Genet. Res., Camb. (1993), **61**, *pp.* 117–126 *With 2 text-figures*

Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. II. Distribution of mutant effects on the trait and fitness

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

Starting from a completely homozygous population of *Drosophila melanogaster*, lines have been derived and subjected to 47 generations of divergent selection for abdominal bristle number (20 lines selected in each direction) or to 60–67 generations of inbreeding (100 B lines maintained by a single brother-sister mating, 100 C lines maintained by two double first cousin matings). In the selected lines, 25 were identified carrying at least 30 mutations affecting bristle number. A large fraction of these mutations (42%) were lethals. Non-lethal mutations had smaller effects on the trait, were predominantly additive and had no detectable pleiotropic effects on fitness. In the inbred lines, 21 mutations affecting bristles were individually analysed. Deleterious mutations had the largest effects on the trait (irrespective of sign) and showed recessive gene action (complete or incomplete). The rest were predominantly additive and had smaller effects. Thus, both procedures identify a quasi-neutral class of additive mutations which should be close to that responsible for standing variation in natural populations. Moreover, the results indicate a leptokurtic distribution of mutant effects, consistent with a model of natural selection acting on bristles through pleiotropic effects of pertinent loci on fitness. Consequently, neutral additive alleles of considerable effect can be found segregating at intermediate frequencies in natural populations.

1. Introduction

Morphological microevolution is currently viewed as a process in which variation, continuously supplied by mutation, is incessantly eroded by selection and drift. Recent theoretical analyses have emphasized the role of mutation in determining both the amount of polygenic variation maintained in populations and the genetic divergence between them (see Hill, 1990, for a review). In finite population theory, the joint action of mutation and selection (stabilizing or directional) critically depends on the shape of the bivariate distribution of mutant effects on the quantitative trait of interest and fitness (Hill & Rasbash, 1986; Keightley & Hill, 1987, 1988, 1990).

The distribution of mutant effects on a quantitative trait can be characterized by its moments. The variance is linearly related to the mutational input of genetic variation per zygote and generation (σ_m^2), which is usually given in dimensionless form (scaled by the environmental variance σ_E^2), as the mutational heritability (h_m^2). For spontaneous mutation, a typical value of 10^{-3} has been advanced for the mutational heritability of additive traits commonly used in artificial selection experiments, i.e. bristle number in *Drosophila* (Hill, 1982) and pupal weight in *Tribolium* (Enfield & Braskerud, 1989). Notwithstanding, mutational heritabilities vary between characters and species. Thus, information reviewed by Lynch (1988) indicates that *Drosophila* viability and mouse skeletal traits have mutational heritabilities of the order of 10^{-5} and 10^{-2}, respectively. Moreover, traits related to body size, such as wing measurements in *Drosophila* (Santiago *et al.* 1992) and body weight of mice (Keightley & Hill, 1992), may have larger mutational heritabilities than those found for *Drosophila* bristles.

Information on the third and fourth moments of the distribution of spontaneous mutant effects is restricted to a few traits in *Drosophila melanogaster*. Negative asymmetries have been reported for wing measurements and abdominal bristle number and one positive asymmetry for sternopleural bristle number (Caballero, Toro & López-Fanjul, 1991; Santiago *et al.* 1992). In these studies, a small number of mutations accounted for most of the mutational variance. Therefore, the distribution of mutant effects on those traits had a high variance and may be leptokurtic.

Pleiotropy on fitness of mutations affecting a

* Corresponding author.

quantitative trait can be represented by the correlation of absolute values of mutant effects on the trait and fitness (Keightley & Hill, 1990). For bristle traits and wing measurements in *Drosophila*, there are indications of this correlation being small (Caballero *et al.* 1991; Santiago *et al.* 1992). In this situation, segregation of quasi-neutral genes of relatively large effect can contribute much variance in natural populations, as has previously been found for sternopleural bristle number (Robertson, 1967; Gallego & López-Fanjul, 1983).

This paper reports on the shape of the joint distribution of mutant effects on abdominal bristle number and fitness in *Drosophila melanogaster*. Data have been obtained from inbred and selected lines started from the same homozygous base population. Gene action on the metric trait of individual mutations causing most of the divergence between lines has also been established.

2. Materials and methods

(i) *Base population, selected lines and control*

A description of the isogenic base population and the selected lines derived from it, as well as a specification of the culture conditions on which the flies were reared, is given in the companion paper (López & López-Fanjul, 1993). There were a total of 40 lines, 25 of which were shown to carry at least one mutation affecting abdominal bristle number.

(ii) *Lethal analysis*

Chromosomes II and III of lines showing a significant response to selection after generation 20 were subsequently screened for lethals using the SM5(*Cy*)–TM3(*Ser*) stock. No dysgenic symptoms were observed in crosses between the balancer stock (M strain) and the selected lines (pseudo-M or weak P for the P–M hybrid dysgenesis system). Lethal chromosomes were isolated and their allelic relationships tested by half-diallele crosses within males and between males within lines. In a line carrying a lethal, 130 males were scored for bristles and classified as homozygous non-lethal or heterozygous for the lethal. Thus, the frequency of the lethal can be calculated. The difference between the mean bristle number in the two groups estimates the effect of the lethal on the selected trait in the heterozygote.

Due to accidental losses, six lines responding to selection near the end of the experiment (1^+, 2^+, 3^+, 4^+, 11^+ and 8^-) were not subjected to lethal analyses.

