Genet. Res., Camb. (1993), **61**, *pp.* 107–116 *With 6 text-figures*

Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. I. Response to artificial selection

MARÍA A. LÓPEZ AND CARLOS LÓPEZ-FANJUL*
Departamento de Genética, Facultad de Ciencias Biológicas, Universidad Complutense, 28040 Madrid, Spain

(*Received 10 July 1992 and in revised form 28 October 1992*)

Summary

Divergent selection for abdominal bristle number was carried out for 47 generations, starting from a completely homozygous population of *Drosophila melanogaster*. All lines were selected with the same proportion (20%) but at two different numbers of selected parents of each sex (5 or 25). A significant response to selection was obtained in 25 lines (out of 40). In most cases, it could be wholly attributed to a single mutation of relatively large effect ($> 0{\cdot}3$ phenotypic standard deviations). A total number of 30 mutations were detected. In agreement with theory, larger responses in each direction were achieved by those lines selected at greater effective population sizes. A large fraction of mutations were lethals (10/30). Thus, the observed divergence between lines of the same effective size selected in opposite directions was smaller than expected under neutrality. The ratio of new mutational variance to environmental variance was estimated to be $(0{\cdot}52 \pm 0{\cdot}09) \times 10^{-3}$.

1. Introduction

Response to artificial selection has usually been considered the result of induced directional changes in the frequency of the alleles initially present in the population, mutations occurring in the course of the process usually being ignored. In the last decade, however, theory has been expanded to include the contribution of new variation to the response. The simplest situation envisages a completely homozygous base population and a constant input of new genetic variance per generation (σ_m^2) due to neutral independent mutations, with small effects on the selected trait symmetrically distributed about zero. In this case, the expected cumulative response to selection is an increasing function of the effective size of the selected line (N) and the between-line variance is proportional to the response (Hill, 1982*b*; Hill & Rasbash, 1986).

The shape of the distribution of mutant effects can substantially modify these predictions. With an asymmetrical distribution, the response in each direction becomes dependent on the mean square of the effects of mutations with positive and negative effects, respectively. When all mutations have large effects such that they are fixed essentially instantaneously, both the expected cumulative response and its variance will be larger than predicted under the infinitesimal model. The first becomes proportional to N and the second to the kurtosis of the distribution (Hill, 1982*b*).

Antagonism between natural and artificial selection forces can be taken into account by considering the bivariate distribution of mutant effects on the metric trait and on fitness. Under the infinitesimal model, when the effects of mutations are additive and symmetrical for the trait and unconditionally deleterious for fitness, the expected rate of response will be lower than predicted in the neutral case, as fixation probabilities are smaller and fixation times larger (Hill & Keightley, 1988).

Direct evidence on spontaneous mutations affecting the response to selection comes from: (i) identification of genes responsible which were not present in the segregating base population (see Mackay, 1990, for a review); (ii) estimation of mutational heritabilities of metric traits in lines derived from a genetically homogeneous base population (Enfield & Braskerud, 1989; Keightley & Hill, 1992; Mackay *et al.* 1992; earlier work reviewed by Lynch, 1988).

More detailed information has been reported by Caballero, Toro & López-Fanjul (1991). Starting from a homozygous population of *Drosophila melanogaster*, a number of lines were subjected to 20 generations of divergent selection for abdominal

* Corresponding author.

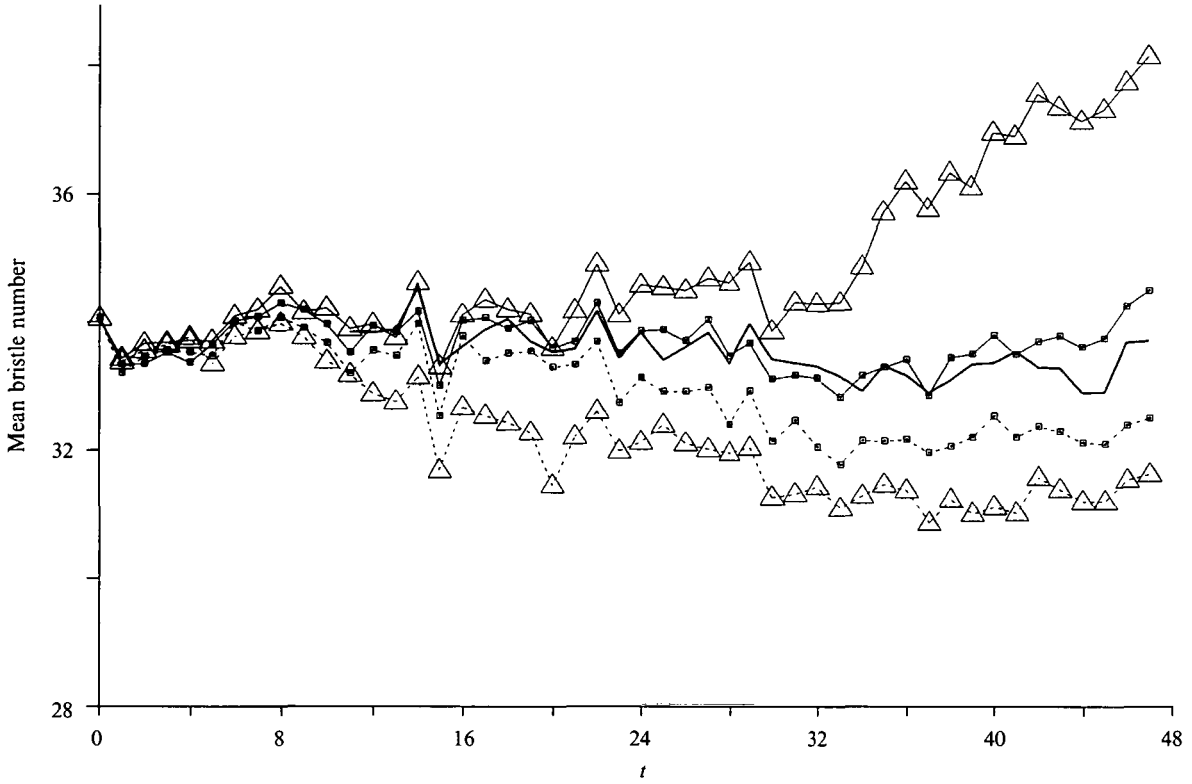

Fig. 1. Average bristle score plotted against generation number for each type of line and the control line. ——, Upward selected lines; – – –, downward selected lines; ——, control line; △, L lines; ⊡, S lines.

