Genet. Res., Camb. (1999), **73**, *pp.* 263–273. *With 3 figures.* Printed in the United Kingdom © 1999 Cambridge University Press

An experimental method for evaluating the contribution of deleterious mutations to quantitative trait variation

JOHN K. KELLY*

Department of Biology, University of Oregon, Eugene, OR 97403, USA

(*Received 3 August 1998 and in revised form 27 October 1998 and 8 December 1998*)

Summary

Unconditionally deleterious mutations could be an important source of variation in quantitative traits. Deleterious mutations should be rare (segregating at low frequency in the population) and at least partially recessive. In this paper, I suggest that the contribution of rare, partially recessive alleles to quantitative trait variation can be assessed by comparing the relative magnitudes of two genetic variance components: the covariance of additive and homozygous dominance effects (C_{ad}) and the additive genetic variance (V_a). If genetic variation is due to rare recessives, then the ratio of C_{ad} to V_a should be equal to or greater than 1. In contrast, C_{ad}/V_a should be close to zero or even negative if variation is caused by alleles at intermediate frequencies. The ratio of C_{ad} to V_a can be estimated from phenotypic comparisons between inbred and outbred relatives, but such estimates are likely to be highly imprecise. Selection experiments provide an alternative estimator for C_{ad}/V_a, one with favourable statistical properties. When combined with other biometrical analyses, the ratio test can provide an incisive test of the deleterious mutation model.

1. Introduction

A primary objective of population genetics is to determine the factors maintaining genetic variation (Robertson, 1967; Lewontin, 1974). Natural populations generally exhibit substantial genetic variation for morphological, behavioural and life history traits (Roff & Mousseau, 1987; Mousseau & Roff, 1987; Houle, 1992). While mutation is the ultimate source of this variation, the role of other evolutionary forces (selection, migration and random genetic drift) in maintaining variation is generally unknown. This is unfortunate because the nature and intensity of natural selection will largely determine the adaptive significance of standing variation and hence the 'evolutionary potential' of populations.

Deleterious mutations could be one important source of genetic variation in quantitative traits. Such mutations occur at a fairly high rate in most organisms and could generate substantial variation in life history traits (Mukai *et al.*, 1972; Simmons & Crow, 1977; Lynch, 1988; Houle *et al.*, 1994, 1996; Drake *et al.*, 1998; but see Keightley, 1996, 1998; Keightley & Caballero, 1997). Deleterious mutations may also be responsible for variation in morphological or behavioural traits via pleiotropy (Keightley & Hill, 1990; Barton, 1990; Santiago *et al.*, 1992; Mackay *et al.*, 1992; Kondrashov & Turelli, 1992; Caballero & Keightley, 1994). Variation caused by unconditionally deleterious mutations is qualitatively different from that maintained by other mechanisms (e.g. balancing selection) because the former may be effectively irrelevant to adaptive evolution (Keightley & Hill, 1990; Houle *et al.*, 1996).

Two important predictions of the deleterious mutation model can be tested empirically. First, quantitative trait variation should be due to rare alleles. Deleterious mutations should not reach substantial frequencies unless selection is very weak. Secondly, the rare allele at each quantitative trait locus should usually be at least partially recessive in its effects on fitness. Mutations with large deleterious effects (e.g. lethals) tend to be almost completely recessive (Simmons & Crow, 1977). Gene action is more variable among mutations with smaller effects (individual mutations may be partially recessive,

* Present address: Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045, USA. e-mail: jkk@eagle.cc.ukans.edu.

additive or partially dominant). However, recessive or partially recessive alleles are likely to dominate standing variation because they will persist longer than additive or dominant mutations under antagonistic selection.

Partial recessivity of deleterious mutations is supported by direct estimates of dominance coefficients for alleles affecting life history traits. Charlesworth & Hughes (1998) summarize many studies of *Drosophila melanogaster* and suggest that the heterozygous effect of deleterious alleles is typically 20% of their homozygous effect ($h = 0{\cdot}2$). Dudash & Carr (1998) and Willis (1999) have obtained similar estimates for a range of life history characters in the annual plant *Mimulus guttatus*. Morphological variation caused by the pleiotropic effects of deleterious mutations should also be caused by rare, partially recessive alleles *if* alleles have consistent dominance relations across all traits they affect. Consistency implies that alleles that are recessive in their effects on fitness will also be recessive in their effects on other traits. Genetic data from *Drosophila* provide support for consistency of dominance relations (Keightley & Kacser, 1987).

Quantitative genetic methods can be used to assess the contribution of rare, partially recessive alleles (e.g. Charlesworth & Hughes, 1998). The change in the genetic variance with inbreeding is particularly informative (Robertson, 1952; Jacquard, 1974). In the next section, I review genetic variance components that emerge with inbreeding. Rare recessives inflate the magnitude of these 'inbreeding components' relative to the additive genetic variance.

2. Genetic statistics

Consider the standard model of quantitative trait inheritance in which the phenotype is the sum of statistically independent genetic and environmental components (Falconer, 1989). We assume that the genotypic value of an individual is the sum of genotypic effects at each of its quantitative trait loci (there is no epistasis). With random mating and linkage equilibrium among quantitative trait loci, the genetic variance is the sum of genetic variances at individual loci.

Consider a particular locus segregating for a high allele (A_1) and a low allele (A_0). Following Falconer (1989, ch. 7), the average phenotypic values of genotypes A_0A_0, A_0A_1 and A_1A_1 are $-a$, d and a, respectively. Let p denote the frequency of A_1 and q denote the frequency of A_0. It follows that the genotypic variance (V_g) at this locus equals the additive variance (V_a) plus the dominance variance (V_d), where $V_a = 2pq[a+d(q-p)]^2$ and $V_d = 4(pqd)^2$. I will subsequently assume that $-a \leqslant d \leqslant a$ (no phenotypic over- or under-dominance).

Inbreeding can change both the mean and genetic variance of a quantitative character. The extent to which the mean changes with inbreeding is characterized by B, the directional dominance. This equals the difference in mean phenotype between an outbred population and a completely inbred population with the same allele frequencies. (Here, I use the term directional dominance instead of the more familiar 'inbreeding depression' because the latter is often formally defined as a ratio.) Under the model outlined above, the directional dominance associated with a single locus is $-2pqd$ (Cockerham & Weir, 1984). The overall value for B is the sum of this quantity across all quantitative trait loci. Using the subscript k to denote locus, we can define the standard phenotypic mean of a randomly mating population (M) and the directional dominance (B) in terms of allele frequencies and genotypic effects:

$$M = \sum_k a_k(p_k - q_k) + 2d_k p_k q_k \tag{1}$$

and

$$B = -\sum_k 2d_k p_k q_k, \tag{2}$$

where the sums are taken across all loci affecting the trait. With inbreeding, the mean phenotype is $M + FB$, where F is the mean inbreeding coefficient (Jacquard, 1974; Cockerham & Weir, 1984).