(iii) *Reverse selection*

This analysis refers only to selected lines carrying a non-lethal mutation detected after generation 20. After the response ceased, selection was reversed and carried out with proportion 5/25 of each sex for 6–9 generations. All forward and reverse selected lines and the control line were kept simultaneously and at the same culture density. Two lines responding to selection in late generations (4^- and 16^-) were accidentally lost before they could be studied.

(iv) *Inbred lines*

From the same isogenic base population mentioned above, two groups of one hundred inbred lines each were started. In each generation, a line was maintained either by a single brother–sister mating (lines B, denoted B1–B100) or by two double-first-cousin matings (lines C, denoted C1–C100). From generation 47, all C lines were each maintained by a single brother–sister mating (see Santiago *et al.* 1992 for further details).

Abdominal bristle number was scored simultaneously in samples of 34 females per line in each of three consecutive generations (lines B, generations 60–62; lines C, generations 65–67). At these times 94 B lines and 89 C lines survived.

Lines showing an extreme mean for abdominal bristle score of females were chosen for further analysis (seven B lines and six C lines from each tail of the distribution). Its number exceeded that of lines significantly departing from their group average in both directions for the two sets of lines ($P < 0{\cdot}05$).

(v) *Mutant effects and action*

This analysis refers only to the extreme 26 inbred lines and the selected lines carrying non-lethal mutations. Reciprocal crosses were made between each line (P_1) and the control (P_0). In the next generation, the trait was simultaneously scored in the reciprocal F_1s (50 individuals of each sex or 75 females per reciprocal formed by crossing selected or inbred lines, respectively), and the corresponding parental lines (inbred, 150 females per line; selected, 35 or 125 individuals of each sex per S or L line, respectively; control, 100 individuals of each sex or 300 females for comparison with selected or inbred lines, respectively). Assuming that the observed divergence can be attributed to a single fixed mutation per line, estimates of additive [$a = (P_1 - P_0)/2$] and dominance [$d = F_1 - (P_0 + P_1)/2$] effects can be obtained.

The presence of mutations in the X chromosome was tested by the difference D between the mean bristle number of males from the two reciprocal F_1 crosses between a line and the control (20 or 50 males simultaneously scored per reciprocal formed by crossing inbred or selected lines, respectively).

(vi) *Pleiotropic effects on fitness*

All F_1s referred to in the preceding section (reciprocals mixed in equal proportions) were kept under crowded

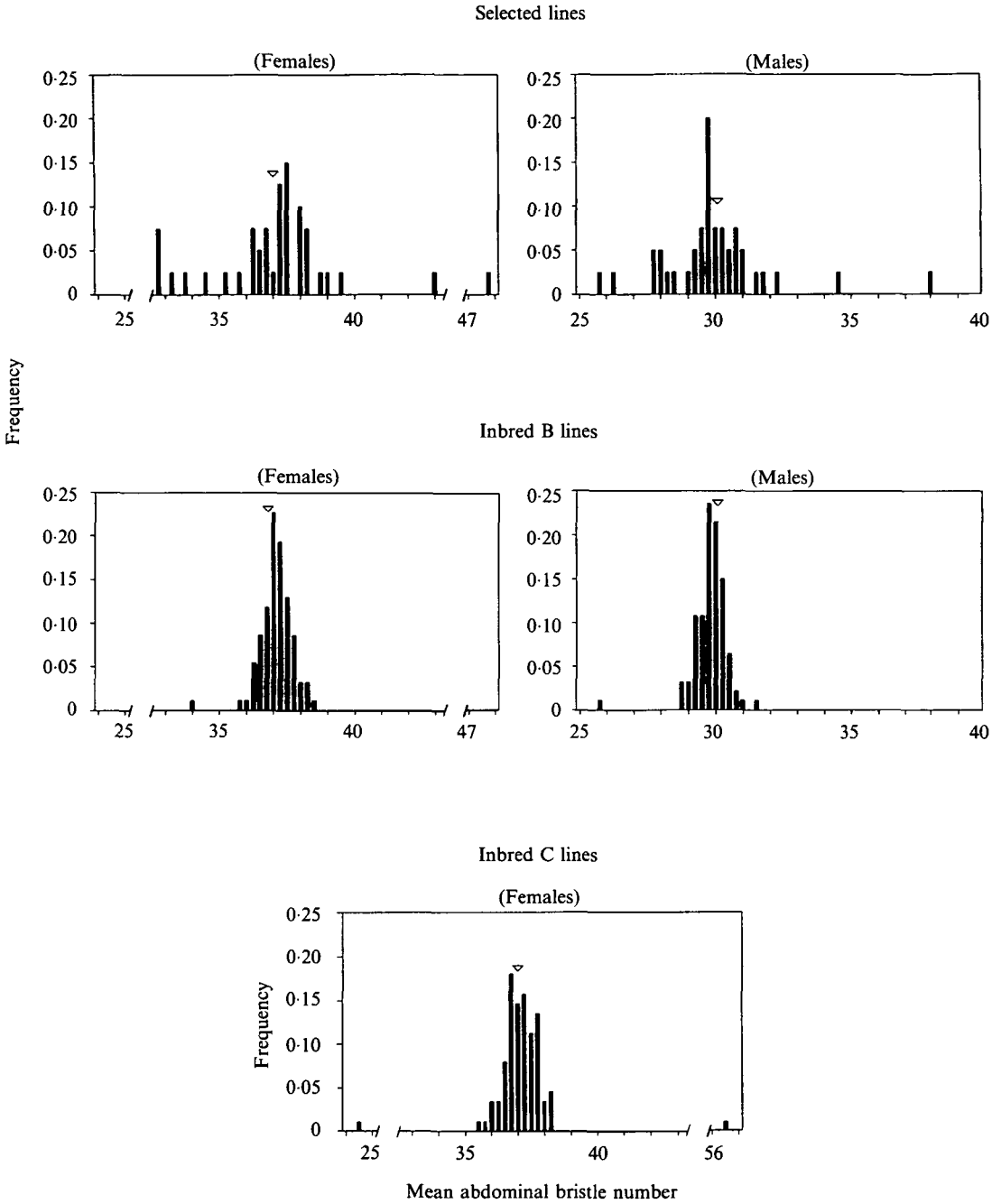

Fig. 1. Distributions of the means of the lines after 47 generations of selection or 62–67 generations of inbreeding (distribution means indicated by open triangles).

