. For instance, when the shape parameter of the mutational distribution is 0.5, the average scaled fixed mutational effect is 1.80 in simulations, which is in agreement with the value (1.83) predicted by the equation presented in the APPENDIX. Although the average fixed effect increases, the frequency of negative effects also increases ~3% to 19.2% in simulations (19.4% by the equations presented in the APPENDIX). The increase in average fixed effect, despite an increase in the frequency of negative effects, is due to the fact that larger mutations arise at higher probabilities when mutation is more leptokurtically distributed, conditioned on the same average random effect, and these larger mutations, if beneficial, have a high probability of fixation.

When N_e is small, but there is no stabilizing selection, an increasing fraction of negative effects occur, the average size of a fixed effect decreases, and the average magnitude of a fixed effect decreases (Table 1). When stabilizing selection occurs because adaptation begins closer to the optimum, the average fixed effect size decreases 62% for the conditions presented in Table 2. Correspondingly, the distribution becomes more balanced because of the sequential overshooting of the optimum such that ~26% of the mutations are in one direction and 74% in the other. The magnitude of fixed effects also decreased by 53%.

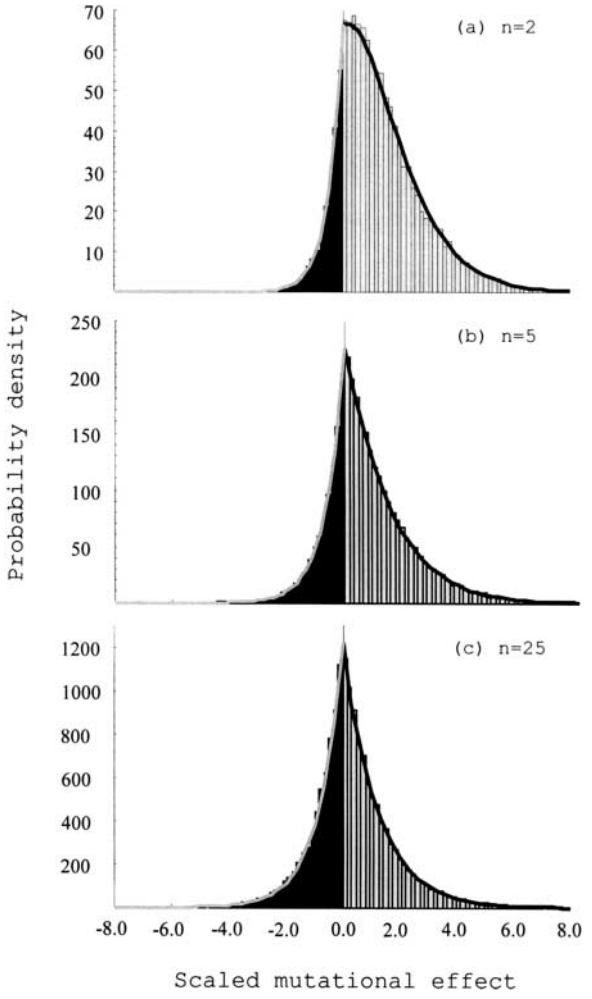

FIGURE 6.—The distribution of fixed effects after adaptation for mutations affecting 2, 5, and 25 characters. The lines are based on the analytical results and the histograms are based on simulations. Shaded bars correspond to cases in which the mutation has a positive pleiotropic effect and solid bars indicate when it has a negative pleiotropic effect. The scale for the *x*-axis is the average magnitude of a random mutational effect on a particular character. In the simulations, each character begins adaptation at a scaled value of 100 units below its optimum. The effective and census population sizes are both 2×10^5. The magnitude of the slope of the fitness function is 1.0. Simulations stopped when the overall phenotype evolved to 90% of the distance to the optimum. The graphs show the results for mutations pleiotropically affecting (a) 2, (b) 5, and (c) 25 characters. In each case, $\alpha = 1$ and $\beta = 0.01/d$. There were 1000 replicates for a and 100 each for b and c.

