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Response to selection from new mutation and effective size of partially inbred populations. II. Experiments with *Drosophila melanogaster*

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Summary

Divergent artificial selection for abdominal bristle number in *Drosophila melanogaster* has been carried out starting from a genetically homogeneous base population. Lines with two different systems of mating, random (*P* lines) or between full sibs whenever possible (about 50%), random otherwise (*I* lines) were compared. Responses after 40 generations of selection were mostly due to one or two mutations of large effect (0·2 to 2 phenotypic standard deviations) per line. Ten mutations affecting the selected trait were individually studied (five lethal and five non-lethal, these being predominantly additive). These mutations satisfactorily explain the response attained, although some minor mutations may also be involved. No evidence of epistasis for bristle number was found. The average final divergence was 57% larger in the *P* lines, but it was mostly due to lethals or highly deleterious mutations. Thus, after relaxation of selection, the ranking reversed and the mean divergence became significantly larger in the *I* lines (14%). Analysis of inbreeding showed that the very small amount of variation created by spontaneous mutations (a heritability for the selected trait of about 3%) was responsible for a reduction in the effective size of about 50% in the *I* lines (relative to the case with random selection), but only about 10% in the *P* lines. Mutational heritabilities estimated from the response to selection (0·05–0·18%) were within the range usually found for this trait in previous experiments. REML estimates account for correlations between relatives, and were much larger in those lines where the response was due to lethal mutations, as these do not contribute to response after reaching maximum frequency.

1. Introduction

In the last decade, theoretical models have been developed to establish the relative importance of the contribution of new variation arising from mutation to artificial selection response (Hill, 1982*a*, *b*; Hill & Rasbash, 1986; Keightley & Hill, 1987). It has been shown that this contribution increases steadily with time and it may eventually become very large, particularly in the long term. The simplest situation is that of a totally homozygous base population and assumes a simplified version of the infinitesimal model, i.e. an average input of new genetic variance per generation (σ^2_M) due to neutral additive mutations with small effects symmetrically distributed about zero. In this instance, the response to selection accumulates approximately quadratic. However, departures from the infinitesimal model assumptions may substantially modify the predictions.

Pertinent data on spontaneous mutations affecting quantitative traits, mainly referring to abdominal and sternopleural bristle number in *Drosophila melanogaster*, have been obtained by Caballero, Toro & López-Fanjul (1991), Santiago *et al.* (1992), Mackay *et al.* (1992, 1994), López & López-Fanjul (1993*a*, *b*). Data from P-element induced mutations affecting those traits have been published by Lai & Mackay (1990) and Mackay, Lyman & Jackson (1992). Results are concordant in showing: (i) a leptokurtic distribution of mutant effects on the metric trait, with genes of very large effect predominantly in the low tail; (ii) mutations of large effect (larger than one-half phenotypic standard deviation of the trait) are usually close to recessive, but those of smaller effect show a variable degree of dominance being mainly additive;

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(iii) an intermediate correlation between mutant effects on the trait and fitness, with mutations of large effect being highly deleterious. Based on this information, Keightley, Mackay & Caballero (1993) found that the prediction from the infinitesimal model will usually overestimate actual response to selection, typically by a factor of three.

Given the characteristics of the new mutants summarized above, it is interesting to investigate systems of mating optimizing the response to artificial selection from these mutations. Using computing simulation, Caballero, Keightley & Hill (1991) have considered the response to selection from recessive mutations with different population structures. They concluded that a scheme in which local inbreeding is enhanced by performing full-sib mating whenever possible increases the probability of fixation of recessive mutations without effectively reducing that of non-recessive mutations or delaying mean time to fixation. Thus, this strategy may result in larger selection responses than those obtained in random mating populations, if favourable recessives are frequent. Moreover, fitness deterioration with selection is expected to be smaller as inbreeding allows a more efficient elimination of deleterious mutations. This may be useful in selection programmes, where natural selection opposing artificial selection can be an important cause of selection limits (see e.g. Hill & Caballero, 1992).

In the companion paper (Caballero & Santiago, 1995) it has been shown, however, that the above considerations depend on the distribution of effects of new mutations and the amount of background genetic variation for the selected trait. The effective population size of partially inbred populations can be severely reduced with selection, decreasing the fixation probability of mutants of small effect. The theory predicts the largest reductions in effective size with partial inbreeding and selection when the amount of background genetic variation is very small, as the cumulative effect of selection on the effective size is then largest. This raises the question of whether the variation generated by spontaneous mutation in a short period of time is in itself enough to drastically reduce the effective size under partial inbreeding.

In the experiment reported in this paper, divergent artificial selection for abdominal bristle number in *Drosophila melanogaster*, starting from a highly inbred base population, was carried out under two mating systems, random mating and partial full-sib mating. The main objectives were to compare the response to selection from new mutations obtained under both systems of mating and to check the effect of selection and partial inbreeding on effective size. In addition, information was obtained on the mutational variance for the selected trait, the gene action of mutations responsible for most of the response attained, and their pleiotropic effects on fitness.

2. Materials and methods

(i) *Base population*

Starting from the *D. melanogaster* isogenic line for all chromosomes obtained by Caballero, Toro & López-Fanjul (1991), a group of 100 inbred lines (B lines) was established. Each of them was maintained by a single brother–sister mating (Santiago *et al.* 1992). After 65 generations, one of these lines (B43) was randomly chosen as the base population of this experiment. At that moment, the mean abdominal bristle score ($\pm$standard error) of line B43 was $33{\cdot}38 \pm 0{\cdot}19$, not significantly different from the group average ($33{\cdot}29 \pm 0{\cdot}50$).

The B lines carried the recessive eye-colour marker sepia (*se*) on chromosome III, as an indicator of possible contamination with exogenous flies. They were classified as Q (weak P) or M′ (pseudo-M) for the P–M system of hybrid dysgenesis.

(ii) *Selected and control lines*

Starting from the B43 line after two generations of multiplication, 40 generations of divergent individual selection were carried out on the sum of the bristle numbers on the 4th and 5th (5th and 6th) abdominal sternites of males (females). There were two groups of lines differing only in their mating system: random pair mating (*P* lines) or full-sib mating whenever available, otherwise random (*I* lines). For each treatment, two replicates (subscripts 1 and 2) were selected in each direction (superscripts + and −) with proportion 20/100 of each sex (20 individuals selected out of 100 scored). Each of the 20 selected mating pairs contributed five male and five female offspring to the scored population. In each generation, random mating was achieved by using a table of random numbers.

Selection was relaxed at different times and the lines were maintained thereafter with as many parents as possible. Their performance was evaluated after a period of 5–13 generations of relaxation. To do this, a random sample of 20 males and 20 virgin females was taken from each relaxed line and individually mated at random in vials, the trait being scored in five offspring of each sex per mating.

An unselected control line (*C*) was also maintained with 20 pairs of randomly mated parents per generation, each contributing five offspring of each sex for evaluation. Control, selected and relaxed lines were evaluated strictly contemporarily and kept under the same environmental conditions.

(iii) *Culture conditions*

Flies were reared at 25 ± 1 °C on the standard medium formula of this laboratory (brewer's yeast–agar–sucrose). In all selected lines and the control line, each pair of parents was kept individually and allowed to

mate and lay eggs in a vial (2 cm diameter, 9 cm height, 10 ml medium added) for 7 d. Relaxed lines were kept in 250-ml bottles with 50 ml medium added, but vials were used for evaluation. Therefore, the same culture density was achieved in all selected and relaxed lines, and the control line.

The generation interval was 3 weeks, both in the control and the selected lines, and 2 weeks in the relaxed lines. Handling was performed at room temperature, using CO_2 anaesthesia.