The genetic variance with inbreeding depends on V_a, V_d and several additional quantities (Harris, 1964; Jacquard, 1974; Cockerham & Weir, 1984; Cornelius, 1988; de Boer & Hoeschele, 1993). Let C_{ad} denote the covariance of additive effects with homozygous dominance effects and V_{hd} denote the variance of homozygous dominance effects. The 'homozygous dominance effect' is the dominance deviation associated with a particular allele when that allele is in homozygous form. As shown by Cockerham & Weir (1984), the values for these quantities at a single diallelic locus are

$$C_{ad} = 2pq(p-q)\,d[a+d(q-p)] \quad \text{and}$$
$$V_{hd} = 4pq(q-p)^2d^2.$$

The ratios of C_{ad} and V_{hd} to V_a are given as a function of the frequency of a partially recessive allele in Fig. 1. If the additive variation in a trait is due primarily to rare recessive alleles, we should observe relatively large values for C_{ad} and V_{hd}. If alleles with intermediate frequencies predominate, however, V_a should be substantially greater than C_{ad} or V_{hd}. In fact, C_{ad} may be negative when recessive alleles occur at high frequencies (greater than 0·5). All these quantities can be estimated from phenotypic comparisons among relatives if inbred individuals are included in the pedigree (Cockerham & Weir, 1984; Cornelius, 1988;

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

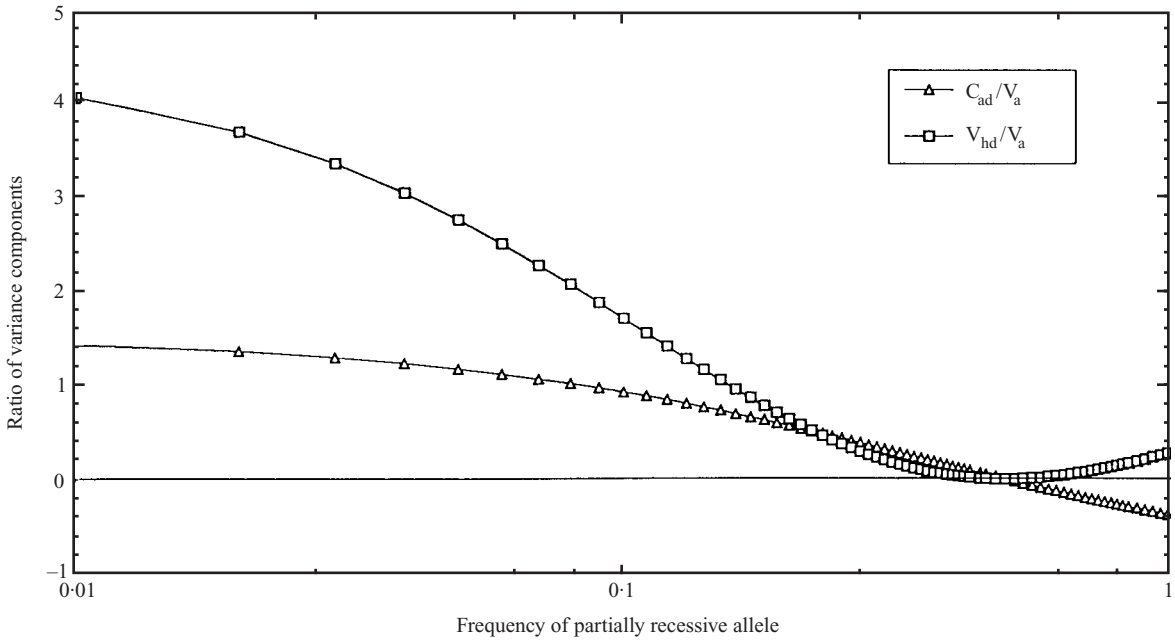

Fig. 1. The ratio of C_{ad} or V_{hd} to the additive genetic variance given the frequency of a partially recessive allele. In this case, the heterozygous effect of the allele is 20% of its homozygous effect ($h = 0{\cdot}2$; $d = -0{\cdot}6a$).

de Boer & Hoeschele, 1993; Shaw & Woolliams, 1998; Shaw *et al*., 1998).

Drosophila geneticists have estimated V_a and the variance among homozygous genotypes (hereafter denoted V_{hg}) for numerous life history traits of *Drosophila melanogaster* (Takano *et al*., 1987; Hughes, 1995, 1997; summarized by Charlesworth & Hughes, 1998). In the terminology of the present paper, $V_{hg} = 2V_a + 4C_{ad} + V_{hd}$. If quantitative trait alleles are at intermediate frequencies or if there is no dominance, V_a/V_{hg} should approach 0·5 (Charlesworth & Hughes, 1998). Lower values for V_a/V_{hg} are expected under the deleterious mutation model. For variation in male longevity attributable to the third chromosome of *D. melanogaster*, Hughes (1995) obtained an estimate for V_{hg} that was only slightly greater than V_a. This suggests that at least some intermediate frequency alleles contribute to variation in the trait (Charlesworth & Hughes, 1998).

Unfortunately, statistical difficulties hinder the broad application of this method. Estimates for V_{hg}, V_a, C_{ad} or V_{hd} from any single experiment will generally have large sampling errors. As a consequence, the ratio of estimated values is a biased estimator of the true ratio and it will typically have a high sampling variance. To illustrate, let $V_a{*}$ and $C_{ad}{*}$ denote estimators for V_a and C_{ad}. By the 'delta method' (Rice, 1988, pp. 142–147; Lynch & Walsh, 1998, pp. 807–809), we find the approximate bias and sampling variance of the ratio of $V_a{*}$ to $C_{ad}{*}$:

$$E\left[\frac{C_{ad}{*}}{V_a{*}} - \frac{C_{ad}}{V_a}\right] \approx \frac{1}{V_a^2}\left[s_{aa}\left(\frac{C_{ad}}{V_a}\right) - s_{ad}\right] \tag{3}$$

and

$$V\left[\frac{C_{ad}{*}}{V_a{*}}\right] \approx \frac{1}{V_a^2}\left[s_{aa}\left(\frac{C_{ad}}{V_a}\right)^2 + s_{dd} - 2s_{ad}\left(\frac{C_{ad}}{V_a}\right)\right], \tag{4}$$

where s_{aa} is the sampling variance of V_a, s_{dd} is the sampling variance of $C_{ad}{*}$, and s_{ad} is the sampling covariance of $V_a{*}$ and $C_{ad}{*}$. If s_{aa}, s_{dd} and s_{ad} are small relative to V_a^2, then the bias and sampling variance are small. Unfortunately, prohibitively large sample sizes may be required to ensure that s_{aa}, s_{dd} and $s_{ad} \ll V_a^2$.

The sampling covariance, s_{ad}, has a particularly important effect on the accuracy of ratio estimates. If $V_a{*}$ and $C_{ad}{*}$ are estimated from distinct experiments then $s_{ad} = 0$. However, if they are estimated simultaneously from the same data (e.g. Shaw *et al*., 1998), the sampling covariance will generally be non-zero. A negative sampling covariance between $V_a{*}$ and $C_{ad}{*}$ ($s_{ad} < 0$) will usually inflate both the bias and the sampling variance. This is noteworthy because sampling covariances between genetic variance component estimators are typically negative (Shaw, 1987; Cornelius, 1988).