Mutations pleiotropically affecting multiple characters: The relative frequency of negative effects increases as the degree of pleiotropy increases (Figure 6, b and c). Both simulation and mathematical analyses show that, for exponentially distributed mutation, the average scaled fixed mutational effect decreases as pleiotropy increases: from 2.0 with no pleiotropy, to 0.8 when mutations affect 5 characters, to 0.35 when mutations affect 25 characters. When mutations affect 5 characters, simulations show that 30.1% of fixed mutations have negative effects, agreeing with the analytical expectation of 30.0%. For 25 characters, 40.0% were negative, in agreement with the analytical expectation of 40.1%. When random mutation is more leptokurtic such that the shape parameter is 0.5, the average scaled fixed effect is 1.0 in simulations (1.0 by the equations in the APPENDIX) when mutations affect 5 characters and 0.44 (0.44 by the equations in the APPENDIX) when mutations affect 25 characters. Again, despite the increase in the average fixed effect, the frequency of negative effects also increases such that when mutations affect 5 characters it is 32.2% (32.5% by the equations in the APPENDIX) and when they affect 25 characters it is 42.6% (42.6% by the equations in the APPENDIX).

TABLE 2

The effects of stabilizing selection on the distribution of fixed mutational effects

No. of characters	Average scaled fixed effect	Average scaled fixed effect magnitude	% negative effects[a]
2	0.50	0.70	26.4
5	0.29	0.60	37.0

Under stabilizing selection, adaptation began the magnitude of two random mutations away from the optimum for each character and stopped once the phenotype traveled 90% of the distance to the optimum. The effective and census population sizes were both 2×10^5. One thousand replicates of the adaptive process occurred for each value. Random mutation was bilaterally symmetric and exponentially distributed.

[a] A character began adaptation to one side of the optimum, and the direction of effects is standardized relative to this initial positioning. Negative effects correspond to mutations that fixed in the opposite direction of the optimum relative to the initial positioning of a character.

When random genetic drift occurs in the absence of stabilizing selection, the average fixed effect size decreases, the average magnitude of a fixed effect decreases, and the frequency of negative effects increases (Table 1). Stabilizing selection drops the average size of a fixed effect 64% under the conditions presented in Table 2 for mutations affecting five characters, and the distribution of fixed effects becomes more balanced with 37% of the effects being negative. The overall magnitude of fixed effects also drops, this time by 50%.

CONSEQUENCES FOR INFERENCE

The following analyses are applicable to QTL studies that cross individuals from isolated populations or species that differ phenotypically. Under these circumstances, fixed mutational differences are one source of the phenotypic difference between populations or species.

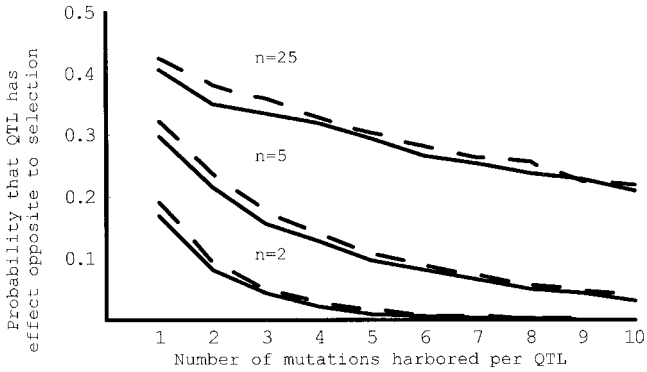

FIGURE 7.—The probability that a QTL has an effect on a character that is opposite to the direction of selection *vs.* the number of mutations harbored per QTL. From bottom to top, results are plotted for mutations pleiotropically affecting 2, 5, and 25 characters, respectively. Solid lines indicate when random mutations are exponentially distributed and dashed lines when the mutational distribution is more leptokurtic (gamma distributed with $\alpha = 0.5$). The fractions are based on 1000 replicate samples from the distribution of fixed mutational effects for each case.

