

Temporal uniformity of the spontaneous mutational variance of quantitative traits in *Drosophila melanogaster*

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Summary

Spontaneous mutations were allowed to accumulate over 209 generations in more than 100 lines, all of them independently derived from a completely homozygous population of *Drosophila melanogaster* and subsequently maintained under strong inbreeding (equivalent to full-sib mating). Traits scored were: abdominal (AB) and sternopleural (ST) bristle number, wing length (WL) and egg-to-adult viability (V). On two occasions – early (generations 93–122) and late (generations 169–209) – ANOVA estimates of the mutational variance and the mutational line \times generation interaction variance were obtained. Mutational heritabilities of morphological traits ranged from 2×10^{-4} to 2×10^{-3} and the mutational coefficient of variation of viability was 0.01. For AB, WL and V, temporal uniformity of the mutational variance was observed. However, a fluctuation of the mutational heritability of ST was detected and could be ascribed to random genotype \times environment interaction.

1. Introduction

For quantitative traits, the amount of variation produced each generation by mutation (mutational variance, σ_m^2) is a fundamental parameter of the models analysing the maintenance of genetic variability in populations and the response to selection. To compare σ_m^2 values, dimensionless quantities are obtained by scaling, either with the environmental variance of the trait σ_e^2 (mutational heritability, $h_m^2 = \sigma_m^2/\sigma_e^2$) or with the trait mean (mutational coefficient of variation, $CV_m = \sigma_m/\bar{X}$). For spontaneous mutation in *Drosophila melanogaster*, a recent review by Houle *et al.* (1996) indicated higher CV_m values for life-history traits (average: 0.021, range: 0.009–0.045) than for morphological traits (average: 0.004, range: 0.001–0.012). On the other hand, no clear difference in h_m^2 between classes of traits was detected (average: 5×10^{-3} ; range: 10^{-4} – 10^{-2}).

Many of those σ_m^2 estimates have been obtained from the divergence between unselected lines, independently derived from an inbred or isogenic base population, after t generations of mutation accumulation. In all cases, neutral mutations with non-

epistatic effects on the metric trait have been assumed to occur uniformly through time. In this situation, the steady rate of increase of the between-line variance per generation equals $2\sigma_m^2$ (Lynch & Hill, 1986). For *Drosophila* abdominal and sternopleural bristle number, no significant departure from a linear temporal increase of the between-line variance was detected during an initial period of about 50 generations (Santiago *et al.*, 1992; López & López-Fanjul, 1993). However, when a longer time horizon was considered (over 100 generations), Mackay *et al.* (1995) found that the between-line variance did not increase substantially and the corresponding σ_m^2 estimate actually declined with time. This result was mainly ascribed by the authors to deleterious effects (direct or pleiotropic) of new mutations affecting bristle number and/or to diminishing epistatic effects on the trait of the mutations involved.

In this paper, we test for the generality of the temporal decline of σ_m^2 reported by Mackay *et al.* (1995). To do that, we compared estimates of σ_m^2 obtained from a set of *D. melanogaster* inbred lines derived from the same isogenic base population, after 93–122 (early evaluations) and 169–209 (late evaluations) generations of mutation accumulation. We considered a fitness-component trait (egg-to-adult

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viability) as well as three morphological traits (wing length and the two bristle traits studied by Mackay *et al.*, 1995). Results corresponding to early evaluations have already been published (Fernández & López-Fanjul, 1996; Monedero *et al.*, 1997; García-Dorado & Marín, 1998) but those for late evaluations have not been reported previously.

2. Materials and methods

(i) Base population, inbred lines and control

The *D. melanogaster* line isogenic for all chromosomes obtained by Caballero *et al.* (1991) was used as the base population. From this, 200 inbred lines were started and subsequently maintained under strong inbreeding (equivalent to full-sib mating). Spare matings were used when the first failed to reproduce (for experimental details see Santiago *et al.*, 1992; Fernández & López-Fanjul, 1996). Some lines were lost at different times and, by generation 209, only 111 lines survived. The isogenic line carried the recessive eye-colour marker *sepia* (*se*) in chromosome III, as an indicator of possible contamination from exogenous flies. It was maintained as a control (synchronous to the lines) in eight bottles and a circular mating scheme was used to ensure a large population size, sufficient to allow the elimination of most mutations (about 800 potential parents per generation).

(ii) Culture conditions and traits scored

Flies were reared in the standard medium formula of this laboratory (brewer's yeast–agar–sucrose). All cultures were incubated at 25 ± 1 °C and 40 ± 10 % relative humidity and maintained under continuous lighting. Flies were handled at room temperature under CO₂ anaesthesia. Each inbred line was main-

tained in a glass vial (20 mm diameter, 100 mm height) with 100 ml medium added.

Traits considered were: (1) the total number of bristles on the fifth and sixth sternites (AB), (2) the total number of bristles on the right and left sternopleural plates (ST), (3) the length of the right wing (WL), and (4) egg-to-adult viability (V) measured as the proportion of adults emerging from one day's egg-laying of a single female (for experimental details see Santiago *et al.*, 1992; López & López-Fanjul, 1993; Fernández & López-Fanjul, 1996). All traits were individually scored on females from the lines and generations specified in Table 1. Samples of 10 (AB, ST and WL) or 4 or 5 (V) females per line and generation were used. Line C59 had a very large effect on AB and ST (about 15 and 6 σ_e , respectively) and was excluded from the analyses concerning bristle traits.