An alternative means of estimating the relative magnitudes of C_{ad} and V_a is from the rate of evolution in selection experiments. In the short term, the expected change in the mean phenotype (M) equals the product of the cumulative selection differential and the narrow-sense heritability (Falconer, 1989). The latter is V_a divided by V_p, the phenotypic variance. The expected change in the directional dominance (B) is the product of the cumulative selection differential and C_{ad}/V_p (Kelly, 1999*a*, *b*; Appendix). Thus, the ratio of the cumulative change in B to the cumulative

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

change in M provides an estimate of the ratio of C_{ad} to V_a.

Estimation of C_{ad}/V_a from a selection experiment has two principal advantages. The first is simplicity: the cumulative change in the directional dominance can be measured simply by generating inbred progeny from the base population (or a non-selected control population) and from the selected population. The difference in mean values of inbred and outbred individuals provides an estimate of B (Cockerham & Weir, 1984). Secondly, sampling errors in estimates of C_{ad} and V_a, which are caused by genetic drift in this type of experiment, should be positively correlated when quantitative trait variation is caused primarily by rare recessive alleles. Positive values for s_{ad} allow the ratio of C_{ad} to V_a to be estimated with much greater precision (Eq. 4).

This can be illustrated with a highly simplified version of the deleterious mutation model. We assume that all mutations segregating in the population are equally deleterious and have equivalent fitness effects in heterozygotes. The fitnesses at a particular locus are 1, $1-hs$ and $1-s$ for the wild-type homozygote, the heterozygote and the mutant homozygote, respectively. As a consequence of their equivalent fitness effects, we assume that all deleterious mutations have the same frequency (ρ).

Pleiotropic effects of deleterious alleles may either increase or decrease trait values. I assume that there are n_1 loci where the deleterious allele increases the trait value and n_2 loci where the deleterious allele decreases the trait value. I further assume that dominance relations for phenotype and fitness are consistent (the rare allele is always partially recessive). Thus, $d = a(1-2h)$ for rare 'low alleles' and $d = a(2h-1)$ for rare 'high alleles'. With these assumptions,

$$C_{ad} = 2(n_1+n_2)\rho(1-\rho)(1-2\rho)a^2(1-2h)(1-(1-2h)(1-2\rho)) \quad (5)$$

and

$$V_a = 2(n_1+n_2)\rho(1-\rho)a^2(1-(1-2h)(1-2\rho))^2. \quad (6)$$

Thus,

$$\frac{C_{ad}}{V_a} = \frac{(1-2\rho)(1-2h)}{1-(1-2h)(1-2\rho)}, \quad (7)$$

which converges on $(1-2h)/2h$ as ρ gets small. With $h = 0{\cdot}2$, $C_{ad}/V_a \approx 1{\cdot}5$. If $h < 0{\cdot}2$ (see Watanabe & Ohnishi, 1975; Sved & Wilton, 1989), then C_{ad}/V_a may be substantially greater.

Equation (7) gives the ratio of C_{ad} to V_a as a function of allele frequencies (ρ) in the population as a whole, i.e. the 'reference' or 'base' population about which we hope to make inferences. After a selection experiment is conducted, allele frequencies will differ from ρ due to both selection and genetic drift. Drift occurs first when an experimental population is established by sampling the initial set of individuals and also during each subsequent round of selection and reproduction. Let M_0 and B_0 denote the mean phenotype and directional dominance of the base population, respectively. Let M_t and B_t denote the values for these quantities in a particular experimental population after t generations of selection. To a first approximation,

$$M_t - M_0 \approx 2a(1-(1-2h)(1-2\rho))\left[\sum_i^{n_1}\Delta p_i + \sum_j^{n_2}\Delta p_j\right] \quad (8)$$

and

$$B_t - B_0 \approx 2a(1-2h)(1-2\rho)\left[\sum_i^{n_1}\Delta p_i + \sum_j^{n_2}\Delta p_j\right], \quad (9)$$

where Δp_i is the cumulative change in the frequency of the high allele at locus i (among the set of loci where the high allele is rare) and Δp_j is the cumulative change in the frequency of the high allele at locus j (among the set of loci where the low allele is rare). Equations (8) and (9) neglect terms of order $(\Delta p)^2$ and are thus only valid for short-term response to selection.

In this simple model, the ratio of $(B_t - B_0)$ to $(M_t - M_0)$ is:

$$\frac{B_t - B_0}{M_t - M_0} \approx \frac{(1-2h)(1-2\rho)}{1-(1-2h)(1-2\rho)}. \quad (10)$$

Equation (10) indicates that $(B_t - B_0)/(M_t - M_0)$ invariably equals C_{ad}/V_a. While genetic drift will cause $(B_t - B_0)$ and $(M_t - M_0)$ to differ among replicate populations, these random effects cancel out of the ratio. In fact, the evolutionary forces determining allele frequency changes (Δp_i and Δp_j in (8) and (9)) are completely arbitrary. In statistical terms, $(B_t - B_0)$ and $(M_t - M_0)$ have a positive sampling covariance because random fluctuations in allele frequency affect each quantity in the same way.

3. Stochastic simulations

The cancellation of random variables in (10) stems from the simplicity of this particular deleterious alleles model. However, we expect a high, positive sampling covariance under a range of models in which variation is due to rare, partially recessive alleles. To test this expectation, I performed stochastic simulations of truncation selection in which the frequencies, effects and dominance of alleles varied among loci.

In these simulations, the genotypic value of the trait under selection is determined by contributions from

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

Table 1. *Simulation results for the ratio test under a variety of genetic models* (*as described in the text*)

Case	Model parameters				Selection up $\Delta B/\Delta M$ (SD)	Selection down $\Delta B/\Delta M$ (SD)
	a	q	h	C_{ad}/V_a		
Model 1						
1	1	0·02	0·2	1·36	1·44 (0·01)	1·04 (0·04)
2	1	0·02	0·1	3·31	3·67 (0·04)	2·11 (0·11)
3	1	0·01	0·2	1·43	1·45 (0·01)	1·13 (0·04)
4	1	0·01	0·1	3·63	3·83 (0·03)	2·41 (0·12)
5	1/−1	0·02	0·2	1·36	1·17 (0·04)	1·17 (0·04)
Models 2 and 3						
6	Varies	0·02	Varies	1·48	1·83 (0·21)	1·18 (0·23)
7	Varies	Varies	0·2	1·40	1·47 (0·02)	1·09 (0·04)
8	Varies	Varies	0·1	3·51	3·84 (0·09)	2·24 (0·15)
Model 4						
9	1	0·01	0·1	3·63	3·83 (0·03)	2·32 (0·17)
10	1/−1	0·01	0·1	3·63	2·69 (0·19)	2·69 (0·19)
Model 5						
11	10/1	0·005/0·02	0·02/0·2	2·02	2·17 (0·13)	1·52 (0·46)
12	10/1	0·01/0·02	0·02/0·2	2·79	2·76 (0·14)	1·84 (0·47)
13	10/1	0·01/0·02	0·02/0·3	1·38	1·63 (0·16)	1·08 (0·44)

The standard deviation among replicates for $\Delta B/\Delta M$ is given in parentheses next to the mean for each direction of selection. Parameter values for a particular simulation are list prior to the results. Here, q denotes the frequency of the rare allele at each locus and h denotes its dominance coefficient. Multiple values (separated by /) are given when the value of a particular parameter varies among loci. For model 5, the first listed value for each parameter refers to loci harbouring lethals.