conditions in bottles with a large number of parents. After 4–7 generations, all inbred lines, their F_{5-8} crosses (75 females scored per line or cross) and the control (150 females scored) were simultaneously evaluated. Crosses involving selected lines were also evaluated after five generations (F_6) in the above conditions (the number of individuals scored of each sex was 50 for each F_6 cross, 100 for the control, and 35 or 125 for each of the S or L lines, respectively). Thus, the performance of the crosses (F_t) can be compared with their expected value at Hardy–Weinberg equilibrium (HW). Significant departures from this expectation ($\Delta = F_t - \mathrm{HW}$) were interpreted as evidence of a deleterious pleiotropic effect of the corresponding mutations. When gene action could not be unambiguously established, the largest of the two Δ values corresponding to the limiting possibilities was tested for significance. However, no significant deleterious effects were found in these instances. 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.

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

3. Results

(i) *Selected lines*

The distribution of the final means of the lines (averaged over the last three generations of selection) is shown in Fig. 1 for each sex. The variance and the coefficients of asymmetry and kurtosis (sexes pooled) are given in Table 1. This distribution was significantly asymmetrical (positive) and leptokurtic, even when those lines not showing a significant response to selection were excluded from the analysis. However, the sign of the asymmetry was consistently negative up to generation 35 and the subsequent change can be ascribed to three late appearing mutations in lines B^+ and D^+ (López & López-Fanjul, 1993).

Most lines showing a significant response to selection after generation 20 were screened for lethals on chromosomes II and III. Information on the frequencies and pleiotropic effects on bristles of these lethals is summarized in Table 2, together with that previously reported by Caballero *et al.* (1991) for lethals detected before generation 20.

Five lines carried one lethal each on chromosome II, four on chromosome III and two on both. Lethals in lines 2^-, 13^-, A^- and B^+ had a significant effect on bristles ($> 0{\cdot}6\sigma$; $\sigma = 2{\cdot}05$ bristles, being the phenotypic standard deviation of the trait estimated in the control population). A permanent increase of the phenotypic variance associated with the response to selection was observed in lines 9^-, 14^+ and D^+ (López & López-Fanjul, 1993). Thus, one could also assume that the corresponding lethals have a pleiotropic effect on bristles, although it was not directly estimated. Finally, those in lines C^+, 3^- and 15^- had no significant effect. In one case (3^-), the lethal had been lost by generation 43, but the response was maintained. Therefore, it can be attributed to a different mutation originally linked to the lethal and later losing its association.

Most lines showing a significant response to selection after generation 20 but not carrying lethals were studied in detail. None of those responded to selection carried out in the opposite direction (Fig. 2), the regression coefficient of the divergence on generation number not being significantly different from

Table 1. *Variance (V) and coefficients of asymmetry (g_1) and kurtosis (g_2) of the distributions of the means of the lines*

Type of line	No. of lines	V	g_1	g_2
Selected (sexes pooled)				
All	40	5·28	1·63*	6·50*
Mutation carriers	25	8·55	1·36*	3·31*
Non-carriers	15	0·06	−0·07	0·93
Inbred B (males)				
All	94	0·42	−2·51*	15·87*
Mutation carriers	11	2·38	−1·26	2·85*
Non-carriers	83	0·17	−0·34	−0·17
Inbred B (females)				
All	94	0·60	−2·58*	13·01*
Mutation carriers	11	3·71	−0·99	0·43
Non-carriers	83	0·20	−0·43	0·53
Inbred C (females)				
All	89	6·46	4·13*	47·63*
Mutation carriers	11	54·96	1·53*	5·81*
Non-carriers	78	0·21	0·10	−0·25

* $P < 0{\cdot}05$.

Table 2. *Summary of information on lethal mutations found in selected lines*

Line	Final response[a]	Chromosome	Frequency	Effect[b]
$2^{-,c}$	−0·97±0·13*	II	0·33±0·02*	−0·67+0·19*
9^-	−0·93±0·14*	II		
$13^{-,c}$	−0·37±0·13*	III	0·42±0·02*	−0·62±0·25*
15^-	−0·27±0·13	III	0·24±0·04*	−0·18±0·34
$A^{-,c}$	−0·82±0·07*	II	0·32±0·02*	−0·62±0·19*
$C^{-,c}$	−1·84±0·07*	III		−2·10±0·52*[d]
D^-	−0·65±0·08*	II		
14^+	1·21±0·14*	III		
B^+	2·28±0·09*	II	0·35±0·05*	1·59±0·73*
B^+	2·28±0·09*	III	0·25±0·05*	0·65±0·73
C^+	0·37±0·08*	II	0·25±0·03*	0·10±0·33
D^+	3·98±0·09*	II		
D^+	3·98±0·09*	III		

[a] Average deviation from control over last three generations of selection in phenotypic standard deviation units of males ($\sigma = 1{\cdot}98$).
[b] Deviation from control in phenotypic standard deviation units of males.
[c] From Caballero *et al.* (1991).
[d] Semilethal, complete recessive for bristle score.
* $P < 0{\cdot}05$.