(iv) *Lethal analysis*

Chromosomes II and III of all lines showing a significant response to selection were screened for lethals using the SM5(*Cy*)-TM3(*Ser*) stock. In each line, the five extreme males in the direction of selection out of 100 were used, and a random sample of five second and five third chromosomes from each male was tested for lethality. Lines were tested once (except when explicitly otherwise). The probability of a single lethal carried by one male not being detected was thus 1/32. Lethal chromosomes were isolated and their allelic relationships tested by half-diallel crosses within males and between males within lines. A minimum of 40 offspring per cross was examined.

In each line carrying a lethal, 100–150 males were scored for bristles and classified as homozygous nonlethal or heterozygous for the lethal. The difference between the mean bristle number in the two groups estimates the effect of the lethal on the selected trait in the heterozygote.

(v) *Non-lethal mutation effects and gene action*

This analysis refers only to selected lines carrying nonlethal mutations (those where no significant changes of the mean previously attained by selection were observed after a period of relaxation) and was always carried out after response ceased. Reciprocal crosses were made between each line (L) and the control (C). In the next generation, bristle number was simultaneously scored in reciprocal F_1's (50 individuals of each sex per reciprocal) and both parental lines. Assuming that the observed divergence can be attributed to a single fixed mutation per line, estimates of additive ($a = [\bar{L} - \bar{C}]/2$) and dominance ($d = \bar{F}_1 - [\bar{L} + \bar{C}]/2$) effects can be obtained (bars denote performance).

The presence of mutations in the X chromosome was tested by the difference between the mean score of males from the two reciprocal F_1 crosses between a line and the control. In this case, hemizygous effects of mutations were estimated as $\bar{L} - \bar{C}$ in males.

(vi) *Pleiotropic effects on fitness*

All F_1's referred to in the previous section (reciprocal mixed in equal proportions) were kept in bottles under crowded conditions. After 5–15 generations, all selected lines, the control line and their F_{5-15} crosses (100 individuals of each sex scored per line or cross) were evaluated simultaneously. Thus, the performance of those crosses ($\bar{F}_{5-15}$) can be compared with their expected value at Hardy–Weinberg equilibrium ($\overline{HW} = 1/4\bar{L} + 1/2\bar{F}_1 + 1/4\bar{C}$). Significant departures from this expectation ($\Delta = \bar{F}_{5-15} - \overline{HW}$) were interpreted as evidence of a deleterious pleiotropic effect of the corresponding mutations. Of course, the probability of detecting a significant difference will decrease with the magnitude of the mutational effect and with recessive gene action. Thus, there may be a tendency to classify as *quasi*-neutral those mutations with small effect on the metric trait.

(vii) *Fecundity and viability evaluations*

Each selected line and the control line were simultaneously evaluated for fecundity and egg-to-pupa viability every five generations, starting at generation 5. At generations 35 and 40, pupa-to-adult viability was also evaluated. The three traits were simultaneously scored in the control and each selected line after seven generations of relaxed selection started at generation 40.

These traits were scored as follows. First, 4-d-old virgin females were individually mated in vials to males of the same age and line (20 pairs per line). On the second day, each pair was transferred to a new vial with fresh medium and the number of eggs laid after 24 h was recorded. After a 9-d (16-d) incubation period, the number of pupae (adults) obtained from each individual lay was also recorded.

(viii) *F-statistics and effective population size*

Each generation, the inbreeding coefficient of individuals (F_{IT}) and the average conacestry among individuals (F_{ST}) were obtained from pedigrees. From these, the coefficient F_{IS}, which represents the magnitude of the deviation from Hardy–Weinberg proportions, was obtained from the relationship $(1 - F_{IS}) = (1 - F_{IT})/(1 - F_{ST})$ (Wright, 1969, pp. 294–295).

The average rate of increase in F_{ST} was calculated from the regression coefficient of $\ln(1 - F_{ST})$ on generation number between generations 10 and 40. The effective population size (N_e) was then obtained as $N_e = 1/2\Delta F_{ST} - 1/2$ (Falconer, 1989, p. 71).

Effective size can be predicted under selection and partial full-sib mating (Santiago & Caballero, 1995). For equal numbers of male and female parents constant over generations,

$$N_e = \frac{4N}{2(1 - F_{IS}) + (S_k^2 + 4Q^2 C^2)(1 + 3F_{IS})},$$

where $F_{IS} \approx \beta/(4 - 3\beta)$ and β is the average proportion of full-sib matings per generation. S_k^2 is the variance of

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the number of offspring per family after random selection, and the term $4Q^2C^2$ accounts for selection. $4C^2$ is the variance of the expected number of offspring per family based on its genotypic value for the selected trait. Q^2 is the term accounting for the cumulative effect of selection (Robertson, 1961), and can generally be obtained as

$$Q = \frac{2}{2-G(1+\beta)},$$

where G is the proportion of the genetic variance remaining after selection has been practiced. Under random mating F_{IS} and β are assumed to be zero, for simplicity.

For a quantitative trait controlled by an infinitesimal model of gene effects and subject to truncation selection, $G = 1-kh^2$ (Bulmer, 1980), where $k = i(i-x)$, i is the selection intensity, x is the truncation point in the Normal distribution and h^2 is the heritability of the trait. Finally,

$$C^2 \approx i^2\frac{h^2G}{2-\beta G}$$

(cf. equation [20] of Caballero & Santiago [1995]).

Parameters for the predictions of effective size were obtained as follows. The total response between generations 10 and 40 was calculated for each line as the difference between the mean of the line in the last three generations (38–40) and the control mean in the same period. The first 10 generations were not considered in order to allow F_{IS} to reach its asymptotic value (see Fig. 5) and because no line (with the exception of P_2^-) had responded appreciably before generation 10 (see Figs. 1, 2). Realized heritabilities were calculated as the ratio of the total response to cumulative selection differential over the 30-generation period. The proportion of full-sib matings carried out per generation (β) was recorded and averaged over the same period. In the absence of selection this proportion for the I lines can be predicted from equation (17) of Caballero & Hill (1992), and this was the value used to predict effective size under partial full-sib mating but in the absence of selection. Predicted values of i and x were obtained from standard statistical tables (Falconer, 1989, pp. 354–355). Finally S_k^2 was predicted by $2(1-1/n)$, where $n = 5$ is the number of scored individuals per family, sex and generation.

(ix) *Estimation of mutational variances*

Under the infinitesimal model, the expected cumulative response R_C after t generations of selection in an initially homozygous line is given by

$$R_C = 2N_e i\sigma_M^2\{t-2N_e[1-\exp(-t/2N_e)]\}/\sigma$$

(Hill, 1982*b*), where σ is the phenotypic standard deviation. For genes of very large effect which are assumed to be fixed instantaneously, the cumulative response is given by

$$R_C = 2t\,N_e i\sigma_M^2/\sigma.$$

From these equations, estimates of the mutational variance of the selected trait under each hypothesis were obtained for each line. The following parameters were used: observed effective population size, phenotypic variance equal to the average of the control line, observed average selection intensity, and cumulative response estimated by the average deviation from the control over the last three generations of selection.

The mutational variance was also estimated in each line by restricted maximum likelihood (REML) using an animal model, as implemented by Meyer (1989). We assumed the initial genetic variance to be insignificant (an arbitrary value of 10^{-9} was used). The model adjusted was

$$y_{ijkl} = s_i + g_j + f_{ijk} + a_{ijkl} + e_{ijkl},$$

where s_i and g_j are fixed sex and generation effects, respectively ($i = 1, 2$; $j = 1, 40$), f_{ijk} is a random family effect nested to sex and generation ($k = 1, 20$), y_{ijkl}, a_{ijkl} and e_{ijkl} are, respectively, the phenotypic, additive genetic and environmental values corresponding to the $ijkl$th individual ($l = 1, 5$). The a_{ijkl} values represent a variance–covariance structure described by the numerator relationship matrix, that can account for the increased covariance between relatives by mutation (Wray, 1990). Thus, in contrast with estimates obtained from selection response, the estimates obtained from REML considered the covariances between relatives within lines generated by mutation but ignored response, as lines were analysed separately and generation effects were fitted.