QTL effects: Provided that a QTL consists of one mutational effect, our results would predict the distribution of QTL effects as well as mutational effects. QTL may consist of more than one mutation in which the overall effect of a QTL is a function of the total mutational effects within that region of DNA (NOOR *et al.* 2001). If there is more than one effect in a QTL region, the QTL will have a total effect that could mask some of the individual effects. For example, a negative effect on a character could be hidden by a larger positive effect in the same region. The probability that the overall effect of a QTL is negative decreases as the number of mutations per QTL increases (Figure 7). Although the overall effect of the QTL may be positive, by summing over the effects of two or more mutations, negative effects would be masked. Because mutations with negative effects are on average smaller than those with positive effects, they have less of a masking effect than those with positive effects. When the mutational distribution is more leptokurtic, there is a higher probability that a QTL will have a negative effect because as was shown earlier, there is a higher frequency of fixed mutations with negative effects on a character.

Of the detected QTL that affect a character, there is a bias for them to contain proportionally fewer mutations with negative pleiotropic effects than the true proportion if all of the regions of DNA that contain mutations that affect the character were detected (Figure 8). As the detection threshold of a QTL study becomes worse, *i.e.*, the average magnitude of an effect that can be detected increases, the bias against detecting mutations with negative pleiotropic effects is magnified. The bias is magnified because positive fixed effects are on average larger than negative effects (on an absolute scale) and given that only mutations of larger absolute effect are detected, they are more likely to be positive.

Inferring directional selection on QTL loci: ORR (1998b) proposed that a sufficient number of unidirectional QTL effects would be evidence of directional selection acting on a character, while bidirectional effects would neither refute nor provide evidence for directional selection. When this method is tested against our results, in which only directional selection occurred and it is assumed that one fixed mutation is present per QTL, the ability to infer directional selection is weak (Table 3). The power of the sign test is <50% except when the random mutational distribution is more leptokurtic, mutations affect very few characters, and detection thresholds are poor. Power increases, despite poorer detection thresholds, because as the magnitude of an effect that is able to be detected increases, the probability the effect will be positive as opposed to negative increases. Unfortunately, it is not possible to correctly evaluate Orr's method when several mutations are fixed per QTL because the sign test assumes the distribution of random QTL effects is gamma distributed. When random mutational effects are gamma distributed and more than one mutation is present per QTL, the expected random QTL effect cannot be closely approximated by a gamma distribution. Qualitatively, though, power improves as the number of mutations fixed per QTL increases because as the number of fixed mutations increases per QTL, the probability that the overall effect of the QTL is positive increases.

DISCUSSION

This study quantified the degree to which pleiotropy contributes to the fixation of mutational effects opposite to the direction of selection for a character and compared it to the contributions of stabilizing selection and random genetic drift. As may be expected, the results have shown that pleiotropy can be a major cause of the fixation of effects opposite to the direction of selection even when the degree of pleiotropy is minimal; *i.e.*, a random mutation affects merely two phenotypic characters. The results also show that despite an increase in the frequency of negative effects when the random mutational distribution is more leptokurtic, the average scaled size of a fixed effect does not decrease—it actually increases.

That the fraction of negative effects increases with the degree of pleiotropy is perhaps unsurprising given that pleiotropy clearly allows mutations with weakly deleterious effects to fix because there is the possibility that they also have stronger beneficial effects. Given a set of mutations that have a certain probability of having a positive effect on a character, as the degree of pleiotropy increases, the fraction of negative fixed effects will increase because there is a better chance that a negative effect will be counterbalanced by a positive effect. What