bristle number with the same proportion selected but at two different effective sizes. In agreement with theory, larger responses in each direction were achieved by those lines selected at greater effective sizes. For the most part, the new variance on which selection acted could be attributed to a few mutations of large effect (0·5–2 phenotypic standard deviations of the trait, σ), asymmetrically (negative) distributed and generally deleterious (lethal). In this paper, we present the selection results from generation 20 to 47. Data referring to the distribution of mutant effects on the trait and fitness will be given in a companion paper (López & López-Fanjul, 1993).

2. Materials and methods

(i) *Base population*

The *D. melanogaster* isogenic line for all chromosomes obtained by Caballero *et al.* (1991) was used as the base population. This line carried the recessive eye-colour marker sepia (*se*) on chromosome III, as an indicator of possible contamination with exogenous flies.

(ii) *Selected lines*

Starting from the isogenic line, 47 generations of divergent mass selection were carried out on the sum of the bristle numbers on the 4th and 5th sternites of males and the 5th and 6th sternites of females. There were two groups of lines: small (16 replicates selected in each direction with proportion 5/25 of each sex per generation; lines S^+, denoted 1^+ to 16^+; lines S^-, denoted 1^- to 16^-) and large (four replicates selected in each direction with proportion 25/125 of each sex per generation; lines L^+, denoted A^+ to D^+; lines L^-, denoted A^- to D^-). In the S lines, the five pairs selected were allowed to mate and lay eggs in a vial for 4 days. The 25 pairs selected in the L lines were randomly divided into five groups of equal size, each group placed in a different vial and treated as above. Each of these vials contributed 25 offspring of each sex to the scored population. Therefore, selection was carried out with the same proportion in all lines (20%), but the effective size of the L lines was five times greater than that of the S lines. Data pertaining to the first 20 generations of selection have already been reported by Caballero *et al.* (1991), where more detailed information can be found. Due to a break-down in the cooling system, all lines were lost at generation 48.

(iii) *Control line*

The isogenic base population was maintained as a control line in eight bottles. A circular mating scheme was used to ensure a sufficiently large population

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

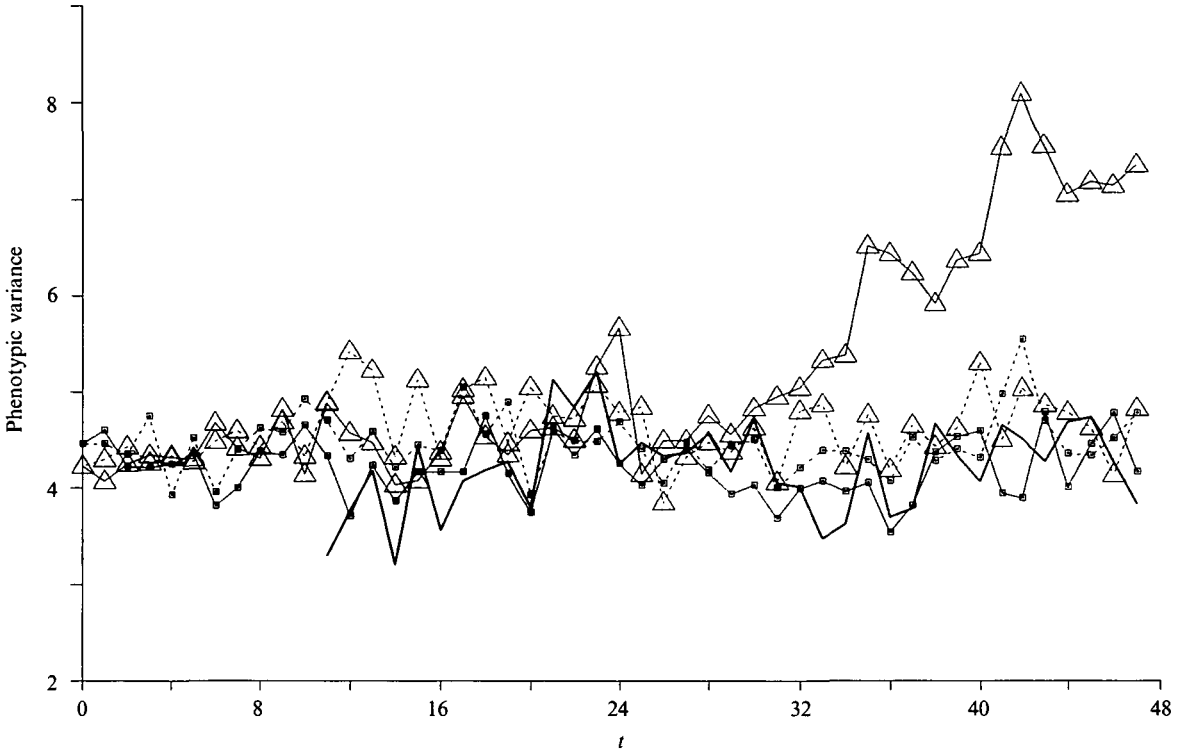

Fig. 2. Phenotypic variance of bristle score plotted against generation number for each type of line and the control line. ——, Upward selected lines; – – –, downward selected lines; ——, control line; △, L lines; ⊡, S lines.

(about 800 parents per generation). The performance of the control line was evaluated on 100 individuals scored per sex and generation, starting with generation 11. Care was taken to ensure a similar culture density in the control and the selected lines. Thus, the control line was kept in bottles but vials were used for evaluation.