Mutational heritabilities (h_M^2) were obtained as the ratio of the mutational variance to the environmental variance (the phenotypic variance in the control line).

3. Results

(i) *Individual replicates*

The evolution of the mean and the variance is presented in Figs. 1 and 2, for each line (both under selection and after a period of relaxation) and the control.

The regression coefficient of the control mean on generation number was $(5{\cdot}83 \pm 5{\cdot}81) \times 10^{-3}$, not significantly different from zero. 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 in both directions of selection. The regression coefficient of the control phenotypic variance on generation number ($[-2{\cdot}32 \pm 7{\cdot}42] \times 10^{-3}$) was also non-significant. In this paper, effects of mutations on the

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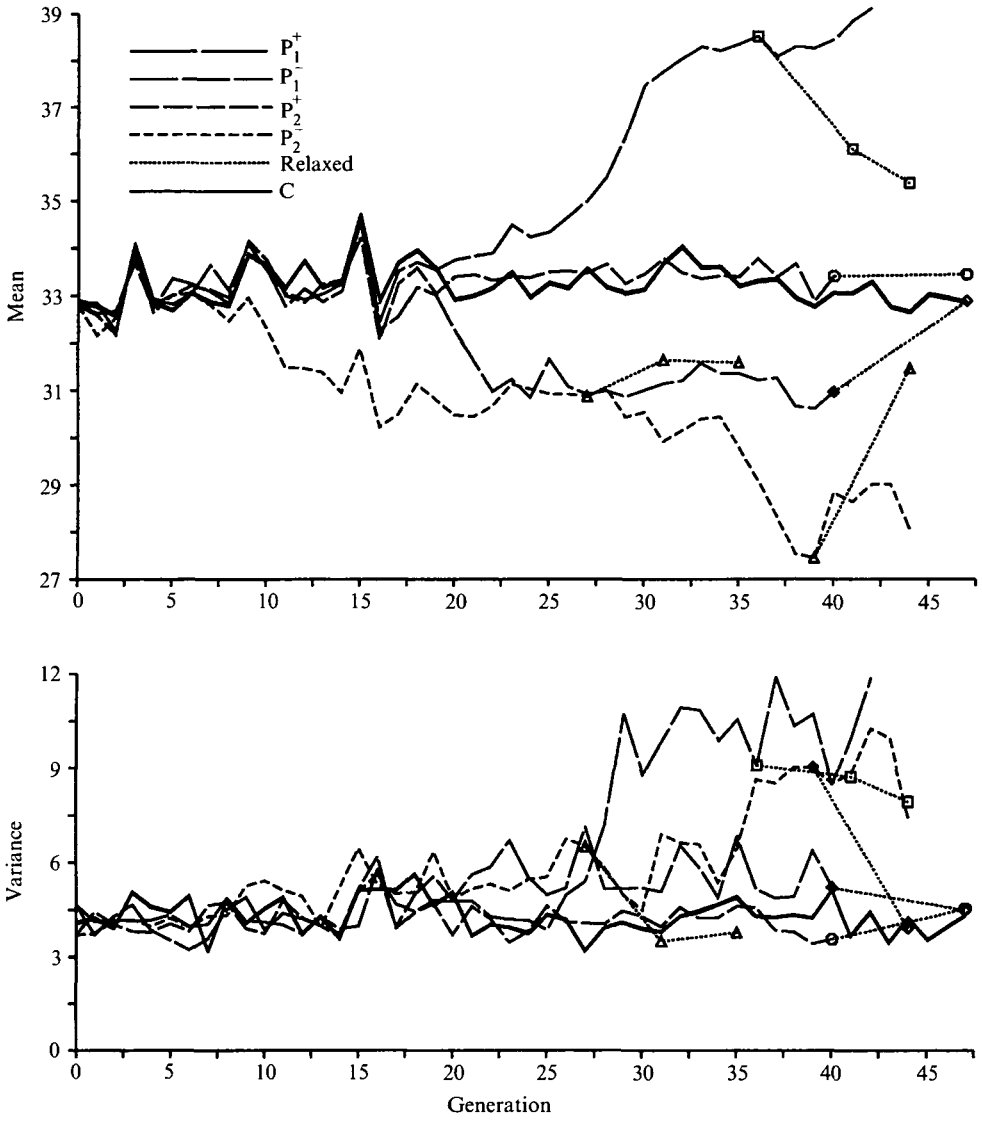

Fig. 1. Mean and phenotypic variance for bristle number plotted over generations for selected and relaxed *P* lines and the control line. (The different symbols identify each relaxed line.)

selected trait will be given in phenotypic standard deviation units of the control line ($\sigma = 1{\cdot}94$ for males and $\sigma = 2{\cdot}24$ for females), obtained from the point of intercept of this regression line in the origin.

In the initial generations, no significant divergence was detected between the mean of a selected line and that of the control. Nevertheless, in the course of the experiment clear differences emerged in all lines but one (P_2^+). Lines departed from the control at different moments, showing changes in the mean, usually in the course of a few 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, reaching its maximum possible frequency. In some lines, there was a pattern suggesting the occurrence of a second mutation (P_1^+ and P_2^-).

A permanent increase of the phenotypic variance associated with the response observed was apparent in all *P* lines responding to selection and, perhaps, in line I_1^-. This is consistent with the presence of mutations that cannot be fixed by selection, i.e. lethals with a pleiotropic effect on bristle number. After a subsequent period of relaxation, the mean of those lines regressed totally (P_1^- and I_1^-) or partially (P_1^+ and P_2^-) to the original level. In the latter, only the fraction of the total response attributable to a second mutation was lost. In the remaining *I* lines the increase in variance was confined to the response period, suggesting the fixation of the mutations involved. In these cases, the response attained was maintained after relaxation.

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

(ii) *Lethal analysis*

Lethals on chromosomes II and III with a significant pleiotropic effect on bristles were detected only in *P*

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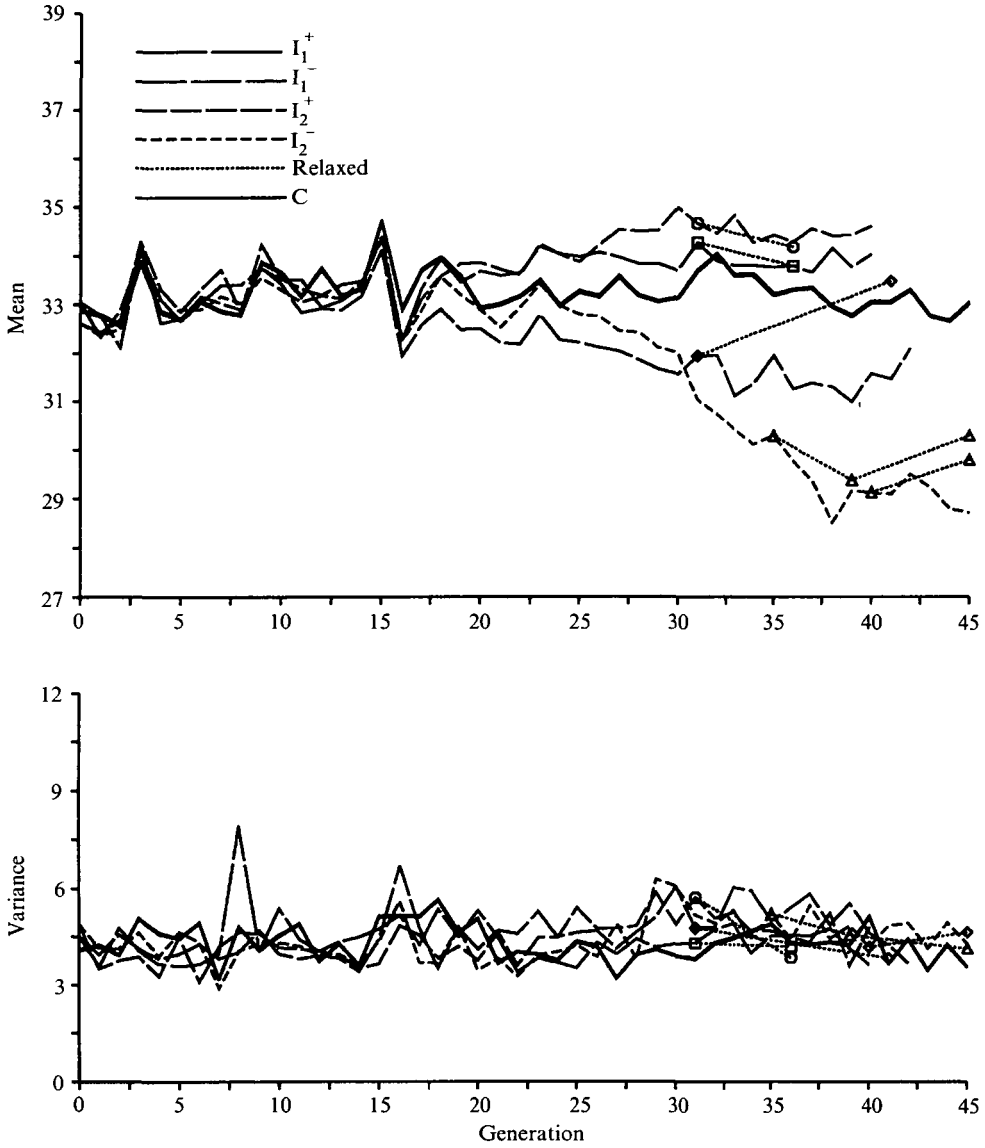