100 unlinked loci. Each locus has two alleles and contributes additively to the genotypic value (there is no epistasis). The three genotypes at locus k (denoted A_0A_0, A_0A_1 and A_1A_1) contribute $-a_k$, d_k and a_k, respectively, to the genotypic value of an individual (as in Falconer, 1989). The frequency of allele A_1 at locus k is p_k ($q_k = 1-p_k$). Different values for d_k, a_k and p_k across loci are considered in different 'models'.

Selection was imposed on populations of 100 individuals. For each particular genetic model, selection was performed for four generations and in both directions (for both high and low values of the trait) at one-half intensity (the top or bottom 50 individuals survive to reproduce). The genotypes of individuals in the initial population for a simulation were obtained by randomly sampling alleles given their respective frequencies in the base population. After the genotypic value of an individual was established, a normally distributed, environmental error was added to determine the phenotypic value. Individuals were then ranked by their phenotypic value and selection was based on those ranks. The 50 selected individuals were randomly paired for mating and each couple contributed four offspring to the next generation.

For each genetic model and direction of selection, evolution was simulated 1000 times. In each simulation, M_t and B_t were calculated from allele frequencies among the progeny of the fourth generation (the last set of selected adults). Subtracting base population values from these quantities, we obtain ΔM, ΔB, and the ratio of ΔB to ΔM. The results of 1000 simulations are summarized by the average $\Delta B/\Delta M$ and the standard deviation among replicates.

(i) *Genetic models*

Model 1 is based on the genetic assumptions of (5)–(10). A rare allele is segregating at 100 loci. The frequency of this rare allele (denoted q_k) is the same at each locus in the base population ($q_k = q$ for all k). The dominance coefficient, h, is also the same for all rare alleles ($d_k = a_k(2h-1)$). Finally, the magnitudes of allelic effects are the same across loci, but the direction of effect may vary ($a_k = 1$ or -1). The rare allele increases the trait value at n_1 loci and decreases the trait value at n_2 loci, where $n_1+n_2 = 100$. Simulation results with $n_2 = 100$ and different values for h and q are given in cases 1–4 of Table 1.

The average ratio of ΔB to ΔM is consistently high in these simulations and the standard deviation among replicates is very low in every case. The joint distribution of ΔB and ΔM in the 1000 replicates of downward selection is given in Fig. 2*a* (for case 3 of Table 1). As expected from (8)–(10), there is a very high correlation between ΔB and ΔM. This correlation reflects the fact that genetic drift changes ΔB and ΔM in the same way under the rare alleles model. This is responsible for the low variance in $\Delta B/\Delta M$ among replicates. In case 4, for example, 95% of the simulations of upward selection yielded ratios between 3·81 and 3·91. Ninety-five per cent of the downward

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

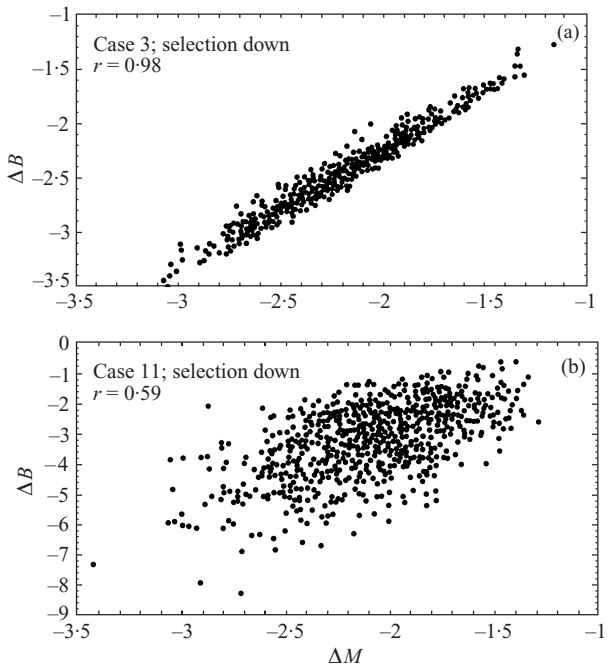

Fig. 2. The joint distribution of ΔB and ΔM in 1000 replications of selection. (*a*) Results from case 3 of Table 1 with selection for lower trait values. (*b*) Results from case 11 with selection for lower trait values. Here, r is the correlation between ΔB and ΔM.

selection simulations gave $\Delta B/\Delta M$ values between 2·17 and 2·63.

A notable feature of cases 1–4 in Table 1 is the difference in mean $\Delta B/\Delta M$ between upward and downward selection. With selection for higher trait values, the observed ratio of ΔB to ΔM is greater than the ratio of C_{ad} to V_a in the base population. With selection for lower values, $\Delta B/\Delta M$ is less than C_{ad}/V_a. There are two causes for this asymmetry. The first is that rare recessive alleles cause non-linearity in parent–offspring regressions (Robertson, 1977; Bulmer, 1985, p. 137). This naturally leads to an asymmetric response to selection. Second, the genetic variance components evolve with selection when the number of loci is finite. The infinitesimal model breaks down very quickly when quantitative trait variation is due entirely to rare alleles. With upward selection in model 1, allele frequencies move towards more intermediate values. This increases V_a and decreases C_{ad}. As a consequence, ΔM is substantially greater (and ΔB is substantially less) in the third or fourth generations of selection than in the first. Thus, the overall ratio of ΔB to ΔM is less than what would be expected given C_{ad}/V_a of the base population. Genetic variance components evolve in the opposite direction with selection for lower trait values (when $n_2 = 100$ in model 1). The ratio of C_{ad} to V_a increases with selection and the final ratio of ΔB to ΔM is greater than expected.

I have also simulated model 1 for a variety of cases in which the rare allele increases trait values at some loci and decreases trait values at others ($n_1, n_2 > 0$). Case 5 in Table 1 describes results from one set of simulations with $n_1 = n_2 = 50$. In this case and others, the average $\Delta B/\Delta M$ invariably exceeds 1 and the standard deviation is small.

Models 2 and 3 allow the frequency and dominance coefficient of the rare allele to vary among loci. In model 3, the frequency of the rare allele is fixed ($q_k = q$ for all k), but a_k varies uniformly between 0·02 and 2 ($a_1 = 0{\cdot}02, a_2 = 0{\cdot}04, \ldots, a_{99} = 1{\cdot}98, a_{100} = 2{\cdot}00$). The dominance coefficient varies among loci such that alleles with larger effects tend to be more recessive: $h_k = 0{\cdot}5 - 0{\cdot}25a_k$. A negative relationship between effect and h is supported by both theoretical arguments and experimental studies (Simmons & Crow, 1977; Caballero & Keightley, 1994). A typical set of results for model 2 are given as case 6 in Table 1. As in the simpler model 1, the average ratio of ΔB to ΔM is consistently greater than 1. The standard deviation among replicates is slightly higher in these simulations, but it remains small relative to the mean.