(iv) *Culture conditions*

Flies were reared on the standard medium formula of this laboratory (brewer's yeast–agar–sucrose). All cultures were incubated at 25 ± 1 °C under continuous lighting. All selected lines and the control were kept strictly contemporary and under the same environmental conditions.

3. Results

(i) *Overall behaviour of selected lines*

The evolution of the mean and the phenotypic variance of each group of selected replicates and the control line is shown in Figs 1 and 2. Pertinent statistics are presented in Table 1. On the whole (generations 11–47), the control mean decreased significantly (regression coefficient on generation number $-0{\cdot}022 \pm 0{\cdot}005$). However, upward and downward trends were apparent and should be attributed to environmental fluctuations, as they were paralleled by similar changes in the selected lines irrespective of the direction of selection. The phenotypic variance of the control increased with time but not significantly (regression coefficient on generation number $0{\cdot}009 \pm 0{\cdot}007$). Its average value over generations was $4{\cdot}19 \pm 0{\cdot}47$ ($\sigma = 2{\cdot}05$ bristles).

In each group of lines, selection intensities were estimated as the applied selection differentials averaged over lines and generations, in phenotypic standard deviation units. These values agreed well with their expectations (Becker, 1967). From generation 10 onwards, the means of the four groups of lines ranked as expected ($L^- < S^- < S^+ < L^+$). The final response of a group of replicates, calculated as deviation from the control averaged over the last three generations of selection, was significant in all cases. However, the rates of response were not constant. Thus, most of the final response was attained during the first (L^- lines), second (S^- lines) or last third of the experiment (L^+ and S^+ lines). These intermittent patterns resulted in asymmetries of response of varying sign. Initially, the asymmetry was negative in both groups of lines. It remained so in the S lines during the whole experiment but, from generation 32, the sign of the asymmetry changed to positive in the L lines.

The average phenotypic variance of a group of replicates departed from the control value only in the case of the L lines and this was solely observed during those periods in which most of the final response was achieved (first and last thirds of the experiment for the L^- and L^+ sets, respectively). The final phenotypic

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

Table 1. *Response to selection and associated parameters for each type of line*

	Type of line			
	S^+	S^-	L^+	L^-
Intensity of selection				
Expected	1·34	1·34	1·39	1·39
Observed[a]	1·32	1·35	1·31	1·33
Response to selection[b]	0·66 ± 0·15*	−1·11 ± 0·15*	4·30 ± 0·12*	−1·99 ± 0·14*
Divergence:				
Expected[c]	3·09		16·05	
Observed[b]	1·77 ± 0·21*		6·30 ± 0·18*	
Between-line variance[d]	0·60 ± 0·03*	2·28 ± 0·24*	14·53 ± 0·81*	1·89 ± 0·12*
Phenotypic variance[d]	4·43 ± 0·17	4·51 ± 0·13	7·18 ± 0·06	4·49 ± 0·20

[a] Average over 47 generations of selection.
[b] Deviation from control averaged over generations 45–47.
[c] Expectation for mutations of large effect (see text for explanation).
[d] Average over generations 45–47.
* $P < 0·05$.

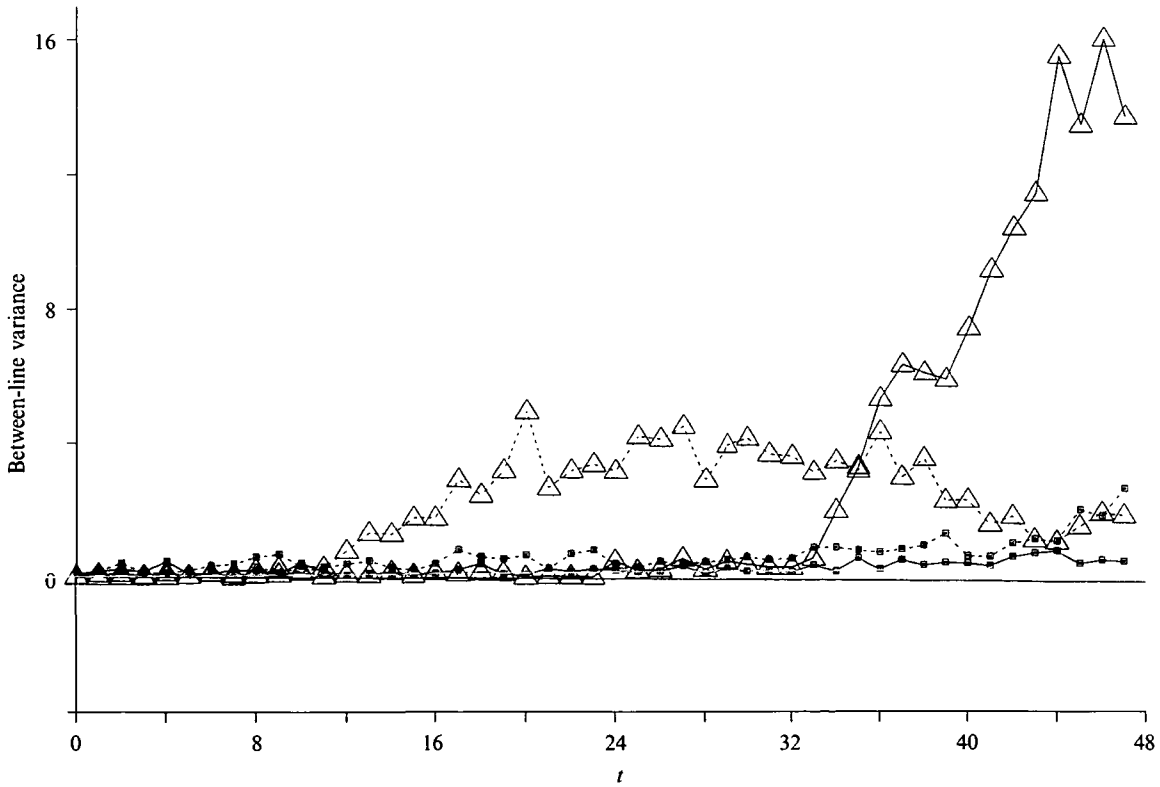

Fig. 3. Between-line variance plotted against generation number for each type of line. ——, Upward selected lines; – – –, downward selected lines; △, L lines; ⊡, S lines.

variance (averaged over the last three generations) of groups S^+, S^- and L^- slightly exceeded the control value but the difference was not significant. Nevertheless, that of the L^+ group experienced a pronounced upward trend during the last third of the experiment and attained a much larger final value than the control line (Fig. 2).