Fig. 2. Mean and phenotypic variance for bristle number plotted over generations for selected and relaxed *I* lines and the control line. (The different symbols identify each relaxed line.)

Table 1. *Summary of information on lethal mutations*

Line	Chromosome	Frequency + *S.E.*	Effect[a] ± *S.E.*
P_1^+	III	0·35 ± 0·06*	1·87 ± 0·31*
P_1^-	II	0·27 ± 0·04*	−0·54 ± 0·21*
P_1^-	III	0·27 ± 0·04*	−0·62 ± 0·21*
P_2^-	II	0·32 ± 0·05*	−1·69 ± 0·28*

[a] Deviation from control in phenotypic standard deviation units of males (σ = 1·94).
* $P < 0·05$.

lines. Pertinent information on those lethals is given in Table 1. The signs of the effects were as expected from the direction of selection, their values ranging from 0·5 to 1·9σ. Frequencies were not significantly different from 1/3, this being the expected value when artificial selection favours the heterozygous form of a recessive lethal with a large effect on the trait. A lethal was also found segregating in line I_2^- at a frequency of 0·11 ± 0·03 (generation 33) and 0·19 ± 0·05 (generation 44). However, its effect on bristles was non-significant at both times (−0·16 ± 0·19 and −0·19 ± 0·21, respectively). The behaviour of line I_1^- (see previous section) suggests a lethal mutation being responsible for the response observed. If at frequency 1/3, its effect would be about 1.3σ. Nevertheless, it passed undetected when pertinent analysis was carried out at generation 24.

(iii) *Non-lethal mutations*

In some lines, the response to selection achieved was wholly or partially retained after a subsequent period of relaxation (Figs. 1, 2). This result is consistent with non-lethality of the mutations involved. Five such

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Table 2. *Summary of information on non-lethal mutations in phenotypic standard deviation units (males = 1·94, females = 2·24)*

Line	a	d	Action	D	Δ
Males					
P_1^+	0·69 ± 0·08*	0·19 ± 0·15	Add[b]	0·92 ± 0·49	0·34 ± 0·16
P_2^-	−0·32 ± 0·08*	−0·04 ± 0·12	Add[b]	−0·42 ± 0·39	0·85 ± 0·13*
I_1^+	0·13 ± 0·06*			1·72 ± 0·40*	−0·02 ± 0·11
I_2^+	0·30 ± 0·08*	0·14 ± 0·12	Add-Dom[a]	0·48 ± 0·36	−0·26 ± 0·11
I_2^-	−0·54 ± 0·11*			−2·34 ± 0·40*	0·14 ± 0·13
Females					
P_1^+	0·60 ± 0·08*	0·08 ± 0·14	Add[b]		0·25 ± 0·15
P_2^-	−0·58 ± 0·06*	−0·08 ± 0·11	Add[b]		1·38 ± 0·13*
I_1^+	0·21 ± 0·02*	−0·03 ± 0·12	Add[b]		0·03 ± 0·12
I_2^+	0·21 ± 0·07*	0·29 ± 0·12*	Dom[c]		−0·23 ± 0·12
I_2^-	−0·88 ± 0·07*	−0·06 ± 0·12	Add[b]		0·37 ± 0·13*

a: half the difference between homozygotes (half the hemizygous effect for males of lines I_1^+ and I_2^-). d: heterozygous effect deviated from the average of the homozygous effects. D: difference between the mean bristle score of males from reciprocal F_1 crosses between each line and the control. Δ: difference between the relaxed F_1 mean bristle score and its Hardy–Weinberg expectation.

[a] Not significantly different from zero and a.

[b] Not significantly different from zero and significantly different from $|a|$.

[c] Significantly different from zero and not significantly different from a.

* $P < 0·05$ based on the sequential Bonferroni test.

mutations have been identified, relevant information being shown in Table 2, separately for each sex.

In two instances (I_1^+ and I_2^-), significant differences (D) were found between the mean bristle number of males from the two reciprocal F_1 crosses between the line and the control. This implies that the mutations involved were located on chromosome X.

Estimates of additive (half the difference between homozygous effects or half the hemizygous effects for males in X-linked mutation lines) and dominance (heterozygous) effects are given in Table 2 for each sex separately. All effects were smaller than σ. Gene action could be established in all cases, four mutations being approximately additive and one dominant.

The differences Δ between the performance of the F_{5-15} crosses between each line and the control and their Hardy–Weinberg expectations are shown in Table 2. Generally, Δ and a had opposite signs, as expected for mutations less fit than the original allele. However, significant Δ values were obtained only for lines P_2^- (both sexes) and I_2^- (females). The number of spare matings used per line and generation can be taken as a measure of viability after adult emergence. This number increased in all lines after a response to selection was observed (data not shown), but the increment was significant only in the P_2^- line, going from 3·0 ± 0·9 to 8·3 ± 1·0, and due to the non-lethal deleterious mutation fixed in the line.

(iv) *Differential effects of mutations by sex*

All mutations detected significantly affected both sexes, but not necessarily equally. Estimates of the effects of non-lethal mutations in both sexes are given in Table 2. Those corresponding to lines I_2^- and P_2^- were significantly higher in females. The remaining non-lethal mutations did not significantly affect sexes differentially. The effect of lethal mutations was only calculated in males. In these cases, however, the mean of each sex (deviated from control) before a response was detected can be compared to that after response ceased (data not shown). This comparison indicates that the two lethals in line P_1^- and the one in line P_2^- affected both sexes equally, but that carried by line P_1^+ had a larger effect on females.

For each sex, the final response attained in each line is presented in Table 3, together with the expected response when only those mutations individually analysed are considered. In the absence of epistasis, the contribution to the expected response is $2a$ for nonlethal mutations (Table 2) and two-thirds of the effect for lethals (Table 1). No significant difference between observed and expected responses was detected for males. In females, a strictly valid comparison is restricted to lines carrying non-lethal mutations, because lethal effects were only measured in males. Nevertheless, the effects of lethals on males can be used in the calculations, although they may underestimate the expected response. Only in two lines (I_1^+ and I_2^+), each carrying a single non-lethal mutation, did observed responses significantly exceed their expectations, suggesting that undetected mutations with a larger effect on females could also be involved. This is compatible with an increase in the female's mean (but not in the male's) observed at later generations, after effects were calculated. As expected and observed responses were not shown to be different in lines carrying two mutations, significant epistatic effects between these can be ruled out.