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

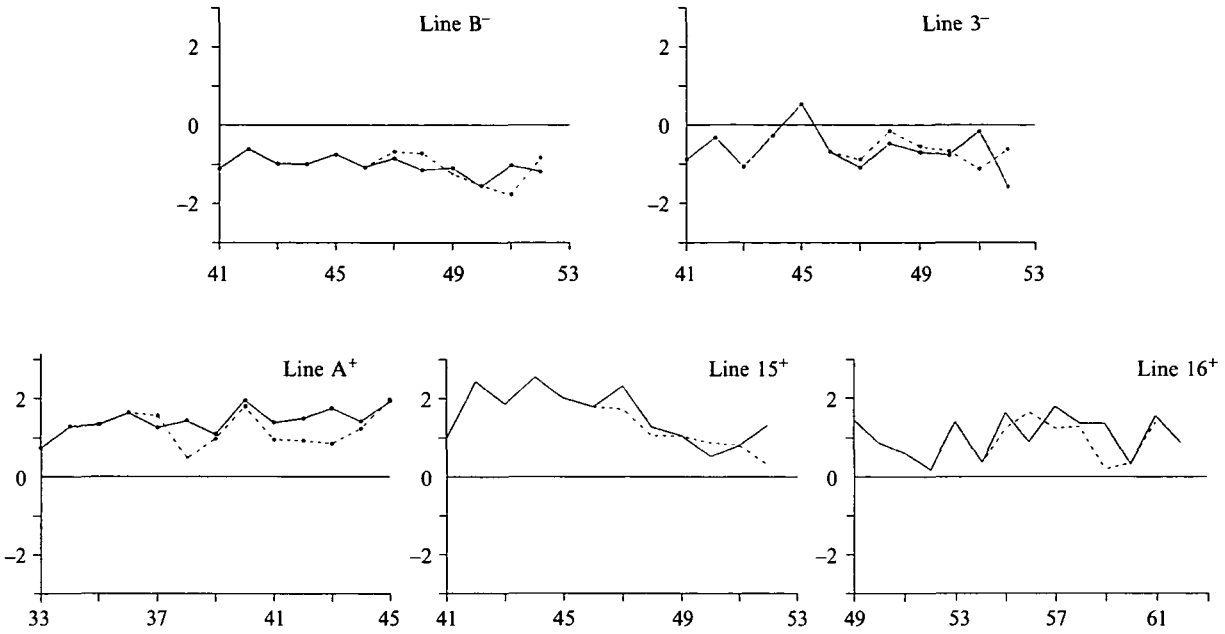

Fig. 2. Response to forward (——) and reverse (– – –) selection in lines carrying non-lethal mutations (in deviations from control). Abscissa is generation number.

Table 3. *Summary of information on non-lethal mutations found in selected lines*

Line	a^a	d^a	Action	D^b	$\Delta^{a,c}$
3^-	$-0{\cdot}97 \pm 0{\cdot}07$*	$0{\cdot}55 \pm 0{\cdot}11$*[d]	Incomplete recessive	$-0{\cdot}12 \pm 0{\cdot}22$[e]	$0{\cdot}27 \pm 0{\cdot}12$
11^{-}[f]	$-0{\cdot}58 \pm 0{\cdot}07$*	$0{\cdot}10 \pm 0{\cdot}29$[g]	Additive		$0{\cdot}10 \pm 0{\cdot}38$
11^{-}[h]	$-0{\cdot}24 \pm 0{\cdot}06$*				
B^-	$-0{\cdot}54 \pm 0{\cdot}04$*	$-0{\cdot}13 \pm 0{\cdot}10$[g]	Additive	$-0{\cdot}35 \pm 0{\cdot}23$	$0{\cdot}09 \pm 0{\cdot}12$
15^+	$0{\cdot}25 \pm 0{\cdot}08$*	$0{\cdot}02 \pm 0{\cdot}11$[g]	Additive	$-0{\cdot}08 \pm 0{\cdot}23$	$-0{\cdot}34 \pm 0{\cdot}12$*
16^+	$0{\cdot}41 \pm 0{\cdot}06$*	$-0{\cdot}18 \pm 0{\cdot}17$[i]	Additive-Recessive	$0{\cdot}06 \pm 0{\cdot}13$	$-0{\cdot}06 \pm 0{\cdot}12$
A^+	$0{\cdot}90 \pm 0{\cdot}06$*	$0{\cdot}02 \pm 0{\cdot}14$[g]	Additive	$-0{\cdot}04 \pm 0{\cdot}30$	$-0{\cdot}92 \pm 0{\cdot}12$*
B^{+}[j]	$0{\cdot}18 \pm 0{\cdot}03$*				

[a] In phenotypic standard deviation units (sexes pooled, $\sigma = 2{\cdot}05$).
[b] Difference between the mean bristle number of males from reciprocal F_1 crosses between each line and the control (described in the text).
[c] Difference between the F_6 mean bristle score and its Hardy–Weinberg expectation (described in the text).
[d] Significantly different from zero and $-a$.
[e] On chromosome III (Caballero *et al.* 1991).
[f] On chromosome II (Caballero *et al.* 1991).
[g] Not significantly different from zero and significantly different from $|a|$.
[g] Deleterious (Caballero *et al.* 1991).
[i] Not significantly different from zero and $-a$.
[j] Quasi-neutral (Caballero *et al.* 1991).
* $P < 0{\cdot}05$ based on the sequential Bonferroni test (Rice, 1989).

zero in all cases. This result is consistent with the mutation responsible for the previous forward response being already fixed.