The evolution of the variances of the means of the replicates is shown in Fig. 3 for each group of lines. They clearly increased with time, gradually in the S

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

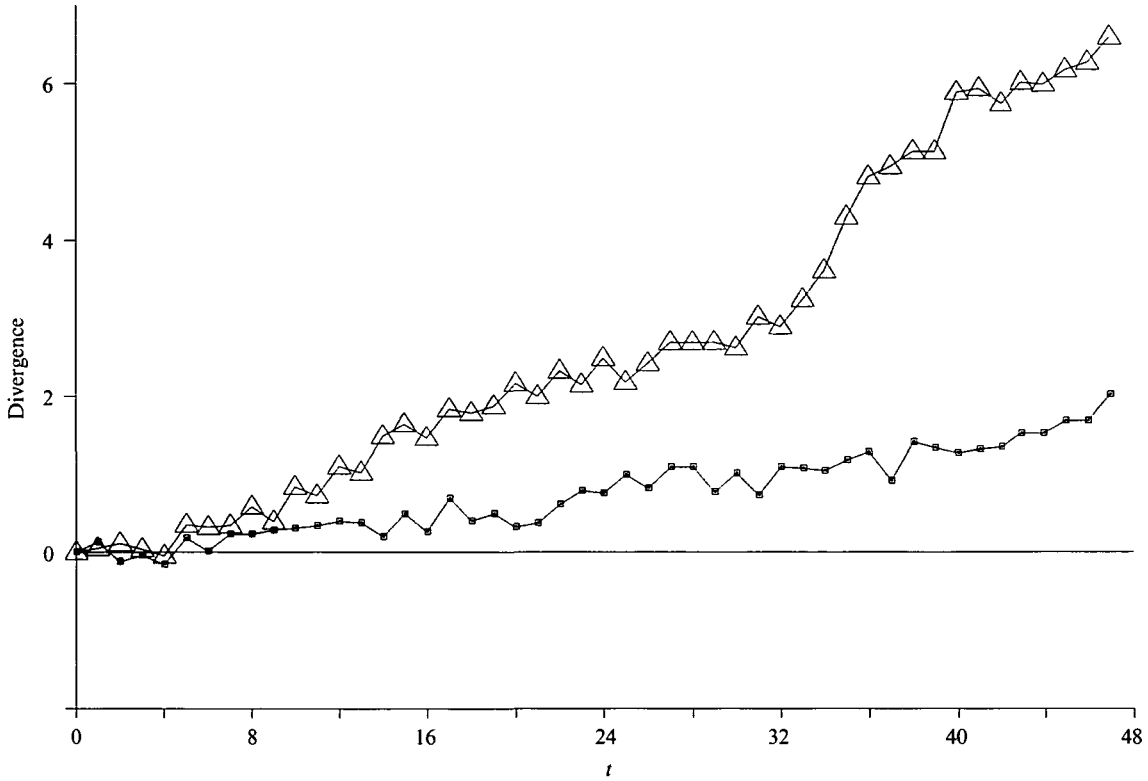

Fig. 4. Divergence between lines of the same effective size selected in opposite directions plotted against generation number. △, L lines; ⊡, S lines.

lines and speedily in the L lines. The variance of the means of the 15 S lines in which no response was detected (see section (ii) below) decreased with time (regression on generation number $-0{\cdot}009 \pm 0{\cdot}003$). Its final value, averaged over the last three generations ($0{\cdot}18 \pm 0{\cdot}07$), was larger than expected from random sampling in the same period (0·08), but the difference was not significant. However, this result should be interpreted with caution as the 15 S lines are not a random sample from the whole set of lines.

The evolution of the divergence between groups of lines of the same effective size selected in opposite directions is shown in Fig. 4. A significant rate was found in both cases (regression coefficient on generation number $0{\cdot}035 \pm 0{\cdot}002$ and $0{\cdot}140 \pm 0{\cdot}005$ for the S and L lines, respectively). However, a significant acceleration of the rate of divergence was apparent in the last third of the experiment (quadratic regression coefficient on generation number $(0{\cdot}30 \pm 0{\cdot}12) \times 10^{-4}$ and $(1{\cdot}96 \pm 0{\cdot}28) \times 10^{-3}$ for the S and L lines, respectively).

All flies scored were sepia homozygotes, indicating that no genetic contamination from external sources occurred in any of the lines.

(ii) *Behaviour of the individual replicates*

To evaluate the final divergence between each selected line and the control line, the average difference over the last three generations was tested for significance ($P < 0{\cdot}05$). Empirical standard errors were computed from the divergences calculated in each generation. The Bonferroni sequential comparison method was used (Rice, 1989). Eleven lines selected downwards and twelve selected upwards departed significantly from the control, these including all L lines. In addition, lines 3^- and 15^- also showed a clear response, their means being always below that of the control from generation 11 and 26, respectively (excepting generation 45).

The evolution of the selected replicates is presented in Figs 5 and 6, only those lines with final significant response being individually identified. In general, the S lines showed a rapid change of the mean in the course of two or three generations, with subsequent maintenance of the new level. This suggests that the response can be attributed in each case to a single mutation of relatively large effect, rapidly reaching its maximum possible frequency. A more gradual response was observed in the L lines, possibly due to the initial frequency of mutations being one-fifth of that in the S lines. In lines D^+, 16^- and 11^-, there was a clear pattern of a second mutation occurring within the same line. Three mutations were detected in line B^+. A detailed analysis of line effects is given in the companion paper (López & López-Fanjul, 1993).