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Table 3. *Averaged observed final response*[a] *and its expectation* (±S.E.) *from detected mutations*

	Males		Females	
Line	Observed	Expected	Observed	Expected
P_1^+	2·42±0·10	2·63±0·26	2·70±0·10	2·45±0·26
P_1^-	−1·18±0·09	−0·77±0·20	−0·92±0·08	−0·77±0·20
P_2^-	−1·95±0·10	−1·77±0·25	−2·76±0·10	−2·29±0·22
I_1^+	0·30±0·08	0·26±0·14	0·67±0·08	0·42±0·04[b]
I_2^+	0·56±0·08	0·60±0·16	0·89±0·08	0·42±0·14[b]
I_2^-	−1·68±0·09	−1·08±0·22	−2·11±0·08	−1·76±0·14

[a] Average deviation from control over last three generations of selection in phenotypic standard deviation units (males = 1·94, females = 2·24).
[b] Significantly smaller than observed ($P < 0·05$, based on the sequential Bonferroni test), otherwise not significant.

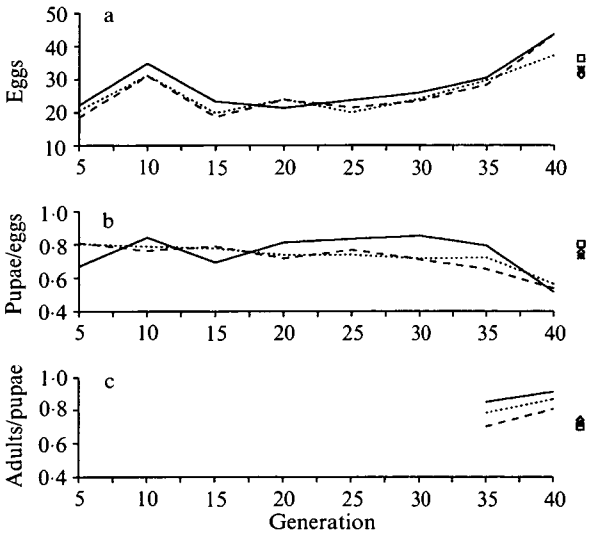

Fig. 3. Average fecundity (a), egg-to-pupa viability (b) and pupa-to-adult viability (c) for each type of lines (*P* and *I*) and the control line. ——, Control; – – –, *P* lines; . . . ., *I* lines; ◇, relaxed C line; □, relaxed *P* lines; *, relaxed *I lines.*

(v) *Evolution of fitness components*

The evolution of fecundity and viability (egg-to-pupa and pupa-to-adult) is given in Fig. 3 for each group of lines and the control. For each trait, possible differences between the control and the selected lines (altogether) or between both groups of lines were investigated. Thus, the variance was partitioned into sources arising from variation between generations, among groups (control *v.* selected lines or *P v. I* lines), generation × group interaction, and within groups of lines. A significant superiority of the control over the selected lines was detected both for fecundity and pupa-to-adult viability but not for egg-to-pupa viability. Differences in fecundity, however, were present from the start of the experiment (see Fig. 3). Therefore, they cannot be due to the appearance of mutations and can only be ascribed to environmental causes. Significant differences between *P* and *I* lines were only found for pupa-to-adult viability, the *I* lines being more viable.

At the end of the experiment, all selected lines and the control line were subjected to a seven-generation period of relaxation, fecundity and both viability components being scored per line immediately after this period was completed. For each trait, no significant differences were detected between the control and the selected lines, suggesting a complete recovery in the latter.

(vi) *Overall behaviour of selected lines*

Response to selection and relevant statistics for each group of selected lines is shown in Table 4. Selection intensities were estimated per line and generation as the applied selection differentials in phenotypic standard deviation units. The average response of a group of lines, given as deviation from the control over the last three generations of selection, was significant in all cases. Responses in the downward direction were always larger, pointing to a parallel asymmetry of the distribution of mutant effects.

The evolution of the divergence between groups of lines of the same mating system selected in opposite directions (*P v. I*) is shown in Fig. 4. *P* lines started to diverge earlier. Subsequently, a similar acceleration of the rate of divergence was observed in both groups of lines, as indicated by significant quadratic regression coefficients on generation number (*P* lines: 0·006±0·001, *I* lines: 0·007±0·001). Thus, divergence was always larger in the *P* lines. The final average phenotypic variance of the *I* lines did not significantly differ from the control value averaged over all generations (4·40±0·17). However, that of the *P* lines was significantly larger (Table 4).

After 40 generations of selection, a seven-generation period of relaxation was imposed on all lines. In general, this resulted in severe losses of the response previously attained. More interestingly, the ranking for divergence of the groups of lines was reversed, as

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Table 4. *Response to selection and associated parameters* ($\pm$ S.E.) *for each type of line*

Type of line	P^+	P^-	I^+	I^-
Selection intensity[a]	1·25	1·13	1·21	1·21
Selection response[b,e]	2·76 $\pm$ 0·09	−3·42 $\pm$ 0·09	1·24 $\pm$ 0·08	−2·69 $\pm$ 0·08
Divergence[b,e]	6·18 $\pm$ 0·10		3·93 $\pm$ 0·08	
Phenotypic variance[c]	6·72 $\pm$ 0·35	7·16 $\pm$ 0·24	4·35 $\pm$ 0·29	4·74 $\pm$ 0·17
Response retained[d,e]	1·66 $\pm$ 0·12	−0·62 $\pm$ 0·11	1·14 $\pm$ 0·11	−1·47 $\pm$ 0·11
Divergence retained[d,e]	2·28 $\pm$ 0·13		2·61 $\pm$ 0·11	
Phenotypic variance retained[f]	6·07 $\pm$ 1·54	4·29 $\pm$ 0·19	4·04 $\pm$ 0·24	4·47 $\pm$ 0·52

[a] Average over 40 generations of selection.
[b] Deviation from control averaged over generations 38–40.
[c] Average over generations 38–40 (control value 4·40).
[d] Deviation from control after seven generations of relaxed selection.
[e] In phenotypic standard deviation units (sexes pooled, $\sigma = 2{\cdot}09$).
[f] After seven generations of relaxed selection (control value 4·31).

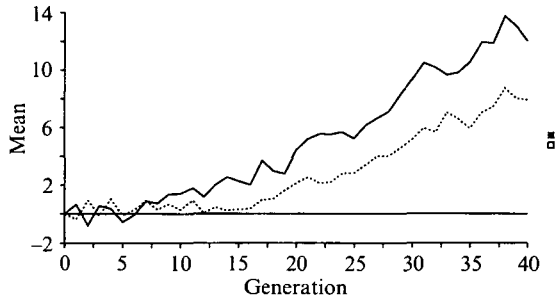

Fig. 4. Average divergence (in bristle number) between high and low selected lines for P and I lines. ——, P lines; . . . . , I lines; □, relaxed P lines; *, relaxed I lines.

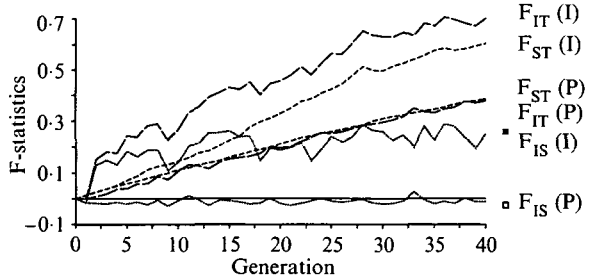

Fig. 5. Average F-statistics (F_{IT}, F_{ST} and F_{IS}; see text for definitions) for each type of lines (P and I). The asterisk and the square represent the expected values of F_{IS} for I and P lines, respectively.