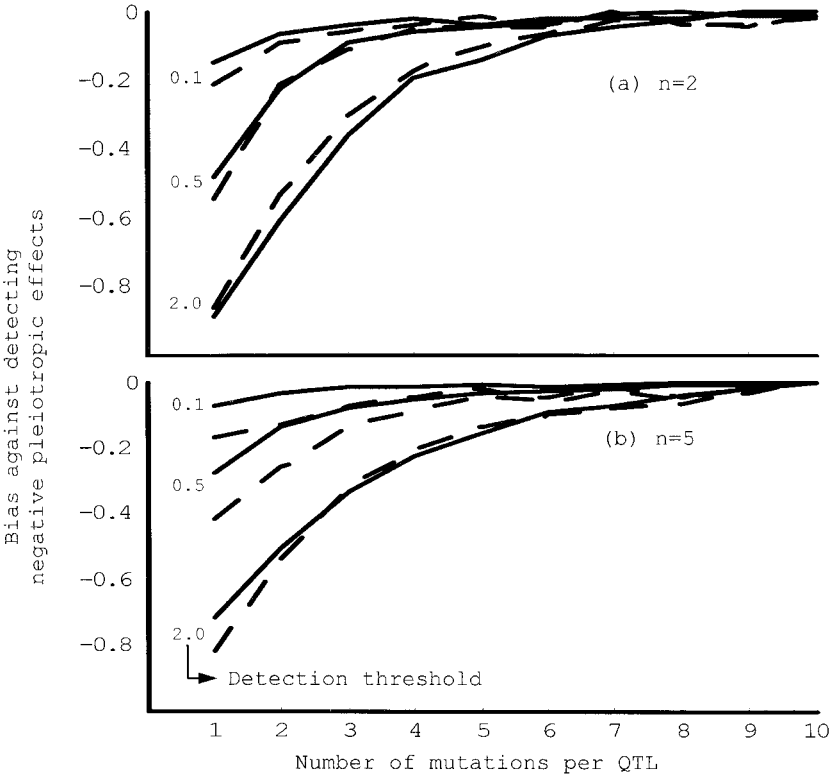

FIGURE 8.—The bias in detected QTL to contain fewer negative pleiotropic effects than expected. A bias of zero represents the case when the observed number of negative pleiotropic effects is equal to the true number when the detection threshold is zero. A bias of −0.2 represents the case when the number of negative pleiotropic effects is reduced by 20% of the true value. A detection threshold of zero corresponds to the case when a QTL study can potentially detect all mutations affecting a character. A detection threshold of 2.0 corresponds to a QTL study that can detect mutations as small as twice the average magnitude of a random mutation affecting a character. Solid lines are for exponentially distributed mutations and dashed for more leptokurtic mutations drawn from a gamma distribution ($\alpha = 0.5$). The plots are based on 1000 replicate samples from the distribution of fixed mutational effects.

is perhaps surprising is that mutations with deleterious effects, which are fixed by pleiotropic selection, can be so frequent.

Implications of model assumptions: Our model implies that the distribution of fixed effects is independent of the strength of selection (σ). This will not be strictly true if we relax the assumption that the mutation rate to beneficial mutations is slow relative to the rate at which they fix. When mutations cosegregate, there is the potential for selective interference (HILL and ROBERTSON 1966). A consequence may be that beneficial mutations of larger effect may outcompete beneficial mutations of smaller effect.

The model assumes that random mutation is equally likely to be in one direction as another for a character. Results from MACKAY *et al.* (1992) and LYMAN *et al.* (1996) suggest this assumption may be reasonable, but more empirical study is necessary. Note that modeling mutation this way does not assume that it is equally likely to have a mutation arise that is beneficial overall *vs.* one that is deleterious. Deleterious mutations still occur at higher probability. Furthermore, in the APPENDIX an alternate approach that allows for the possibility that negative effects occur at a high frequency is presented.

The distributions of fixed effects derived here are for a set of mutations that pleiotropically affect the same number of characters. Some characters may be affected by mutations that pleiotropically affect different numbers of characters. The resulting effects for such characters would be a mixed distribution over different degrees of pleiotropy.