Model 3 assumes the same distribution for a_k, but allows the frequency of the rare allele to vary among loci instead of the dominance coefficient. I assume that initial allele frequencies are uniformly distributed (on a log scale) between 0·005 and 0·05: $q_k = 0{\cdot}05 - \log_{10}(4{\cdot}5\, a_k + 1)$. The negative relationship between q_k and a_k reflects the biological intuition that deleterious mutations with larger effects will be rarer. The results for model 3 (cases 7 and 8) are entirely consistent with previous cases. Standard deviations are comparable to model 1.

In models 1–3, genetic variation affects only the phenotype. There is no direct effect of deleterious mutations on 'fitness', which is determined entirely by phenotypic value in the selection experiment. This kind of model applies most directly to deleterious mutations of 'small effect'. However, lethal and sterile mutations make a substantial contribution to inbreeding depression in some traits (Crow & Simmons, 1977). Such 'large effect' mutations will affect fitness even in a selection experiment. Model 4 considers lethal alleles. If an individual is homozygous for a lethal at any locus, it is eliminated and replaced by a second individual from the same family. Two examples of model 4 are given as cases 9 and 10 in Table 1. Rare alleles invariably decrease the trait in case 9, while half increase and half decrease in case 10. The average ratio of ΔB to ΔM is very high under both these models and the standard deviations among replicates are small relative to the average ratios. The results are strikingly similar to the non-lethal case with similar phenotypic effects (case 4).

The final pure 'rare allele' model considers a mixture of loci with large and small effects. In model

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

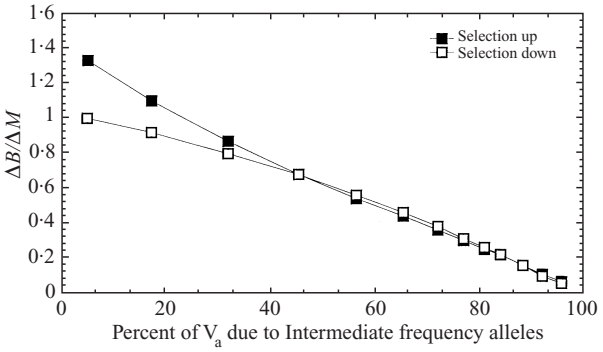

Fig. 3. The average ratio of ΔB to ΔM as a function of the percentage of additive variance contributed by loci with intermediate-frequency alleles. Results were obtained from simulations of model 6 by increasing a_1 from 0·1 (where loci 1–9 contribute 4·9% of V_a) to 2·0 (where loci 1–9 contribute 95·6% of V_a). Open symbols denote ratios obtained with selection for lower trait values; filled symbols denote ratios obtained with selection for higher trait values. In this example, $h_1 = 0{\cdot}5$, $q_{10} = 0{\cdot}02$, $a_{10} = 1$, $h_{10} = 0{\cdot}2$.

5, lethal alleles with large phenotypic effects segregate at loci 1–10. Non-lethal alleles with minor phenotypic effects segregate at loci 11–100. I assume that all loci within a 'category' are equivalent ($a_k = 10$, $q_k = q_1$, and $h_k = h_1$ for $k \leqslant 10$; and $a_k = 1$, $q_k = q_{11}$, and $h_k = h_{11}$ for $k > 10$). Results for different parameter values are given as cases 11–13 in Table 1. The relative contribution of lethal alleles to phenotypic variation differs among these cases. Lethals contribute 33% of the initial directional dominance in case 11, 47% in case 12 and 58% in case 13. As with previous models, the average ratio of ΔB to ΔM is near to or greater than 1. However, the standard deviation among replicates is substantially greater. This is due primarily to a lower sampling covariance of ΔB and ΔM. The bivariate distribution is given for downward selection of case 11 in Fig. 2*b*.

For comparison, I also simulated models in which at least some portion of quantitative trait variation is caused by alleles at intermediate frequencies (between 0·1 and 0·9). In model 6, allele frequencies are intermediate (in the base population) at loci 1–9 ($q_1 = 0{\cdot}1$, $q_2 = 0{\cdot}2$, $q_3 = 0{\cdot}3, \ldots, q_8 = 0{\cdot}8$, $q_9 = 0{\cdot}9$). I assume that effects and dominance coefficients are the same across these loci ($a_k = a_1$ and $h_k = h_1$ for $k = 1$–9). Loci 10–100 harbour rare alleles with equivalent parameters ($q_k = q_{10}$, $a_k = a_{10}$ and $h_k = h_{10}$ for $k =$ 10–100). Simulations were conducted by fixing all other parameters and allowing a_1 to vary from 0·1 to 2·0. With $a_{10} = 1$, the contribution of loci 1–9 to V_a increases from about 5% of the total (when $a_1 = 0{\cdot}1$) to about 95% of the total (when $a_1 = 2{\cdot}0$). The average ratio of ΔB to ΔM is given as a function of the relative contribution of loci 1–9 (to V_a) in Fig. 3. As expected, the average $\Delta B/\Delta M$ is high when V_a is caused primarily by rare recessive alleles and declines towards zero as intermediate-frequency alleles make a larger contribution.

In all the preceding simulations the environmental variance was adjusted so that the trait heritability (V_a/V_p) was 0·5 in the base population. I have also simulated all these cases assuming that the heritability was 0·2. The absolute magnitudes of ΔB and ΔM were smaller in these simulations, but ratios of ΔB to ΔM were generally similar to the results in Table 1. In fact, $\Delta B/\Delta M$ was generally closer to C_{ad}/V_a in cases with lower heritability.

4. Discussion

(i) *The nature of genetic variation*

The ratio test is a method for assessing the contribution of rare, partially recessive alleles to quantitative trait variation. Rare recessives should be the principal cause of variation under the deleterious mutation model. In contrast, a large contribution by alleles at intermediate frequencies suggests that quantitative genetic variation is either actively maintained by selection or 'quasi-neutral'. The difference between these two scenarios has important implications for both quantitative trait evolution and the methods we use to investigate it.

Consider a plant population that has recently experienced a change in selection regime such that smaller flowers are favoured. If genetic variation in flower size is not caused by unconditionally deleterious mutations, there should be an immediate response to selection. Alleles that reduce flower size should increase in frequency and the rate of phenotypic evolution can be predicted from quantitative genetic parameters (the selection differential and trait heritability; Falconer, 1989). In contrast, we expect very limited phenotypic change (in the short term) if standing variation is caused by deleterious mutations. Small flower alleles will increase in frequency only if positive selection on their phenotypic effects is strong enough to overwhelm their direct deleterious effect on fitness. As a consequence, the rate of phenotypic evolution will deviate from the quantitative genetic prediction.

Genetic variances and covariances are frequently used to assess the evolutionary potential of populations (Arnold, 1992; Houle, 1992). Such studies implicitly assume that observed genetic variation is not inherently deleterious, because deleterious mutations probably will not contribute to adaptive evolution (Keightley & Hill, 1990). In contrast, investigations of life history traits (survival, fecundity, etc.) frequently assume that genetic variation is due *entirely* to deleterious mutations (Deng, 1998). This assumption allows mutational parameters to be estimated from phenotypic data (Morton *et al.*, 1956; Charlesworth *et al.*, 1990; Deng & Lynch, 1996;

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

Willis, 1999). This striking difference in empirical presumptions indicates that experimental studies are necessary to assess the contribution of deleterious mutations to standing genetic variation.