I lines retained a significantly larger proportion of previous response than P lines (Table 4, Fig. 4).

(vii) *F-statistics and effective population size*

Figure 5 shows the evolution of the observed F-statistics for each type of line (averaged among replicates and direction of selection). The expected asymptotic values of F_{IS} are also shown in the figure. In the P lines F_{IS} is not exactly zero (as was assumed for simplicity in the predictions of N_e), but about $-1/(N-1)$ (Kimura & Crow, 1963). In the I lines, the average observed β among lines was 0·58, and the expected F_{IS} is approximately $\beta/(4-3\beta) = 0{\cdot}26$. The rate of increase in F_{ST}, which gives a measure of the effective population size, is much larger for the I lines than for the P lines. The evolution of F_{ST} in all P lines was very similar and rather linear (data not shown). However, that of the I lines varied substantially among lines, with periods of higher rates of increase. In general, these periods were approximately coincident with those of response, but on some occasions (e.g. in lines I_1^+ and I_2^+, which carried the mutations of smallest effects), there were sharp increases in F_{ST} several generations previous to response being detected. This suggests that in these latter cases, the mutation or mutations responsible for the response appeared before the mean bristle number of the lines clearly departed from that of the control line.

Table 5 shows the observed and predicted values of effective size, as well as other relevant parameters used in their calculations. Predicted values of N_e with random selection (assuming $C^2 = 0$) were 44·44 for random mating and 37·95 for partial full-sib mating (assuming that the number of full-sib matings per generation is 10·68, the expected value with random selection). The observed N_e in the unselected random mating control line was 45·20, in good agreement with its expectation. When selection is accounted for using the realized heritabilities from the table, the average predicted N_e are 39·07 for the P lines and 18·72 for the I lines (11 and 51% smaller than that with random selection, respectively). Observed effective sizes averaged over lines were very close to the expectations though values for individual lines differed substantially, particularly for I lines. This is not at all unexpected, however, as predictions are based on an infinitesimal model of gene effects, which clearly cannot represent accurately the genetic constitution of the lines. I^- lines carried mutations with larger effects than those of I^+ lines. The larger the effect of a

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Table 5. *Observed (N_{eo}) and predicted effective population size with (N_{es}) and without selection (N_{ens}) for each selected line and the control*

Line	*NFS*	h^2	C^2	Q^2	N_{es}	N_{ens}	N_{eo}
P_1^+	1·5	0·054	0·050	3·52	36·89		38·21
P_2^+	0·9	0·005	0·005	3·77	43·48		41·42
P_1^-	1·2	0·026	0·024	3·66	40·31		41·42
P_2^-	0·4	0·068	0·062	3·45	35·59		38·21
Average *P*					39·07 ± 1·78	44·44	39·81 ± 0·93
I_1^+	11·3	0·013	0·017	19·70	24·12		16·99
I_2^+	11·4	0·020	0·026	19·52	20·26		14·90
I_1^-	11·8	0·021	0·028	21·08	18·67		20·58
I_2^-	12·1	0·050	0·065	19·18	11·83		19·75
Average *I*					18·72 ± 2·56	37·95	18·05 ± 1·30
C	0·4	—	—	—	44·44		45·20

Predictions use the following parameters: $S_k^2 = 1·6$, $i = 1·386$, $x = 0·832$. *NFS*: average number of full-sib matings carried out per line and generation over generations 10 to 40. h^2: realized heritability between generations 10 and 40. C^2 and Q^2: see text for definitions.

Table 6. *Mutational heritabilities* ($\times 10^{-3}$) *estimated from the response to selection assuming the infinitesimal model (INF) or mutations of large effect (LAR), or by REML*

Line	LAR	INF	REML
P_1^+	0·81	3·63	64·00
P_2^+	0·04	0·21	1·00
P_1^-	0·28	1·33	5·54
P_2^-	0·85	3·83	21·50
I_1^+	0·30	0·73	1·00
I_2^+	0·52	1·15	1·80
I_1^-	0·42	1·15	2·30
I_2^-	0·99	2·64	2·04
Average	0·53 ± 0·12	1·83 ± 0·48	12·40 ± 7·76

mutation, the faster it will be fixed or reach maximum frequency, and the shorter will be the period of time in which it affects the effective size of the population. This is in agreement with the observed N_e for I^- lines being larger than for I^+ lines. The infinitesimal model, however, predicts smaller N_e in the former (see Table 5), as the total response was larger, Nevertheless, average predictions for both types of lines were exceptionally good despite the clear violation of infinitesimal model assumptions.

The main cause in the large reduction in N_e in the *I* lines compared to the *P* lines is the higher magnitude of the value Q^2 (Table 5), which determines the cumulative effect of selection and is about six times larger in the *I* lines than in the *P* lines.

(viii) *Mutational heritabilities*

Estimates of the mutational heritability based on the cumulative response to selection, as well as REML estimates, are given in Table 6. The former assume either the infinitesimal model or all mutations having large effects. The latter account for correlations between relatives within lines, assuming also the infinitesimal model.

Estimates from response to selection assuming large mutational effects were of course smaller than those based on the infinitesimal model and, in turn, these were smaller than REML estimates. Striking between-line differences were found for the latter and were restricted to those lines showing a quick and pronounced response due to the increase in frequency of lethal mutations of large effect. Thus, average REML estimates of mutational heritabilities were one order of magnitude greater but not significantly different from zero, contrasting with significant average estimates obtained by the other procedures. The difference can be ascribed to REML estimates taking into account correlations between relatives due to lethals that reach their maximum frequency or to deleterious mutations which get eventually lost and do not contribute to the final response.

4. Discussion

(i) *Properties of mutations*

Half of the detected mutations with significant effect on the selected trait were lethals (5/10) at the maximum frequency (1/3) with effects between 0.5 and 1.9σ. Lethals of smaller effects will reach lower equilibrium frequencies, making difficult their detection and to prove whether or not they affect the selected trait. This could be the case of the lethal detected in line I_2^- at frequency 0·15 ± 0·03 and non-significant effect $-0·17 \pm 0·14\sigma$ (averages of generation 33 and 44). Computer simulations of the same experimental design used in this experiment show that lethals with effect $0·25\sigma$ reach an equilibrium frequency of 0·15 (data not shown), so it is plausible that

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the lethal observed in line I_2^- had a real effect on the selected trait.

A high frequency of lethal mutations affecting bristle number was also found in a previous study in which mass selected lines were started from the same base population used in this experiment (Caballero, Toro & López-Fanjul, 1991; López & López-Fanjul, 1993*b*). Moreover, lethals contributing to response are a common feature of selection experiments (e.g. Clayton & Robertson, 1957; Frankham *et al.* 1968; Hollingdale, 1971; Madalena & Robertson, 1975; Yoo, 1980; García-Dorado & López-Fanjul, 1983), and are generally considered as mutations that occurred after selection started.

Non-lethal mutations detected in this experiment had generally smaller effects than lethals (a value between 0·1 and 0·9σ), additive gene action and little or no deleterious effects on fitness, although the procedure used to establish pleiotropic mutational effects on fitness is biased towards neutrality, particularly for mutations of small effects on the metric trait. Two of these mutations were X-linked, with homozygous effects ($2a$) on females of $0{\cdot}42 \pm 0{\cdot}04\sigma$ and $-1{\cdot}76 \pm 0{\cdot}14\sigma$. Assuming complete dosage compensation in the X chromosome of the *Drosophila* male, this is the expected value in hemizygotes. The effects observed in hemizygous males were $0{\cdot}26 \pm 0{\cdot}14$ and $-1{\cdot}08 \pm 0{\cdot}22$, respectively, both smaller than $2a$ (but only significantly in the latter), and not significantly different from a, the expected value with no dosage compensation. This, however, may be confounded with possible differential effects of mutations by sex.