Lines departed from the control at different generations. Assuming that each permanent change of the performance of a line is due to a single mutation, a total of 30 mutations were detected. Coincidently,

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

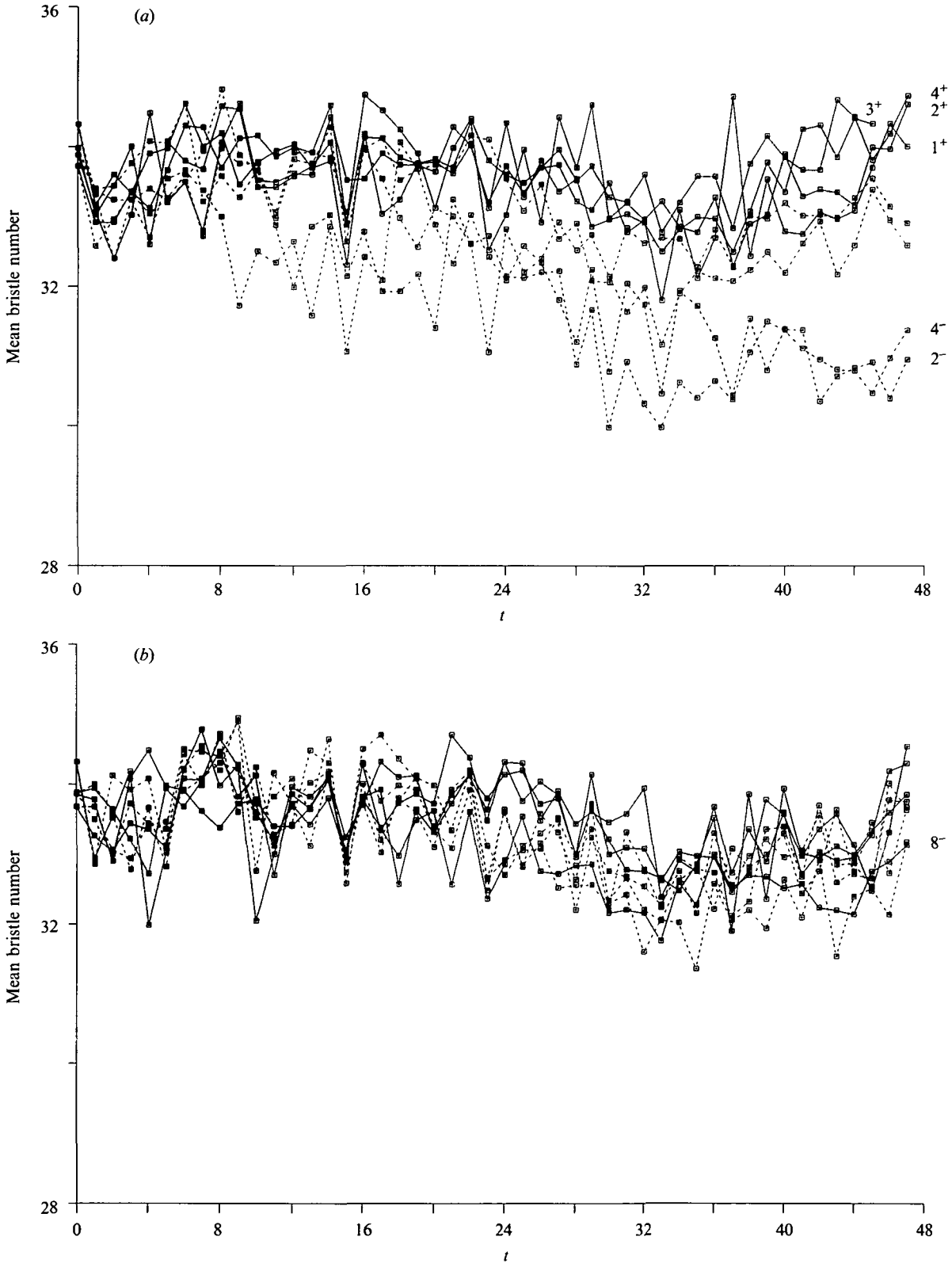

Fig. 5. For legend see opposite.

the number appearing in each third of the experiment was 10.

A change of the phenotypic variance associated with the selection response was only patent in those cases where the inferred mutation had a relatively large effect ($> \sigma$). In some lines (B^+, D^+, A^-, 14^+, 9^- and 2^-) the variance increased permanently, suggesting the presence of mutations that cannot be fixed, i.e.