Estimates of additive and dominant effects are presented in Table 3, where information from Caballero *et al.* (1991) has also been included. Although the procedure followed to establish the significance of these effects rules out recessive (dominant) gene action, it may introduce a bias in favour of additive action. However, it will only be to a very limited extent, i.e. for incomplete recessives (or incomplete dominants) when their additive effect is small. Notwithstanding, the type of gene action was unambiguously established in most cases. Four mutations were additive (15^+, A^+, 11^- and B^-) and one was incompletely recessive (3^-). In the remaining case (16^+), additivity could not be distinguished from complete recessivity. All mutant effects were smaller than σ.

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

Table 4. *Summary of information on mutations found in inbred lines*

Line	a^a	d^a	Action	D^b	$\Delta^{a,c}$
B40	0·41 ± 0·05*	0·04 ± 0·08	Additive[d]	1·50 ± 0·69	0·21 ± 0·13
B62	0·17 ± 0·05*	0·19 ± 0·07*	Dominant[e]	−0·20 ± 0·65	0·06 ± 0·14
B65	0·35 ± 0·05*	0·09 ± 0·08	Additive[d]	0·45 ± 0·69	−0·17 ± 0·14
B72	0·29 ± 0·05*	−0·20 ± 0·11	Additive-Recessive[f]	0·05 ± 0·63	0·28 ± 0·12
B80	0·22 ± 0·05*	0·31 ± 0·07*	Dominant[e]	0·55 ± 0·70	0·24 ± 0·14
C58	0·22 ± 0·06*	−0·05 ± 0·11	Additive[d]	0·50 ± 0·64	0·20 ± 0·13
C59	5·05 ± 0·13*	−2·22 ± 0·20*	Incomplete recessive[g]	0·10 ± 0·80	−2·45 ± 0·24*
C67	0·17 ± 0·05*	−0·10 ± 0·08	Additive-Recessive[f]	−0·10 ± 0·57	0·49 ± 0·14*
C90	0·16 ± 0·05*	−0·04 ± 0·08	Additive[d]	−0·10 ± 0·54	−0·24 ± 0·13
C91	0·11 ± 0·05*	−0·22 ± 0·09*	Recessive[h]	0·10 ± 0·56	0·19 ± 0·12
B24	−0·33 ± 0·05*	0·01 ± 0·07	Additive[d]	2·45 ± 0·56*	0·10 ± 0·12
B29	−0·64 ± 0·06*	0·65 ± 0·09*	Recessive[h]	—	0·57 ± 0·14*
B52	−0·18 ± 0·05*	0·19 ± 0·07*	Recessive[h]	0·60 ± 0·70	0·32 ± 0·13
B64	−0·29 ± 0·05*	−0·12 ± 0·07	Additive-Dominant[i]	−1·50 ± 0·69	0·32 ± 0·13
B79	−1·32 ± 0·05*	0·84 ± 0·07*	Incomplete recessive[g]	0·85 ± 0·66	0·08 ± 0·13
B93	−0·27 ± 0·05*	0·04 ± 0·15	Additive[d]	0·05 ± 0·71	−0·01 ± 0·12
C10	−0·36 ± 0·08*	0·05 ± 0·08	Additive[d]	−0·80 ± 0·59	−0·24 ± 0·19
C24	−0·46 ± 0·06*	0·08 ± 0·10	Additive[d]	0·10 ± 0·46	−0·06 ± 0·13
C40	−0·39 ± 0·13*	0·40 ± 0·14*	Recessive[h]	−0·05 ± 0·71	0·34 ± 0·16
C55	−0·16 ± 0·05*	−0·17 ± 0·08*	Dominant[e]	1·49 ± 0·66	0·48 ± 0·13*
C73	−0·67 ± 0·05*	0·11 ± 0·08	Additive[d]	−0·15 ± 0·57	0·15 ± 0·13
C80	−3·40 ± 0·17*	1·51 ± 0·19*	Incomplete recessive[g]	—	2·31 ± 0·18*

[a] In phenotypic standard deviation units of females ($\sigma = 2{\cdot}14$).
[b] Difference between the mean bristle score of males from the reciprocal F_1 crosses between each line and the control (described in the text).
[c] Difference between the F_{5-8} mean bristle score and its Hardy–Weinberg expectation (described in the text).
[d] Not significantly different from zero and significantly different from $|a|$.
[e] Significantly different from zero and not significantly different from a.
[f] Not significantly different from zero and $-a$.
[g] Significantly different from zero and $-a$.
[h] Significantly different from zero and not significantly different from $-a$.
[i] Not significantly different from zero and a.
* $P < 0{\cdot}05$ based on the sequential Bonferroni test (Rice, 1989).

In some lines, two (11^- and D^+) or three (B^+) mutations were individually studied.

The differences Δ between the performance of the F_6 crosses between each line and the control and their Hardy–Weinberg expectations are also shown in Table 3. A significant discrepancy was found in only two instances. However, all F_6 means were closer to the control value than expected. This result suggests that most mutations were less fit than the original allele.

(ii) *Inbred lines*

The distribution of the means of the inbred lines are shown in Fig. 1. The variance and the coefficients of asymmetry and kurtosis are given in Table 1. The distribution was significantly leptokurtic in both sets of lines, even when the analysis was restricted to those shown to carry mutations. It was also asymmetric but of variable sign, negative for B lines and positive for C lines. This last result can be wholly ascribed to a single mutation of very large effect (C59). The data did not significantly depart from normality after excluding those from lines shown to carry mutations.