In general, lethal and non-lethal mutations affected both sexes but had a larger effect on females. Mutations affecting abdominal bristle number often modify sex dimorphism for this trait (Frankham, 1980; Mackay & Langley, 1990; Mackay, Lyman & Jackson, 1992; Mackay *et al.* 1992, 1994; López & López-Fanjul, 1993*b*). Some genes involved in sex determination with pleiotropic effects on bristle number could be responsible for the sex dimorphism (Mackay, Lyman & Jackson, 1992; Mackay *et al.* 1992).

Both lethal and non-lethal mutations were not allelic, as shown by differences in the sign of the effects, gene action and chromosomal location. In the three lines carrying two mutations, observed and expected responses to selection were not significantly different. As expectations assumed independent gene action between loci, the data suggest epistatic effects between pairs of newly arisen mutations being small or non-existent. Similar results were obtained by Caballero, Toro & López-Fanjul (1991) by analysing the response attained by selecting synthetics formed by crossing pairs of lines which had previously shown a response due to mutation.

(ii) *Distribution of effects*

The total response observed was satisfactorily explained by mutations of relatively large effect. However, the power of resolution of the experiment implies that mutational effects smaller than $0{\cdot}2\sigma$ would pass undetected in practice. There is, in fact, some indication of mutations of small effect contributing to the response: observed responses were generally larger than expected when the mutations individually analysed are considered (but, in most cases, not significantly). These results are also similar to those obtained previously by Caballero, Toro & López-Fanjul (1991) and López & López-Fanjul (1993*b*), and suggest a leptokurtic distribution of mutant effects, as proposed by Robertson (1967).

Variation for bristle number in *Drosophila* is assumed to be mostly neutral and additive (Robertson, 1967). From current data on the distribution of mutant effects, Caballero & Keightley (1994) inferred that most of the additive variance for bristle number in natural populations is due to *quasi*-neutral genes with relatively large effects ($2a$ between 0·1 and $0{\cdot}5\sigma$). A large fraction of the genetic variation of bristle number in natural populations has been shown to be due to segregation of genes of large effect at intermediate frequencies (Robertson, 1967; Gallego & López-Fanjul, 1983). Lai *et al.* (1994) have recently suggested that about 10% of the additive genetic variance for bristle number in a natural population can be explained by allelic variation at intermediate frequencies at the locus *scabrous*. In the present experiment and in previous ones from the same base population we have found additive *quasi*-neutral mutations of substantial effect which could be good candidates to contribute significantly to variation in natural populations.

The number of mutations with positive and negative effects were similar, both in this experiment (four positive, six negative) and in previous ones (16 positive, 14 negative; López & López-Fanjul, 1993*b*). However, higher responses to selection were generally obtained in the downward direction both in those experiments and in that of Mackay *et al.* (1994). Mutations caused by *P*-element insertions also show a negative asymmetric distribution (Mackay, Lyman & Jackson, 1992). These data suggest a negative asymmetry of the original distribution of mutant effects. However, asymmetry of short-term response to selection in lines derived from natural populations has been shown to be positive (Clayton & Robertson, 1957; Latter & Robertson, 1962). These contradictory results can be due to the fact that mutations decreasing bristle number are in general less fit (López & López-Fanjul, 1993*b*; this report). Lines selected for low bristle number from natural populations usually present lower reproductive capacity (Clayton & Robertson, 1957; Latter & Robertson, 1962) and, in our experiment, the asymmetry disappeared after relaxation

as most mutations reducing bristle number were lethals. Therefore, it is possible that the original distribution of mutant effects on bristle number is negatively skewed, but approaches symmetry or even changes its sign after natural selection acts. Nevertheless, the number of mutations on which all these pieces of information are based is small, so that the sign of the asymmetry might be subject to a large sampling error.

(iii) *Mutational heritabilities*

Average estimates of mutational heritability from the response to selection were 0·05% assuming large mutational effects and 0·18% assuming the infinitesimal model. These are within the range of estimates previously found (reviewed by Keightley, Mackay & Caballero, 1993). Estimates from REML were very different among lines. When the response could be ascribed to non-lethal mutations, they were similar to estimates from the selection response (0·15%) but when lethals were involved they were much larger (2·33%). This suggests that REML estimates account for correlations among relatives induced by the segregation of lethals even when these have reached their maximum frequency and do not contribute to further selection response. In addition, correlations induced by deleterious mutants which are eventually lost and, therefore, do not contribute to the selection response, can also be responsible for enhanced estimates of mutational variance from REML (Caballero, Keightley & Hill, 1995).

All estimates of mutational heritability assume that the base population was devoid of genetic variation. Strictly, it was a highly inbred population obtained by many generations of full-sib mating starting from an isogenic line. If the equilibrium variance for a full-sib line (about $5\sigma^2_M$; Lynch & Hill, 1986) is assumed, estimates in Table 6 are reduced by about 15%.

(iv) *Effective population size*

It has been predicted that partial inbreeding can severely reduce effective population size in selected populations (Charlesworth, Morgan & Charlesworth, 1993; Charlesworth, 1994; Santiago & Caballero, 1995). This reduction is expected to be largest for intense selection and small amounts of genetic variation for the selected trait, because the cumulative effect of selection also becomes then largest (Caballero & Santiago, 1995). An extreme case to be tested is that of an initially homogeneous base population where some variation is built up by spontaneous mutation. This is the situation in the present experiment. The realized heritability observed over a period of 40 generations was only about 3%. This was enough, however, to cause a reduction in the effective size relative to the case of random selection of about 50% when half of the matings were between full sibs (F_{IS} = 0·26), but that corresponding to random mating populations was much smaller (about 10%). Indeed, this situation is far from the limiting case. If we were able to produce 100% full-sib matings or selfed offspring ($F_{IS} = 1$), the relative reduction in N_e would have been much larger under the same selection scheme (Caballero & Santiago, 1995).

Equations to predict N_e are general, but the infinitesimal model was assumed in some calculations for mathematical tractability. By contrast, the response observed in the selected lines was mostly due to one or two mutations of large effect, often lethal or deleterious. Despite that, predictions seem to be very reliable and our results can be considered a good example of a situation clearly violating the infinitesimal model assumptions where the model works quite well.

(v) *Response to selection from neutral mutations*

The large reduction in effective size observed has the consequence of reducing the fixation rate of neutral mutations of small effect affecting the trait (Caballero & Santiago, 1995) so that their contribution to the response to selection is expected to be smaller in the inbred lines. All non-lethal mutations detected, however, had a relatively large effect ($2a$ between 0·25 and $1{\cdot}76\sigma$) and there was only indirect evidence of mutations with smaller effects.

For mutations of large effect, for which fixation is decided in the short term, the differences between *P* and *I* lines are not expected to be large if mutations have substantial effect on the heterozygote, but recessives would be more easily fixed in the *I* lines. However, no recessive mutation contributed to the response in both groups of lines. In previous experiments from the same base population (López & López-Fanjul, 1993*b*), a total of five complete recessives were found out of 30 analysed mutations affecting abdominal bristle number, generally deleterious and with variable effects. Thus, the absence of recessives among the four mutations detected in the *I* lines or the six in the *P* lines was not totally unexpected. However, this empirical consideration is based on a small sample of mutations and has little inductive value.

The mutations analysed in lines I_1^+ and I_2^- were X-linked. None of the mutations detected in previous experiments (López & López-Fanjul, 1993*b*) or in the *P* lines were of this class. It is not expected, however, that partial inbreeding favours this class of mutants (Caballero & Santiago, 1995), so their presence in the *I* lines and not in the *P* lines must be fortuitous.

Times to fixation are reduced by partial inbreeding if mutants have small effects or they are completely recessive (Caballero & Santiago, 1995). Thus, in these situations responses should be faster than with random mating. However, for mutants of large effect with substantial effect on the heterozygote (as found in this

experiment), times to fixation should be similar for inbred and random mating lines. Therefore, an earlier response in the *I* lines was not expected and, in fact, *P* lines started to respond first.