The model assumes characters are independently affected by new mutations. Clearly this is not the case, in general. To overcome this problem, empirical studies need to employ statistical methods that orthogonalize their data if they wish to use the results presented here. Incorporating the effects of mutational covariance on the distribution of fixed effects represents a significant challenge for future work.

We have assumed that organisms are haploid. This

TABLE 3

The power of ORR's (1998b) method to detect directional selection

	Detection threshold (%):	No. of characters					
		2		5		25	
		0	50	0	50	0	50
Exponential		17.2	41.4	13.8[a]	33.1	3.9	6.8
Leptokurtic ($\alpha = 0.5$)		39.2	79.9	11.1	33.1	2.8	7.5

In the analysis it was assumed that 10 QTL loci were detected and one fixed mutation occurred per QTL. Power is based on 1000 replicate samples at the $\alpha = 0.05$ significance level, with one exception.

[a] The *P* value associated with 9 positive effects was 0.0526.

assumption will likely not affect our overall conclusions under directional selection with no drift. In a diploid model, under directional selection with drift and recessivity, deleterious mutations have a higher probability of fixation, which may alter the distribution of fixed mutational effects. Likewise, in a diploid model under stabilizing selection and codominance, if mutations cosegregate and one mutation of large effect is paired with a mutation of small effect, they may have a combined effect that is beneficial; *i.e.*, together they bring a phenotype to be closer to the optimum. But when the mutation with a large effect becomes homozygous, it may then overshoot the optimum to such an extreme that it is deleterious because the homozygous effect brings the phenotype to be farther from the optimum. This overdominance and its importance are left for future study.

Evolutionary consequences: Similar to the finding of Orr (2000), our results show that as the degree of pleiotropy increases the average scaled size of a fixed effect per character decreases. The scale is relative to the average magnitude of a random mutational effect per character, so the inference is not an artifact of the possibility that as pleiotropy increases the average magnitude of a random mutation may decrease.

The decrease in the average scaled fixed effect per character and the increase in frequency of negative effects present potential measures that can be used in comparative studies to determine whether characters are pleiotropically associated with more or less characters in one taxa *vs.* another, or whether, within a taxa, one character is pleiotropically associated with more characters than another. The implementation of such measures would assume that the characters of interest are experiencing the same evolutionary forces, that is, the same amount of drift, stabilizing selection, and directional selection.

Implications for inferences: Some studies rely on QTL analyses to narrow down regions of a genome that have mutations that affect a character and then perform positional cloning techniques to ultimately determine the effects of individual mutations on a character. Our results quantify a systematic bias for these studies to miss mutations with negative effects. Of the detected QTL, there is a bias for these to contain fewer negative effects than actually occur in the genome because the QTL that contain negative effects are less likely to be detected. Additionally, in missing regions with negative effects, the studies will also miss some positive effects because of masking by the negative ones.

The ability to tell whether directional selection is shaping the evolution of a character is made more complicated by pleiotropy. The frequency of negative pleiotropic effects is sufficient to cause high type II error rates with Orr's (1998b) method.

This study quantified how pleiotropy leads to the fixation of bidirectional mutational effects even in the absence of random genetic drift and stabilizing selection. Mutations pleiotropically affecting more characters fix more negative effects. The potential prevalence of bidirectional effects caused by pleiotropy leads to biases in QTL studies that seek to determine the genetic basis of phenotypic characters.

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APPENDIX

Here we describe the distribution of fixed effects for mutations pleiotropically affecting two or more characters when the random mutational distribution is leptokurtic such that the shape parameter of the gamma distribution is less than one.