(ii) *The ratio test*

The ratio test is based on the relative magnitudes of two genetic variance components: the covariance of additive and homozygous dominance effects (C_{ad}) and the more familiar additive genetic variance (V_a). It is thus an indirect assay of allele frequencies. Such indirect methods have proved useful in the past, however. An example is the classic genetic studies of heterosis in corn (Reviewed by Moll *et al.*, 1964). These experiments used changes in the ratio of the dominance variance to additive variance to reject single locus overdominance as the primary cause of heterosis.

Rare, partially recessive alleles increase C_{ad} relative to V_a (Fig. 1). Large values of C_{ad}/V_a (equal to or greater than 1) are only likely if the additive variation in a trait is due primarily to rare, recessive alleles. Small or negative values of C_{ad}/V_a will obtain if quantitative trait variation is caused by alleles at intermediate frequency. It is possible to estimate both C_{ad} and V_a from comparisons among relatives (Cockerham & Weir, 1984; Shaw *et al.*, 1998). Unfortunately, such estimates typically have large sampling errors. This makes estimating the ratio of C_{ad} to V_a very difficult.

Selection experiments may provide a statistically effective means to estimate C_{ad}/V_a. The change in the directional dominance of a randomly mating population under selection, ΔB, is approximately proportional to C_{ad} (Kelly, 1999*a*, *b*; Appendix). Analyses presented here indicate that $\Delta B/\Delta M$ can be used as an estimator of C_{ad}/V_a and that this estimator has favourable statistical properties (ΔM is the change in the population mean). In the highly simplified version of the deleterious mutation model considered in (8)–(10), random factors that generate variance in ΔB and ΔM among replicate selection populations cancel out in the ratio (to a first approximation). As a consequence, $\Delta B/\Delta M$ invariably equals C_{ad}/V_a (the sampling variance is zero). This result is surprising in that quantitative genetic estimators typically have high sampling variances.

Stochastic simulations of truncation selection were used to investigate the generality of this result (Figs 2–3; Table 1). These simulations confirm that the sampling variance of $\Delta B/\Delta M$ is relatively low under a broad range of models in which genetic variation is caused by rare, partially recessive alleles. However, they also indicate that $\Delta B/\Delta M$ can be biased. In simulations where rare alleles reduced the trait value, the average $\Delta B/\Delta M$ was typically greater than C_{ad}/V_a with upward selection but less than C_{ad}/V_a with downward selection (Table 1). This is a notable concern for quantitative estimation of C_{ad}/V_a, but it does not hinder use of $\Delta B/\Delta M$ as a test of the deleterious mutation model. Despite bias, $\Delta B/\Delta M$ was uniformly high across replicate populations in simulations with variation caused by entirely by rare alleles. In contrast, $\Delta B/\Delta M$ was generally close to zero or even negative in simulations where variation was caused primarily by intermediate-frequency alleles (see Fig. 3).

(iii) *Inbreeding depression versus genetic variation*

It is important to distinguish the contribution of rare recessives to 'inbreeding depression' from their contribution to the genetic variance in a trait. As emphasized by Charlesworth (1998), rare recessives may be the sole determinant of inbreeding depression (directional dominance) but generate only part of the genetic variance. This will occur if two qualitatively different types of loci contribute to variation in the trait. At the first set of loci, non-deleterious alleles are segregating at intermediate frequencies. The second set of loci harbour rare, partially recessive alleles. Under these circumstances, C_{ad} will be positive but substantially smaller than V_a (because the intermediate-frequency alleles will make large contributions to V_a but not to C_{ad}). Simulation results for this kind of model are given in Fig. 3.

Under very different assumptions, deleterious mutations could be responsible for genetic variation in traits that exhibit no directional dominance. Here, we are primarily concerned with the pleiotropic effects of deleterious mutations on morphological characters because fitness components generally show inbreeding depression (Wright, 1977; Charlesworth & Charlesworth, 1987). Lack of directional dominance may result for at least two different reasons. The first is that the population contains rare recessives that increase the trait value at some loci ($2pqd < 0$) and decrease the trait value at other loci ($2pqd > 0$) so that these two sets of loci cancel in their net effect on B. In fact, theoretical models typically assume that deleterious mutations are equally likely to increase or decrease the value of a morphological character (Keightley & Hill, 1990; Barton, 1990; Kondrashov & Turelli, 1992; Caballero & Keightley, 1994). These conditions will yield high values for C_{ad} and selection will subsequently generate directional dominance in the trait.

There will also be no inbreeding depression if the pleiotropic effects of deleterious mutations are additive. For reasons described previously, deleterious mutations that persist in a population are likely to be at least partially recessive in their effects on fitness. If alleles have consistent dominance relations across all

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

traits they affect, as suggested by some studies (Keightley & Kacser, 1987), then deleterious alleles will also tend to be recessive in their morphological effects. It is possible, however, that deleterious mutations with recessive affects on fitness could have additive pleiotropic effects (Caballero & Keightley, 1994). In this case, we expect small values for C_{ad} even when quantitative variation is caused entirely by deleterious mutations.

Finally, it is necessary to consider the case in which both the inbreeding depression and the genetic variance in a trait are due to intermediate-frequency alleles. Under these circumstances, C_{ad} may actually be significantly negative (although smaller in magnitude than V_a). The contribution of a particular quantitative trait locus to C_{ad} will be negative if the recessive allele at that locus has a frequency greater than 0·5 (Fig. 1; Cockerham & Weir, 1984).

(iv) Experimental design

Consider an experiment in which selection is performed on a self-compatible plant. An initial concern is to choose a scale of measurement in which trait variation conforms approximately to the relevant quantitative genetic model (e.g. Falconer, 1989; Appendix). Most models assume that the effects of deleterious mutations combine multiplicatively across loci (Crow & Kimura, 1970, ch. 6). In this case, a simple logarithmic transformation of the trait will ensure that each locus contributes additively to variation. If the interactions among loci are neither additive nor multiplicative (e.g. Sved & Wilton, 1989; Willis, 1993; Charlesworth, 1998), then a more complicated transformation may be required.

The duration of the selection experiment is a second important issue. The number of generations should be sufficient to generate a significant change in the population mean (say 1–3 phenotypic standard deviations), but not so many that new mutations can contribute to the selection response (e.g. Frankham *et al*., 1968). The purpose of the ratio test is to assess the contribution of rare alleles *in the base population*. Only the immediate response to selection is informative about the genetic composition of the base population. The choice of four generations of selection in the simulation study was arbitrary and longer durations (or more intense selection) may be preferable with low heritabilities.