In summary, given the type of non-lethal mutations observed in the experiment, i.e. large effect and mainly additive gene action, a similar response for *P* and *I* lines would be expected. That was approximately the result achieved if lethal mutations are not considered.

(vi) *Collateral effects on fitness*

The frequency of lethals and less deleterious mutations affecting the selected trait should theoretically be lower in the *I* lines, particularly if their effects are not very large ($< 1\sigma$; Caballero & Santiago, 1995). The response attained in *P* lines could almost totally be attributed to six mutations, four of them being lethal (two with effects smaller than 1σ) and another one strongly depressing viability. In contrast, the response of *I* lines could be ascribed to four mutations, three of them *quasi*-neutral and one due to a putative lethal with effect larger than 1σ. In this situation, the final advantage of *P* lines over *I* lines (57%) was not permanent. After a subsequent period of relaxation, both groups of lines became equally fit but their ranking for the selected trait was reversed, *I* lines becoming superior (14%). In a previous experiment (López & López-Fanjul, 1993*b*), at least one-half of identified mutations responsible for the response to artificial selection in panmictic lines were lethal. Thus, this result is compatible with that obtained in *P* lines (two-thirds), the proportion in *I* lines being smaller (one-fourth). Again, large sampling errors must be attached to these comparisons as the number of mutations was small in all cases.

Analysis of fitness components (fecundity and egg-to-pupa and pupa-to-adult viability) detected significant differences between *P* and *I* lines only for pupa-to-adult viability, this being, on average, about 10% smaller in the *P* lines. Most of the difference, however, was due to line P_2^-, where a lethal as well as a highly deleterious mutation were segregating. This line had a reduced selection intensity relative to the other lines and it was difficult to maintain due to low reproductive performance.

The effects of partial inbreeding on the dynamics of the populations have interest both from an evolutionary and practical point of view. The drastic effects on effective population size will lead to highly reduced variation, for example in highly selfed plants. Response to selection due to new mutations is strongly dependent on the effects of the mutants and the time scale considered. Possibly, the largest advantage of partial inbreeding from a practical point of view may be the purging of deleterious genes contributing to the response and, thus, allowing for enduring responses after selection ceases.

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References

Bulmer, M. G. (1980). *The Mathematical Theory of Quantitative Genetics*. Oxford: Clarendon Press.

Caballero, A. & Hill, W. G. (1992). Effects of partial inbreeding on fixation rates and variation of mutant genes. *Genetics* **131**, 493–507.

Caballero, A. & Keightley, P. D. (1994). A pleiotropic nonadditive model of variation in quantitative traits. *Genetics* **138**, 883–900.

Caballero, A., Keightley, P. D. & Hill, W. G. (1991). Strategies for increasing fixation probabilities of recessive mutations. *Genetical Research* **58**, 129–138.

Caballero, A., Keightley, P. D. & Hill, W. G. (1995). Accumulation of mutations affecting body weight in inbred mouse lines. *Genetical Research* **65**, 145–149.

Caballero, A. & Santiago, E. (1995). Response to selection from new mutation and effective size of partially inbred populations. I. Theoretical results. *Genetical Research* **66**, 213–225.

Caballero, A., Toro, M. A. & López-Fanjul, C. (1991). The response to artificial selection from new mutations in *Drosophila melanogaster*. *Genetics* **127**, 89–102.

Charlesworth, B. (1994). The effect of background selection against deleterious mutations on weakly selected, linked variants. *Genetical Research* **63**, 213–227.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. (1993). The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**, 1289–1303.

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.

Falconer, D. S. (1989). *Introduction to Quantitative Genetics*, 3rd edn. Harlow, Essex: Longman.

Frankham, R. (1980). Origin of genetic variation in selection lines. In *Selection Experiments in Laboratory and Domestic Animals* (ed. A. Robertson), pp. 56–68. Slough, UK: Commonwealth Agricultural Bureaux.

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

Gallego, A. & López-Fanjul, C. (1983). The number of loci affecting a quantitative trait in *Drosophila melanogaster* revealed by artificial selection. *Genetical Research* **42**, 137–149.

García-Dorado, A. & López-Fanjul, C. (1983). Accumulation of lethals in highly selected lines of *Drosophila melanogaster*. *Theoretical and Applied Genetics* **66**, 221–223.

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

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

Hill, W. G. & Caballero, A. (1992). Artificial selection experiments. *Annual Review of Ecology and Systematics* **23**, 287–310.

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

Hollingdale, B. (1971). Analysis of some genes from abdominal bristle number selection lines in *Drosophila melanogaster*. *Theoretical and Applied Genetics* **41**, 292–301.

Keightley, P. D. & Hill, W. G. (1987). Directional selection and variation in finite populations. *Genetics* **117**, 573–582.

Keightley, P. D., Mackay, T. F. C. & Caballero, A. (1993). Accounting for bias in estimates of the rate of polygenic variation. *Proceedings of the Royal Society of London* B **253**, 291–296.

Kimura, M. & Crow, J. F. (1963). The measurement of effective population number. *Evolution* **17**, 279–288.

Lai, C., Lyman, R. F., Long, A. D., Langley, C. H. & Mackay, T. F. C. (1994). Naturally occurring variation in bristle number and DNA polymorphisms at the *scabrous* locus of *Drosophila melanogaster*. *Science* **266**, 1697–1702.

Lai, C. & Mackay, T. F. C. (1990). Hybrid dysgenesis-induced quantitative variation on the X chromosome of *Drosophila melanogaster*. *Genetics* **124**, 627–636.

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*a*). Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. I. Response to artificial selection. *Genetical Research* **61**, 107–116.

López, M. A. & López-Fanjul, C. (1993*b*). 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. & Hill, W. G. (1986). Phenotypic evolution by neutral mutation. *Evolution* **40**, 915–935.

Mackay, T. F. C., Fry, J. D., Lyman, R. F. & Nuzhdin, S. V. (1994). Polygenic mutation in *Drosophila melanogaster*: estimates from response to selection of inbred strains. *Genetics* **136**, 937–951.

Mackay, T. F. C. & Langley, C. H. (1990). Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*. *Nature* **348**, 64–66.

Mackay, T. F. C., Lyman, R. F. & Jackson, M. S. (1992). Effects of *P* elements on quantitative traits in *Drosophila melanogaster*. *Genetics* **130**, 315–332.

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.

Madalena, F. E. & Robertson, A. (1975). Population structure in artificial selection: studies with *Drosophila melanogaster*. *Genetical Research* **24**, 113–126.

Meyer, K. (1989). Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm. *Genetics Selection Evolution* **21**, 317–340.

Robertson, A. (1961). Inbreeding in artificially selected programmes. *Genetical Research* **2**, 189–194.

Robertson, A. (1967). The nature of quantitative genetic variation. In *Heritage from Mendel* (ed. R. A. Brink), pp. 265–280. Madison, USA: University of Wisconsin Press.

Santiago, E., Albornoz, J., Domínguez, A., Toro, M. A. & López-Fanjul, C. (1992). The distribution of effects of spontaneous mutations on quantitative traits and fitness in *Drosophila melanogaster*. *Genetics* **132**, 771–781.

Santiago, E. & Caballero, A. (1995). Effective size of populations under selection. *Genetics* **139**, 1013–1030.

Wray, N. R. (1990). Accounting for mutation effects in the additive genetic variance-covariance matrix and its inverse. *Biometrics* **46**, 177–186.

Wright, S. (1969). *Evolution and the Genetics of Populations. Vol. 2, The Theory of Gene Frequencies*. Chicago, USA: University of Chicago Press.

Yoo, B. H. (1980). Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. 2. Lethals and visible mutants with large effects. *Genetical Research* **35**, 19–31.