Mutations pleiotropically affecting two characters: The derivation of the distribution of fixed effects proceeds as in the exponentially distributed mutational case. The probability that a mutation has an effect of size x on a character and fixes is $g(x; \alpha, \beta) = f(x; \alpha, \beta)\int_{-x}^{\infty} 2((x + y)\sigma)f(y; \alpha, \beta)\,dy$, where y is its effect on the other character. The distribution of fixed effects is $\zeta_2(\delta_i; \alpha, \beta) = g(\delta_i; \alpha, \beta)/\int_{-\infty}^{\infty} g(x; \alpha, \beta)\,dx$. The fraction of fixed effects that are negative and the average fixed effect size can be determined by numerically integrating $\zeta_2(\delta_i; \alpha, \beta)$.

Mutations pleiotropically affecting multiple characters: Here the normal approximation to the sum of the effects on the $n - 1$ other characters is poor. But we can make use of the property of gamma-distributed random variables that given r effects are of the same sign and are drawn from a distribution with the same shape (α) and scale parameter (β); then the distribution of the absolute value of their sum is gamma distributed with shape parameter $r\alpha$ and scale parameter β. In our model up to now, the probability that a random mutational effect on a character is positive is 1/2 and correspondingly the probability that it is negative is also 1/2. We can further generalize and have the probability a random mutation is positive be p and the probability it is negative be q. Then given a mutation that pleiotropically affects n characters, we focus on the effect of that mutation on one character and ask what the probability is that, of the remaining $n - 1$ characters, t are positive. This probability is binomially distributed, or $v_n(t; p, q) = \binom{n-1}{t} p^t q^{n-t-1}$. Given that t effects are positive, the probability that the sum of those effects is equal to y is $f(y; t\alpha, \beta)$ using the same notation as in the main text. Likewise, given that $n - t - 1$ effects are negative, the probability that the sum of those effects is equal to w is $f(w;(n - t - 1)\alpha, \beta)$. The probability that a mutation arises and fixes with effect x on a component character needs to be broken into two parts: when x is negative *vs.* when it is positive. When x is negative, the probability that it arises and fixes is

$$m_n^-(x; \alpha, \beta) = f(x; \alpha, \beta)\int_{-\infty}^{0}\int_{-(x+w)}^{\infty} \sum_{i=0}^{n-1} 2v_n(i; p, q)((x + w + y)\sigma)f(w;(n - i - 1)\alpha, \beta)f(y; i\alpha, \beta)\,dydw$$

and when x is positive the probability is

$$m_n^+(x; \alpha, \beta) = f(x; \alpha, \beta)\int_{0}^{\infty}\int_{-(x+y)}^{\infty} \sum_{i=0}^{n-1} 2v_n(i; p, q)((x + w + y)\sigma)f(w;(n - i - 1)\alpha, \beta)f(y; i\alpha, \beta)\,dwdy.$$

The overall distribution of fixed mutational effects for mutations affecting n characters is

$$\zeta_n^-(\delta_i; \alpha, \beta) = \frac{qm_n^-(\delta_i; \alpha, \beta)}{q\int_{-\infty}^{0} m_n^-(x; \alpha, \beta)\,dx + p\int_{0}^{\infty} m_n^+(x; \alpha, \beta)\,dx}$$

for negative δ_i and

$$\zeta_n^+(\delta_i; \alpha, \beta) = \frac{pm_n^+(\delta_i; \alpha, \beta)}{q\int_{-\infty}^{0} m_n^-(x; \alpha, \beta)\,dx + p\int_{0}^{\infty} m_n^+(x; \alpha, \beta)\,dx}$$

for positive δ_i. As was shown in the RESULTS when mutation is bilateral and exponentially distributed and pleiotropically affects two characters, 16.67% of fixed mutations have negative effects and the average scaled fixed effect is 1.34. In comparison, when 90% of mutations have positive effects, *i.e.*, are beneficial and 10% have negative effects, the fraction of fixed mutations with negative effects is 2.6% and the averaged scaled fixed effect is 1.47. When 10% of mutations have positive effects and 90% have negative effects, 40.9% of fixed mutations are negative and the average scaled fixed effect is 1.10.