After selection is completed, outbred seeds from the selected populations are germinated simultaneously with outbred seeds preserved from the base population (or from an unselected control population). Adults from each population then produce two types of seed: self-fertilized and outbred (each individual is randomly mated to another member of the same population to generate outbred seed). The resulting seeds fall into four categories: base-outcrossed (bo), base-inbred (bi), selected-outcrossed (so) and selected-inbred (si). The seeds are subsequently germinated and measured for the trait. The inbreeding coefficient of selfed progeny is 0·5. Hence, the directional dominance of the base population may be estimated as $2(M_{bi}-M_{bo})$ and the directional dominance of the selected population as $2(M_{si}-M_{so})$. Here the M denotes the mean phenotype of individuals in the category given by the subscript. (If inbred progeny were generated by full-sib mating, then each difference would be multiplied by 4 to estimate B).

The ratio test can be applied by using $M_{so}-M_{bo}$ as an estimator of ΔM and $2(M_{si}-M_{so})-2(M_{bi}-M_{bo})$ as an estimator of ΔB. If ΔM is substantially greater in magnitude than ΔB (across replicates), we can reject the hypothesis that variation is caused primarily by rare, partially recessive alleles. We cannot reject the hypothesis that variation is caused by rare, additive alleles. Fortunately, alternative analyses of data from the same selection experiment can also be used to test this hypothesis (e.g. Curtsinger & Ming, 1997).

Appendix

We consider a slightly more general model than that described in the text. Each quantitative trait locus can have an arbitrary number of alleles. Let p^{jl} denote the frequency of the jth allele (A_j) at locus l. Let α^{jl} denote the additive effect of allele j at locus l and δ^{jkl} denote the dominance deviation associated with genotype A_jA_k at locus l. Additive and dominance effects are defined in the standard way (Cockerham & Weir, 1984; Falconer, 1989). With these definitions,

$$B = \sum_l \sum_j p^{jl}\,\delta^{jjl} \tag{A 1}$$

and

$$\Delta B = \sum_l \sum_j \Delta p^{jl}\,\delta^{jjl} \tag{A 2}$$

where Δp^{jl} is the change in the frequency of A_j at locus l. Assuming that quantitative trait variation is caused by many loci of small effect and that these loci are in linkage equilibrium, allele frequency changes are approximately a linear function of S, the selection differential (Kimura, 1958; Griffing, 1960). In this model,

$$\Delta p^{jl} \approx \left(\frac{S}{V_p}\right) p_{jl}\,\alpha^{jl}, \tag{A 3}$$

where V_p is the phenotypic variance. Substituting (A 3) into (A 2),

$$\Delta B = \left(\frac{S}{V_p}\right) \sum_l \sum_j p^{jl}\delta^{jjl}\alpha^{jl}. \tag{A 4}$$

https://doi.org/10.1017/S0016672399003766 Published online by Cambridge University Press

Noting that the latter sum equals the multi-allele definition for C_{ad} (Cockerham & Weir, 1984), we obtain

$$\Delta B = \left(\frac{S}{V_p}\right) C_{ad}. \quad \text{(A 5)}$$

Linkage disequilibrium will develop among quantitative trait loci if selection is sustained (Bulmer, 1985). This violates the assumptions of the preceding analysis. However, because linkage disequilibrium tends to change V_a and C_{ad} in the same direction, it will have less effect on $\Delta B/\Delta M$ than on either ΔB or ΔM alone. Dynamical recursions for ΔB and ΔM that allow linkage disequilibrium are given in Kelly (1999*a*, *b*).

Another notable assumption of the preceding derivation is that the contribution of each locus is small relative to the phenotypic variation. This assumption is violated by 'large effect' mutations such as lethals. Such mutations can make an important contribution to inbreeding depression (Crow & Simmons, 1977) and their dynamics may not be accurately described by (A 3). However, the derivation of (8) and (9) does not depend on the assumption of small effects. Moreover, the simulation studies of models 5–7 indicate that the ratio test is not invalidated by lethals.

I would like to thank P. McElhany, J. Willis, W. G. Hill and two anonymous reviewers for comments on the manuscript. I am also grateful to Kim Hughes for providing a preprint and discussing her experimental studies. Finally, I would like to acknowledge the support of a NIH postdoctoral fellowship.

References

Arnold, S. (1992). Constraints on phenotypic evolution. *American Naturalist* **140**, S85–S107.

Barton, N. H. (1990). Pleiotropic models of quantitative trait variation. *Genetics* **124**, 773–782.

Beniwal, B. K., Hastings, I. M., Thompson, R. & Hill, W. G. (1992). Estimation of changes in selected lines of mice using REML with an animal model. I. Lean mass. *Heredity* **69**, 352–360.

Bulmer, M. G. (1985). *The Mathematical Theory of Quantitative Genetics*. Oxford: Clarendon Press.

Caballero, A. & Keightley, P. D. (1994). A pleiotropic nonadditive model of variation in quantitative traits. *Genetics* **138**, 885–900.

Charlesworth, B. (1998). The effect of synergistic epistasis on the inbreeding load. *Genetical Research* **71**, 85–89.

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.

Charlesworth, B. & Hughes, K. A. (1998). The maintenance of genetic variation in life history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. B. Krimbas). Cambridge: Cambridge University Press.

Charlesworth, B., Charlesworth, D. & Morgan, M. T. (1990). Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* **347**, 380–382.

Cockerham, C. C. & Weir, B. S. (1984). Covariance of relatives stemming from a population undergoing mixed self and random mating. *Biometrics* **40**, 157–164.

Cornelius, P. L. (1988). Properties of components of covariance of inbred relatives and their estimates in a maize population. *Theoretical and Applied Genetics* **75**, 701–711.

Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. Minneapolis, Minnesota: Burgess Publishing.

Curtsinger, J. W. & Ming, R. (1997). Non-linear selection response in *Drosophila*: a strategy for testing the rare-alleles model of quantitative genetic variability. *Genetica* **99**, 59–66.

de Boer, I. J. M. & Hoeschele, I. (1993). Genetic evaluation methods for populations with dominance and inbreeding. *Theoretical Applied Genetics* **86**, 245–258.

Deng, H.-W. (1998). Characterization of deleterious mutations in outcrossing populations. *Genetics* **150**, 945–956.

Deng, H.-W. & Lynch, M. (1996). Estimation of the genomic mutation parameters in natural populations. *Genetics* **144**, 349–360.

Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. (1998). Rates of spontaneous mutation. *Genetics* **148**, 1667–1686.

Dudash, M. R. & Carr, D. E. (1998). Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* **393**, 682–684.

Falconer, D. S. (1989). *An Introduction to Quantitative Genetics*. New York: Wiley.

Frankham, R., Jones, L. P. & Barker, J. S. F. (1968). The effect of population size and selection intensity in selection for a quantitative character in *Drosophila*. III. Analysis of lines. *Genetical Research* **12**, 267–283.

Goldstein, D. B. & Holsinger, K. E. (1992). Maintenance of polygenic variation in spatially structured populations: roles for local mating and genetic redundancy. *Evolution* **46**, 412–429.

Harris, D. L. (1964). Genotypic covariance between inbred relatives. *Genetics* **50**, 1319–1348.