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

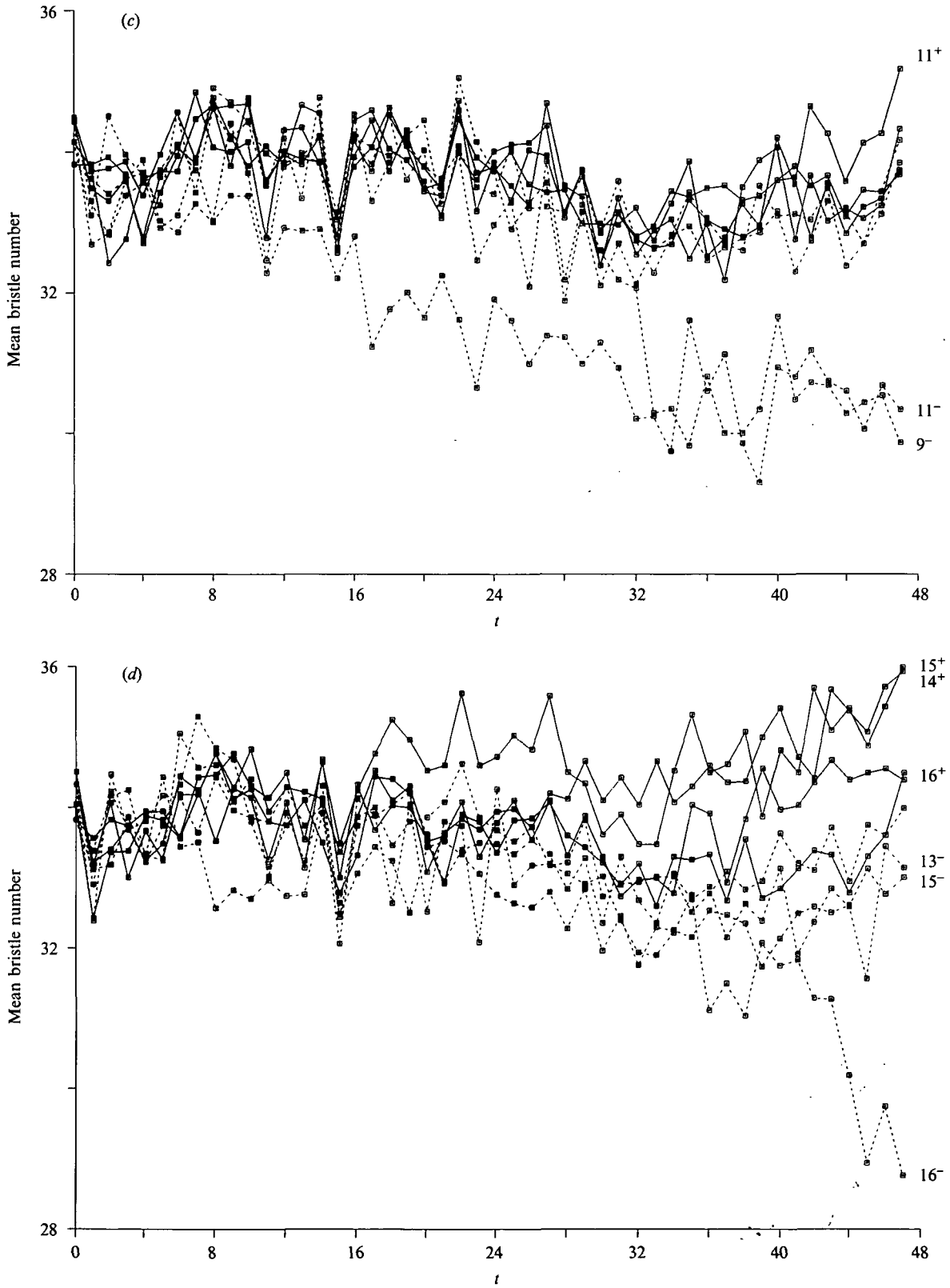

Fig. 5. Mean bristle score plotted against generation number for the S lines (+ and −): (*a*) lines 1–4, (*b*) lines 5–8, (*c*) lines 9–12, (*d*) lines 13–16. ——, Upward selected lines; – – –, downward selected lines.

lethals with a pleiotrophic effect on bristles. In the remaining instances (15^+, 11^- and 16^-) a transient increase of the variance was observed during the period of response, returning afterwards to its previous level. This observation suggests that the mutations responsible for those responses were fixed by selection. In both cases, these conclusions were later confirmed through direct analyses (López & López-Fanjul, 1993).

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

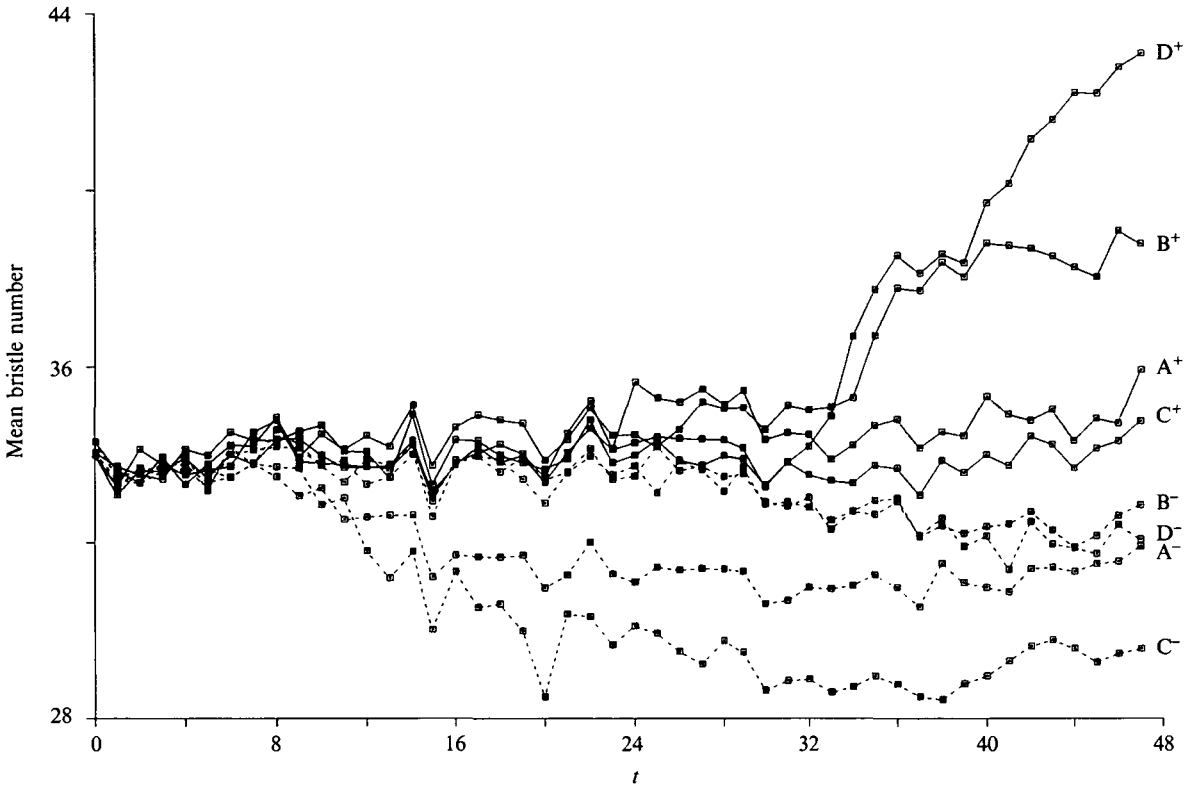

Fig. 6. Mean bristle score plotted against generation number for the L lines. ——, Upward selected lines; – – –, downward selected lines.