Further analysis was restricted to the 26 lines showing maximum divergence from the overall performance of their groups. Assuming that a single mutation affecting bristle number was fixed in each line, estimates of additive and dominant effects were obtained and are given in Table 4. They are subjected to the same restrictions of those calculated for the selected lines above. In four cases (not shown), the additive effect was very small ($< 0{\cdot}03\sigma$ in absolute value) and not significant. Nevertheless, its sign was the same found in three previous evaluations, suggesting that these effects may be real. These results also indicate that a suitable criterion was used to identify lines carrying mutations. At the other end, the effect exceeded σ in three instances. In the remaining 19 lines, the divergence from the control line ranked between 0·1 and $0{\cdot}7\sigma$, in absolute value.

All mutations of very large effect ($> \sigma$) were incomplete recessives, one of them increasing and the remaining two decreasing bristle number. Among the rest, nine were additive, four complete recessive and three complete dominant. In the three remaining cases, the effect of mutations was small ($< 0{\cdot}3\sigma$) and gene action could not be unequivocally established, ranging from additive to dominant or recessive. No indication of directional dominance was found and dominant effects were as common as recessive.

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

Table 5. *Differential effects by sex of mutations detected in inbred lines*[a]

	Positive effects			Negative effects	
Line	Females	Males	Line	Females	Males
B40	0·81 ± 0·16*	0·69 ± 0·17*	B24	−0·28 ± 0·17	−0·53 ± 0·18*
B62	0·71 ± 0·15*	−0·03 ± 0·15	B29	−1·22 ± 0·21*	−0·62 ± 0·18*
B65	0·68 ± 0·17*	0·37 ± 0·17	B52	−0·30 ± 0·17	−0·44 ± 0·17*
B72	0·55 ± 0·18*	−0·05 ± 0·16	B64	−0·27 ± 0·16	−0·71 ± 0·15*
B80	0·69 ± 0·19*	0·23 ± 0·15	B79	−1·97 ± 0·14*	−2·25 ± 0·15*
C58	0·28 ± 0·15	0·24 ± 0·15	B93	−0·31 ± 0·16	−0·80 ± 0·16*
C59	9·13 ± 0·20*	8·32 ± 0·16*	C10	−0·28 ± 0·15	−0·03 ± 0·15
C67	0·50 ± 0·15*	0·90 ± 0·15*	C24	−0·69 ± 0·20*	−0·53 ± 0·20*
C90	0·01 ± 0·14	0·13 ± 0·15	C40	−0·98 ± 0·16*	0·06 ± 0·15
C91	−0·20 ± 0·15	0·02 ± 0·16	C55	0·04 ± 0·16	0·06 ± 0·15
			C73	−0·70 ± 0·16*	−0·69 ± 0·15*

[a] Deviations from control in phenotypic standard deviation units (2·14 or 1·98 bristles for females or males, respectively).
* $P < 0·05$ based on the sequential Bonferroni test (Rice, 1989).

The differences Δ between the performance of the F_{5-8} crosses and their expected values at Hardy–Weinberg equilibrium, are also given in Table 4. Only those lines showing a significant effect on bristles were considered. Those differences only reached significance in 5 out of 22 cases. In seven additional cases, the sign of Δ was that expected when the mutant allele is the less fit (positive for mutations decreasing bristle number and negative otherwise). These 12 cases included most mutations with negative effect, all those with an effect larger than $0·5\sigma$ (irrespective of their sign) and all but one recessive mutations (complete or incomplete). In the remaining instances (10/22), the sign of Δ suggested that the mutant allele had a selective advantage over the original allele (although not necessarily over those segregating in natural populations). This latter group embraced additive mutations of relatively small effects ($< 0·5\sigma$) and non-additive mutations of even smaller effects ($< 0·2\sigma$).

(iii) *Differential effects of mutations by sex*

All mutations detected in selected lines significantly affected both sexes. Typically, the mean bristle number of carrier lines deviated from control was higher in females (in absolute value), irrespective of the direction of selection. In those lines, the variance of bristle number in the last three generations was partitioned into sources arising from variation between sexes, generations, lines and their corresponding interactions. A significant sex-by-line interaction was found, indicating that the effects of the mutations involved were not proportional in males and females.

The behaviour of mutations isolated in inbred lines was different. Lines carrying mutations were identified by their effects on females (Table 4), though results from a later evaluation on both sexes (generation 87) are given in Table 5 (75 individuals of each sex scored per line). There was good agreement between the effects on females found in both evaluations, even though the significance of the smallest ($< 0·3\sigma$) could not be shown in the latter test due to its lower power. There was a clear tendency for positive mutations to have smaller or no effects on males. However, negative mutations affected both sexes and their effects on males were often larger. On the whole, the results indicate that mutations in inbred lines differentially affected sex dimorphism in the expression of the trait according to their sign.

No significant differences (D) were found between the mean bristle number of males from the two reciprocal F_1 crosses between a line (inbred or selected) and the control (Tables 3 and 4). This result implies that none of the mutations involved were located in the X chromosome.

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

(iv) *Estimates of mutational heritability*

For each group of inbred lines, the variance was partitioned, separately for each sex, into sources arising from variation between generations (fixed effect), between lines (V_L), generation × line interaction, and within lines (V_W). The interaction components were not significantly different from zero, so the mean squares corresponding to interaction and variation within lines were pooled.