Heath, S. C., Bulfield, G., Thompson, R. & Keightley, P. D. (1995). Rates of change in genetic parameters of body weight in selected mouse lines. *Genetical Research* **66**, 19–25.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Houle, D., Hoffmaster, D. K., Assimacopoulous, S. & Charlesworth, B. (1992). The genomic mutation rate for fitness in *Drosophila*. *Nature* **359**, 58–60.

Houle, D., Hughes, K. A., Hoffmaster, D. K., Ihara, J. T., Assimacopoulous, S. & Charlesworth, B. (1994). The effects of spontaneous mutations on quantitative traits. I. Variances and covariances of life history traits. *Genetics* **138**, 773–785.

Houle, D., Morikawa, B. & Lynch, M. (1996). Comparing mutational variabilities. *Genetics* **143**, 1467–1483.

Hughes, K. A. (1995). The inbreeding decline and average dominance of genes affecting male life-history characters in *Drosophila melanogaster*. *Genetical Research* **65**, 41–52.

Hughes, K. A. (1997). Quantitative genetics of sperm precedence in *Drosophila melanogaster*. *Genetics* **145**, 139–151.

Jacquard, A. (1974). *The Genetic Structure of Populations*. New York: Springer-Verlag.

Keightley, P. D. (1996). The nature of deleterious mutation load in *Drosophila*. *Genetics* **144**, 1993–1999.

Keightley, P. D. (1998). Inference of genome-wide mutation

rates and distribution of mutation effects for fitness traits: a simulation study. *Genetics* **150**, 1283–1293.
Keightley, P. D. & Caballero, A. (1997). Genomic mutation rate for fitness in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **94**, 3823–3827.
Keightley, P. D. & Hill, W. G. (1988). Quantitative genetic variability maintained by mutation–selection balance in finite populations. *Genetical Research* **52**, 33–43.
Keightley, P. D. & Hill, W. G. (1990). Variation maintained in quantitative traits with mutation selection balance: pleiotropic side effects on fitness traits. *Proceedings of the Royal Society of London, Series B* **242**, 95–100.
Keightley, P. D. & Kacser, H. (1987). Dominance, pleiotropy, and metabolic structure. *Genetics* **117**, 319–329.
Kelly, J. K. (1999*a*). Response to selection in partially self fertilizing populations. I. Selection on a single trait. *Evolution*, in press.
Kelly, J. K. (1999*b*). Response to selection in partially self fertilizing populations. II. Selection on multiple traits. *Evolution*, in press.
Kingman, J. F. C. (1978). a simple model for the balance between selection and mutation. *Journal of Applied Probability* **15**, 1–12.
Kondrashov, A. S. & Turelli, M. (1992). Deleterious mutations, apparent stabilizing selection and the maintenance of quantitative variation. *Genetics* **132**, 603–618.
Latter, B. D. H. (1965). The response to artificial selection due to autosomal genes of large effect. I. Changes in gene frequency at an additive locus. *Australian Journal of Biological Sciences* **18**, 585–598.
Lewontin, R. C. (1974). *The Genetic Basis of Evolutionary Change*. New York: Columbia University Press.
Lindsley, D. L. & Grell, E. H. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Institute of Washington Publication **627**.
Lynch, M. (1988). The rate of polygenic mutation. *Genetical Research* **51**, 137–148.
Lynch, M. & Walsh, B. (1998). *Genetics and Analysis of Quantitative Characters*. Sunderland, Mass.: Sinauer Associates.
Mackay, T. F. C., Lyman, R. & Jackson, M. S. (1992). Effects of P elements on quantitative traits in *Drosophila melanogaster*. *Genetics* **130**, 315–332.
Meyer, K. & Hill, W. G. (1991). Mixed model analysis of a selection experiment for food intake in mice. *Genetical Research* **57**, 71–81.
Moll, R. H., Lindsey, M. F. & Robinson, H. F. (1964). Estimates of genetic variances and level of dominance in maize. *Genetics* **49**, 411–423.
Morton, N. E., Crow, J. F. & Muller, H. J. (1956). An estimate of the mutational damage in man from data on consanguineous marriages. *Proceedings of the National Academy of Sciences of the USA* **42**, 855–863.
Mousseau, T. A. & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity* **59**, 181–197.
Mukai, T., Chigusa, S., Mettler, L. E. & Crow, J. F. (1972). Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 335–355.
Ohta, T. (1996). The current significance and standing of neutral and nearly neutral theories. *BioEssays* **18**, 673–677.
Rice, J. A. (1989). *Mathematical Statistics and Data Analysis*. Pacific Grove, Calif.: Wadsworth and Brooks.
Robertson, A. (1952). The effect of inbreeding on the variation due to recessive genes. *Genetics* **37**, 189–207.
Robertson, A. (1967). The nature of quantitative genetic variation. In *Heritage from Mendel* (ed. R. A. Brink), pp. 265–280. Madison: University of Wisconsin Press.
Robertson, A. (1977). The non-linearity of offspring–parent regression. In *Proceedings of the 2nd International Conference on Quantitative Genetics* (ed. B. Weir), pp. 297–304.
Roff, D. A. & Mousseau, T. A. (1987). Quantitative genetics and fitness: lessons from *Drosophila*. *Heredity* **58**, 103–118.
Santiago, E., Albornoz, J., Dominguez, A., Toro, M. A. & Lopez-fajul, C. (1992). The distribution of effects of spontaneous mutations on quantitative traits and fitness in *Drosophila melanogaster*. *Genetics* **132**, 771–781.
Shaw, R. G. (1987). Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution* **41**, 812–826.
Shaw, F. H. & Woolliams, J. (1998). Variance component analysis of skin and weight data for sheep subjected to rapid inbreeding. GSE, in review.
Shaw, R. G., Byers, D. L. & Shaw, F. H. (1998). Genetic components of variation in *Nemophila menziesii* undergoing inbreeding: morphology and flowering time. *Genetics*, in review.
Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.
Sved, J. A. & Wilton, A. N. (1989). Inbreeding depression and the maintenance of deleterious genes by mutation: model of a *Drosophila* chromosome. *Genetical Research* **53**, 119–128.
Takano, T., Kusakabe, S. & Mukai, T. (1987). The genetic structure of natural populations of *Drosophila melanogaster*. XX. Comparisons of genotype–environment interaction in viability between a northern and a southern population. *Genetics* **117**, 245–254.
Turelli, M. (1984). Heritable genetic variation via mutation–selection balance: Lerch's zeta meets the abdominal bristle. *Theoretical Population Biology* **25**, 138–193.
Turelli, M. & Barton, N. H. (1994). Genetic and statistical analyses of strong selection on polygenic traits: what, me normal? *Genetics* **138**, 913–941.
Watanabe, T. K. & Ohnishi, S. (1975). Genes affecting productivity in natural populations of *Drosophila melanogaster*. *Genetics* **80**, 807–819.
Willis, J. H. (1993). Effects of different levels of inbreeding depression in two populations of *Mimulus guttatus*. *Evolution* **47**, 864–876.
Willis, J. H. (1999). Genetics of mildly deleterious mutations in *Mimulus guttatus*. *Genetics*, in press.
Wright, S. (1977). *Evolution and the Genetics of Populations*, vol. 3, *Experimental Results and Evolutionary Deductions*. Chicago: University of Chicago Press.