Table 2. *Mutational heritabilities* ($\times 10^{-3}$) *calculated under the infinitesimal model or for mutations of large effect in each type of line*[a]

	Mutant effects	
Type of line	Small	Large
S^+	0·63 ± 0·17	0·47 ± 0·12
S^-	0·98 ± 0·32	0·75 ± 0·23
Average	0·81 ± 0·18	0·61 ± 0·13
L^+	1·93 ± 0·85	0·58 ± 0·26
L^-	0·89 ± 0·30	0·27 ± 0·09
Average	1·41 ± 0·46	0·42 ± 0·14
Overall average	1·11 ± 0·25	0·52 ± 0·09

[a] All estimates significantly different from zero at the 5% level (one-tailed t test).

(iii) *Mutational heritabilities*

The expected asymptotic cumulative response R_c after t generations of selection with intensity i in an initially homozygous line of effective size N is given by

$$R_c = 2Ni\sigma_m^2\{t - 2N[1 - \exp(-t/2N)]\}/\sigma$$

(Hill, 1982*a*) under the infinitesimal model or, for genes of large effect (greater than σ/Ni) which are fixed instantaneously, by

$$R_c = 2tNi\sigma_m^2/\sigma.$$

In both cases, mutations are assumed to be neutral and to have additive effects symmetrically distributed around zero. From the equations, the mutational variance σ_m^2 of the selected trait has been calculated for each line. The following parameter values have been used: average phenotypic variance of the line, observed average selection intensity, effective size equal to 60% of the number of parents per generation, and cumulative response estimated by the deviation from the control averaged over the last three generations of selection.

Under both hypotheses, the average mutational heritability (σ_m^2/σ^2) and its empirical standard error were obtained for each group of lines and the corresponding values are presented in Table 2. All estimates were significantly different from zero and not significantly different from those obtained after 20 generations of selection (Caballero *et al.* 1991). Estimates from the L^- and S^+ lines were smaller than those for the L^+ and S^- lines, respectively. This is a reflection of the sign of the asymmetry of the response to selection (positive in the L lines and negative in the S lines). In this respect, more reliable estimates can be obtained by averaging over both directions of selection.

The mutational heritability can also be estimated from the control data under a drift-mutation model. After t generations, the expected increase in variance ΔV_t due to neutral mutations of small additive effects symmetrically distributed is given approximately by

$$\Delta V_t = 2N\sigma_m^2[1 - \exp(-t/2N)]$$

(Lynch & Hill, 1986). At generation 47, its observed value can be calculated as the regression coefficient of

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

the phenotypic variance of the control on generation number times the number of generations elapsed. The effective size of the control was made equal to 60% of the number of parents per generation. Equating observed and expected values, an estimate of the mutational heritability of $(9{\cdot}02 \pm 7{\cdot}02) \times 10^{-3}$ was obtained, assuming temporal stability of the environmental variance of the trait. This was an order of magnitude larger than those calculated from the selection responses. Nevertheless, it was not significantly different from zero, since it was based on the above mentioned regression coefficient that was also non-significant.

4. Discussion

A significant response to selection has been detected in 25 lines. This can be mostly attributed to 30 mutations with an effect greater than $\sigma/4$ (López & López-Fanjul, 1993). Therefore, a discussion of the results within the framework of a model of mutations of large effect is justified.

Starting from a genetically homogeneous base population, the asymptotic rate of response to artificial selection due to new neutral mutations of large additive effects is a function of the selection intensity, the effective population size and the mean square of the effects of mutations affecting the trait in the direction of selection. Therefore, when lines are selected in the same direction and with the same proportion, the largest response will be achieved by those with the greatest effective size. In our situation this implies $L^+ > S^+ > S^- > L^-$, in agreement with the pattern found after generation 10. More precisely, the response attained by the L lines in each direction should be five times larger than that of the corresponding S lines, since the ratio between their effective sizes is also five. However, this prediction either overestimated (upward selection) or underestimated (downward selection) final results. Sudden jumps of response followed by periods of stasis or small response were observed in all groups of lines, particularly in the L lines. Obviously, this could result in biased estimates of the response achieved after t generations of selection as well as introducing noise in the comparisons.

A more suitable check of the theory can be carried out by comparing the divergence between selected lines of the same effective size selected in opposite directions. Essentially, this test implies double replication. In this situation, both the rate of response and the total cumulative response were four times greater in the L lines. Furthermore, the curvilinearity of the response was more pronounced in these lines.

Departures from the model's assumptions must be examined in detail. The choice of the values of i and N entering the prediction equation will not significantly affect the outcome. First, expected and observed intensities of selection were very close to each other. Second, the experimental design ensures that the effective size of the L lines will be five times larger than that of the S lines, even if we cannot be certain of the value of the latter.

Under the infinitesimal model with only mutation and drift (Lynch & Hill, 1986), 27 generations will be needed to reach 90% of the equilibrium variance in the S lines, but only 50% would be achieved by the L lines at the end of the experiment. Those periods will be shortened with selection and for mutations of large effect. Notwithstanding, it is quite likely that L lines had not yet reached the equilibrium variance and, therefore, the asymptotic rate of response will be underestimated.

Additive gene action within and between loci has been assumed. However, departures from additivity will not be important as long as the mutations involved have an effect on the heterozygote (Hill, 1982*b*). Practically all mutations detected were of this kind, the only exception being one deleterious recessive (C^-). Furthermore, no epistatic effects on bristles were found between pairs of mutations present before generation 20 (Caballero *et al.* 1991).