Starting from a completely homozygous population and assuming that all mutations are neutral, additive and of small effect, the mutational variance (σ_m^2) can be obtained from

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

(Lynch & Hill, 1986), where t is generation number and N is effective population size. The mutational heritability was calculated as σ_m^2/V_W. An approximate

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

Table 6. *Estimates of between-line (V_L) and within-line (V_W) components of variance, and mutational heritabilities (h^2_m)*

Type of line (sex)	$V_L(\times 10^{-2})$	V_W	$h^2_m(\times 10^{-5})$
B (male)	$1{\cdot}10 \pm 0{\cdot}20$	$3{\cdot}73 \pm 0{\cdot}08$	$2{\cdot}5 \pm 0{\cdot}5$
B (female)	$1{\cdot}37 \pm 0{\cdot}24$	$4{\cdot}53 \pm 0{\cdot}10$	$2{\cdot}6 \pm 0{\cdot}4$
C (female)	$19{\cdot}18 \pm 2{\cdot}91$	$4{\cdot}64 \pm 0{\cdot}07$	$33{\cdot}8 \pm 0{\cdot}5$
C (female)[a]	$0{\cdot}96 \pm 0{\cdot}17$	$4{\cdot}55 \pm 0{\cdot}07$	$1{\cdot}7 \pm 0{\cdot}3$

[a] Lines carrying mutations with an effect larger than 3σ excluded from the analysis (C59 and C80).

standard error was computed from standard ANOVA techniques, as used by Mackay *et al.* (1992*b*).

Estimates of the between- and within-line components of variance, as well as those of mutational heritabilities are listed in Table 6 for both types of lines. In all cases, no significant differences were detected between the values of these three parameters, once data from lines carrying mutations of very large effects ($> 3\sigma$) were excluded from the analysis (lines C59 and C80). In the B lines, the mutational heritability was not significantly different for the two sexes.

4. Discussion

The two methods used to characterize the joint distribution of mutant effects (inbreeding and selection), could *a priori* result in partially overlapping descriptions, depending on the differential genetic properties of the mutations involved.

First, the fixation probability of neutral mutations is independent of gene action in the inbred lines but not in the selected lines. This discrepancy will not be important for those mutations having an effect in the heterozygote. However, recessive mutations will rarely be fixed by mass selection unless their effect is large and/or population size small. Thus, only one recessive mutation (out of 30) has been isolated in the selected lines and had a very large effect ($2{\cdot}1\sigma$). In contrast, four complete recessive mutations (out of 22) were detected in the inbred lines and their effects were smaller ($< 0{\cdot}7\sigma$).

Second, the chance of fixation of neutral mutations is independent of their effects in the inbred lines but not in the selected lines. Therefore, the description of the distribution of mutant effects obtained from selection results will be biased in favour of major mutations. However, this procedure will be more efficient than inbreeding. Thus, 30 mutations were found in 40 lines after 47 generations of selection, but only 22 mutations in 200 lines after 67 generations of inbreeding.

Third, the fixation probability of deleterious mutations will generally be lower in the inbred lines, as directional selection antagonizes natural selection more effectively than drift.

Thus, although both methods are biased, selection gives a better representation of the original distribution of mutant effects, the description obtained from inbreeding being closer to the genetic variation in natural populations after selection acts. Of course, the last set of mutations is part of the former.

Similar numbers of mutations with positive and negative effects were found in both experiments (16 and 14 in the selected lines and 10 and 12 in the inbred lines, respectively). However, the sign of the asymmetry of the distribution of line means oscillated widely. In the selected lines, it was negative during the first three-quarters of the experiment and positive afterwards. In the inbred lines, it was negative in one set of lines (B) and positive in the other (C). In parallel, the distribution of mutant effects (original or standing) is likely to be leptokurtic, since the coefficient of kurtosis of the distribution of the means of the lines shown to carry mutations (selected or inbred), was significantly larger than zero. Our information is mainly restricted to the tails of the distribution of the means of the lines and, therefore, may lead to underestimates of the true kurtosis of the distribution of mutant effects. However, mutations with an effect as small as $0{\cdot}1\sigma$ were detected. Thus, the data are compatible with a leptokurtic but symmetrical distribution of effects, asymmetry of fluctuating sign being essentially due to sampling. In both experiments, mutations decreasing bristles were in general less fit. This is in agreement with a lower reproductive capacity being observed in lines selected for low bristle number (Clayton & Robertson, 1957; Latter & Robertson, 1962).

In the selected lines, we have identified 25 lines carrying at least 30 mutations affecting bristles. A considerable fraction were lethals. In general, non-lethal mutations had smaller effects on the metric trait, were predominantly additive and, at most, slightly deleterious. In parallel, 21 mutations were detected in the inbred lines. Those with large effects were usually recessive and detrimental. The rest were generally additive and quasi-neutral, non-additive mutations in this group having very small effects. Thus, both methods identify a quasi-neutral class of mutations, essentially including those with not too large additive effects and non-additive of small effect.

https://doi.org/10.1017/S0016672300031220 Published online by Cambridge University Press

This class should be close to that responsible for standing variation in natural populations.

The complementary class of deleterious mutations included most recessives (complete or incomplete) and those with a large effect on the trait (irrespective of their gene action). This kind of variation usually appears in long-term selected lines at unstable plateaux determined by antagonism between natural and artificial selection forces. It is considered to be due to mutations occurring in the course of the selection process (see Mackay, 1990, and Caballero *et al.* 1991, for reviews). The distribution of effects of P-element-induced insertions on abdominal bristle number and viability has been studied by Mackay, Lyman & Jackson (1992*a*). They found this distribution to be negatively skewed and leptokurtic. Furthermore, the effects of extreme lines were mostly recessive but some of them were close to additive. As P-element insertions considerably reduced viability, the previous description is particularly informative on the subset of deleterious mutations affecting bristle score. These results are consistent with those obtained in the present experiment.