The influence of linkage on the response due to neutral mutation is expected to be small (Keightley & Hill, 1983). Nevertheless, selection will maintain at maximum possible frequencies those lethals with a pleiotropic effect on bristles. This will correspondingly reduce the intensity of selection imposed on other mutations later appearing in the line, their fixation probabilities being substantially lowered.

Assumed neutrality, not sustained by the data, can seriously distort the predictions. As reported in the companion paper (López & López-Fanjul, 1993), most mutations had deleterious pleiotropic effects and a large fraction of them were lethals (6 and 4 out of 11 and 19 mutations in the L and S lines, respectively). Therefore, the observed divergence between lines of the same effective size selected in opposite directions will be smaller than expected for neutral mutations yielding the same mutational variance per generation. This reduction will be more pronounced in the L lines as lethals were commoner in this case. This agreed with 57% of the expected final response being achieved in the S lines but only 39% in the L lines.

Previous results from artificial selection for abdominal bristle number starting from variable populations, indicate that long-term response is often due to the incorporation of lethal mutations with an effect on the heterozygote (Clayton & Robertson, 1957; Latter & Robertson, 1962; Frankham, Jones & Barker, 1968; Yoo, 1980). This observation agrees with our finding of one third of the mutations detected being of this kind.

Mutational heritabilities have been calculated under the infinitesimal model or assuming all mutations to have large effects. Under the first assumption, overestimates are likely to be obtained, as the responses in most lines could be attributed to major mutations.

https://doi.org/10.1017/S0016672300031219 Published online by Cambridge University Press

Furthermore, larger estimates are also expected as N increases. Consequently, estimates under the second assumption will be closer to the true value and they will also be practically independent of population size. In agreement with this, mutational heritabilities calculated under the assumption of major mutations were similar for both population sizes and consistently smaller than those based on the infinitesimal model. An average value of $(0{\cdot}52 \pm 0{\cdot}09) \times 10^{-3}$ was obtained, significantly smaller than the consensus value of 10^{-3}, based on the infinitesimal model (Hill, 1982*a*). However, our calculations have been made from the cumulative responses to selection and they are, therefore, subjected to the restrictions mentioned above. Thus, our value may be an underestimate, mainly due to the deleterious condition of the mutations involved.

Although the response to selection could be mostly explained by mutations of relatively large effect, there is some evidence pointing to a parallel contribution of minor mutations, albeit they could not be isolated for further study. First, individual effects detected were as small as the power of resolution of the tests permitted and this was determined by the magnitude of σ and the number of individuals scored per line. Second, the sign of the final divergence from control of those lines showing a non-significant response was generally that expected from the direction of selection practised. Moreover, segregation of lethals with large pleiotropic effects on bristles may also have prevented the incorporation of minor mutations in some lines. This indicates that the analysis of minor mutations requires an experimental effort that would be prohibitive in most cases.

We wish to thank A. Caballero, A. Gallego, A. García-Dorado, W. G. Hill, T. F. C. Mackay and M. A. Toro for helpful discussions and comments on the manuscript. This work was financially supported by a grant from the Comisión Interministerial de Ciencia y Tecnología (GAN 90-0096).

References

Becker, W. A. (1967). *Manual of Procedures in Quantitative Genetics*. Pullman: Washington State University Press.

Caballero, A., Toro, M. A. & López-Fanjul, C. (1991). The response to artificial selection from new mutations in *Drosophila melanogaster*. *Genetics* **128**, 89–102.

Clayton, G. A. & Robertson, A. (1957). An experimental check on quantitative genetical theory. II. The long-term effects of selection. *Journal of Genetics* **55**, 152–170.

Enfield, F. D. & Braskerud, O. (1989). Mutational variance for pupa weight in *Tribolium castaneum*. *Theoretical and Applied Genetics* **77**, 416–420.

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

Hill, W. G. (1982*a*). Predictions of response to artificial selection from new mutations. *Genetical Research* **40**, 255–278.

Hill, W. G. (1982*b*). Rates of change in quantitative traits from fixation of new mutations. *Proceedings of the National Academy of Sciences, U.S.A.* **79**, 142–145.

Hill, W. G. & Keightley, P. D. (1988). Interrelations of mutation, population size, artificial and natural selection. In *Proceedings of the Second International Conference on Quantitative Genetics* (ed. E. J. Eisen, M. M. Goodman, G. Namkoong and B. S. Weir), pp. 57–70. Sunderland, Mass.: Sinauer.

Hill, W. G. & Rasbash, J. (1986). Models of long-term artificial selection in finite population with recurrent mutation. *Genetical Research* **48**, 125–131.

Keightley, P. D. & Hill, W. G. (1983). Effects of linkage on response to directional selection from new mutations. *Genetical Research* **42**, 193–206.

Keightley, P. D. & Hill, W. G. (1992). Quantitative genetic variation in body size of mice from new mutations. *Genetics* **131**, 693–700.

Latter, B. D. H. & Robertson, A. (1962). The effects of inbreeding and artificial selection on reproductive fitness. *Genetical Research* **3**, 110–138.

López, M. A. & López-Fanjul, C. (1993). Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. II. Distribution of mutant effects on the trait and fitness. *Genetical Research* **61**, 117–126

Lynch, M. (1988). The rate of polygenic mutation. *Genetical Research* **51**, 137–148.

Lynch, M. & Hill, W. G. (1986). Phenotypic evolution from neutral mutation. *Evolution* **40**, 915–935.

Mackay, T. F. C. (1990). Distribution of effects of new mutations affecting quantitative traits. *Proceedings of the 4th World Congress on Genetics Applied to Livestock Production* **13**, 219–228.

Mackay, T. F. C., Lyman, R. F., Jackson, M. S., Terzian, C. & Hill, W. G. (1992). Polygenic mutation in *Drosophila melanogaster*: estimates from divergence among inbred strains. *Evolution* **46**, 300–316.

Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Yoo, B. H. (1980). Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. II. Lethals and visible mutants with large effects. *Genetical Research* **35**, 19–31.