Assuming that all mutations are neutral, additive and of small effect, estimates of the mutational heritabilities from response to selection and from divergence among inbred lines should be the same. Nevertheless, many mutations affecting the metric trait also had pleiotropic effects on fitness. Therefore, their probability of fixation in inbred lines will be smaller and, at one end, the extreme lethal class will obviously not be represented. Thus, our estimate from selection data [$(5{\cdot}2 \pm 0{\cdot}9) \times 10^{-4}$; López & López-Fanjul, 1993] exceeded that from inbreeding by an order of magnitude (average: $(2{\cdot}8 \pm 0{\cdot}3) \times 10^{-5}$). This indicates that the total rate of production of spontaneous mutational variance per generation is substantially larger than that corresponding to quasi-neutral mutations. Our estimates are both lower than those previously reported from selection (Hill, 1982; Lynch, 1988) or inbreeding experiments (Mackay *et al.* 1992), perhaps as a reflection of differences in the genetic background of the stocks assayed.

Most mutations increased sexual dimorphism in the expression of the trait. Those in selected lines affected both sexes, but usually had larger effects on the female. However, those isolated in inbred lines tended to have larger effects on the female, when increasing bristles, and on the male otherwise. In principle, this disparity can be attributed to differences in the procedures of detection used, as selection was carried out on both sexes but inbred lines carrying mutations were identified on the basis of female data. Thus, the fixation probability of a mutation in the inbred lines will be independent of its differential effect by sex, but those affecting both sexes will be more easily fixed by selection. Similar results have been reported by Mackay *et al.* (1992*a*) and Mackay *et al.* (1992*b*), studying the effects of P-element induced and spontaneous mutations on abdominal bristle number, respectively. As a possible explanation, Mackay and coworkers hypothesized that genes involved in sex determination with pleiotropic effects on bristle number, might be responsible for sex dimorphism in the expression of this trait.

Our results do not support the classical infinitesimal model of a large number of equivalent loci. Rather, they are in agreement with a leptokurtic distribution of mutational effects (Robertson, 1967). Evidence from other characters and species points in the same direction (see Piper & Shrimpton, 1989, for a review). Excluding mutations with extreme effects on the metric trait (all unconditionally deleterious), no consistent relationship was found between the effects of mutations on bristles and fitness, i.e. mutations with similar effects on the trait may have all sorts of pleiotropic effects on fitness, ranging from lethal to quasi-neutral. Therefore, our data are consistent with a model of natural selection acting on bristles through the pleiotropic effects of pertinent loci on fitness (Robertson, 1967). This will result in apparent stabilizing selection (Barton, 1990; Keightley & Hill, 1990). Our observations suggest that genetic variation for bristles in populations is unlikely to be seriously constrained by direct stabilizing selection balanced with mutation and drift. On the contrary, neutral additive alleles of considerable effect can be found segregating at intermediate frequencies in natural populations, contributing a large fraction of the total variance. This is in agreement with the current view of abdominal bristle number being a neutral additive trait (Robertson, 1967; Latter & Robertson, 1962; López-Fanjul, Guerra & García, 1989).

We wish to thank A. Caballero, A. Gallego, A. García-Dorado, W. G. Hill, T. F. C. Mackay and M. A. Toro for helpful comments on the manuscript. This work was supported by a grant from the Comisión Interministerial de Ciencia y Tecnología (GAN90-0096).

References

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

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.

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.

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

Hill, W. G. (1990). Mutation, selection and quantitative variation. In *Population Biology of Genes and Molecules* (ed. N. Takahata and J. F. Crow), pp. 219–232. Baifukan.

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. (1987). Directional selection and variation in finite populations. *Genetics* **117**, 573–582.

Keightley, P. D. & Hill, W. G. (1988). Genetic variability maintained by mutation-stabilizing selection balance in finite populations. *Genetical Research* **52**, 33–43.

Keightley, P. D. & Hill, W. G. (1990). Variation maintained in quantitative traits with mutation-selection balance: pleiotropic side effects on fitness traits. *Proceedings of the Royal Society of London* B242, 95–100.

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*. I. Response to artificial selection. *Genetical Research* **61**, 107–116.

López-Fanjul, C., Guerra, J. & García, A. (1989). Changes in the distribution of the genetic variance of a quantitative trait in small populations of *Drosophila melanogaster*. *Genetics, Selection, Evolution* **21**, 159–168.

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

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

Mackay, T. F. C. (1990). Distribution of effects of new mutations affecting quantitative traits. In *Proceedings of the 4th World Congress on Genetics Applied to Livestock Production* (ed. W. G. Hill, R. Thompson and J. A. Woolliams), pp. 219–228.

Mackay, T. F. C., Lyman, R. F. & Jackson, M. S. (1992*a*). Effects of P element insertions 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*b*). Polygenic mutation in *Drosophila melanogaster*: estimates from divergence among inbred strains. *Evolution* **46**, 300–316.

Piper, L. R. & Shrimpton, A. E. (1989). The quantitative effects of genes which influence metric traits. In *Evolution and Animal Breeding* (ed. W. G. Hill and T. F. C. Mackay), pp. 147–151. Wallingford, UK: C.A.B. International.

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

Robertson, A. (1967). The nature of quantitative genetic variation. In *Heritage from Mendel* (ed. 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.