A number of single-pair matings between control flies were also established in individual vials and ten progeny females per mating were scored for morphological traits (generations 96–97: 100 matings per generation scored for WL; generations 99–100: 100 matings per generation scored for AB; generations 203–204: 20 matings per generation scored for AB and ST). The control average viability in generations 104–106 was inferred from the values obtained at generations 109 and 116, after adjusting for environmental effects (for detail see García-Dorado, 1997). Unfortunately, control single-pair matings established at generations 208–209 were affected by a bacterial infection and V could not be evaluated. However, the control line could be continued from larvae treated with ethanol.

(iii) Parameter estimation

Using data from two or three consecutive generations, two-way ANOVA were carried out (random main effects: line and generation). Thus, the variance was partitioned into sources arising from variation between generations, between lines (σ_b^2), generation \times line interaction ($\sigma_{1 \times t}^2$) and within lines. The mutational variance σ_m^2 was estimated by $\sigma_b^2/2F_t$, where F_t is the forward cumulative inbreeding coefficient of the lines at generation t . The interaction component of variance ($\sigma_{1 \times t}^2$) can be ascribed to mutational effects varying between consecutive generations ($\sigma_{g \times t}^2$, a type of genotype \times environment interaction) and/or to environmentally caused between-vial differences (σ_{ec}^2 , common environmental variance). Thus, $\sigma_{1 \times t}^2 = \sigma_{g \times t}^2 + \sigma_{ec}^2$ and a separate estimation of these two components could be made only when synchronous evaluation of the control was available, allowing us to obtain an independent estimate of σ_{ec}^2 .

Table 1. Number of lines evaluated at specified generations for each trait

Trait	Early evaluations		Late evaluations	
	Generation	No. of lines	Generation	No. of lines
AB	93–94; 99–100	154 ^a ; 171 ^a	203–204	116 ^d
ST	119–120–122	170 ^b	203–204	116 ^d
WL	93–94; 96–97	153 ^a ; 172 ^a	169–170	148 ^d
V	104–106	176 ^c	208–209	111 ^d

^a García-Dorado & Marín (1998).

^b Monedero *et al.* (1997).

^c Fernández & López-Fanjul (1996).

^d This paper.

3. Results

(i) Mutational parameters

ANOVA estimates of the mutational heritability of morphological traits and of the squared mutational coefficient of variation of viability, obtained in different generations, are shown in Table 2. Over the period of mutation accumulation considered, scaled σ_m^2 estimates remained constant (AB and V) or increased (WL) and only for ST was a significant temporal decrease detected. For earlier periods of mutation accumulation, the h_m^2 of morphological traits was computed in the same set of inbred lines, evaluated in this laboratory (AB: 0.63×10^{-3} , generations 60–62 and 65–67; López & López-Fanjul, 1993) or a different laboratory (ST: 0.76×10^{-3} , WL: 2.0×10^{-3} , generations 0–46; Santiago *et al.*, 1992). These estimates do not differ substantially from those in Table 2.

The mean viability of the lines (relative to that inferred for the control line at generations 104–106) was 0.897 (gen. 104), 0.798 (gen. 105), 0.892 (gen. 106), 0.858 (gen. 208) and 0.797 (gen. 209). Therefore, over the 103 generation period considered, the mutational decline of viability was not significant (0.00034 ± 0.00046). However, this may be an artefact, as accumulation of deleterious mutations will increase the chance of loss of specific lines. During the first 50 generations, about one line was lost every three generations. Assuming this to be the rate of accidental loss, the expected (observed) numbers of surviving lines at generations 104 and 208 were, respectively, 168 (176) and 141 (111). Thus, in the late evaluation, about $30/141 = 0.21$ of the lines could have been lost because of mutation. Assigning zero viability to those lines, the above-mentioned mutational decline could be increased by $0.21/208 = 0.001$, giving an upper bound estimate of 0.0013 (equivalent to a total relative viability decline of 0.27 over the whole experiment). In spite of line losses, CV_m estimates for viability did not change with time. This may also be an artefact, as

Table 3. Line \times generation ($\sigma_{1 \times t}^2$) and genotype \times generation interaction ($\sigma_{g \times t}^2$ in italics) for different traits and generations (as a percentage of the between-line variance)

Generation	Trait			
	AB	ST	WL	V
t_1^a	10	—	88	—
t_2^a	36 (36)	7.3	179 (176)	33
t_3^c	31 (31)	40 (0)	135	50

^a t_1 = generations 93–94 (AB and WL), 104–106 (V) and 119–120–122 (ST).

^b t_2 = generations 96–97 (WL) and 99–100 (AB).

^c t_3 = generations 169–170 (WL), 203–204 (AB and ST) and 208–209 (V).

synergistic epistasis of the mutations involved could result in a temporal increase of the between-line divergence, compensating for line losses. A rise of the between-line variance was interpreted by Mukai (1969) as evidence for synergistic epistasis between viability mutations. However, instability of transposable element copy number has also been proposed as an alternative explanation of that result (Keightley, 1996).

(ii) Mutational genotype–environment interaction

Considerable experimental effort was made to minimize the spatio-temporal variation of environmental agents known to affect the traits considered (temperature, relative humidity, food composition, etc.). However, uncontrolled environmental factors could also vary and, presumably, they will not be the same in different generations. This may generate random genotype–environmental interaction resulting in fluctuating parameter values. Table 3 gives the line \times generation interaction variance component $\sigma_{1 \times t}^2$

Table 2. ANOVA σ_m^2 ($\times 10^3$) estimates^a for different traits at specified generations (see text for explanation)

Generation	Trait			
	AB	ST	WL	V
t_1^b	0.49 \pm 0.11	0.61 \pm 0.09	1.07 \pm 0.26	0.12 \pm 0.02
t_2^c	0.48 \pm 0.11	—	0.62 \pm 0.17	—
t_3^d	0.42 \pm 0.10	0.21 \pm 0.05	2.09 \pm 0.45	0.13 \pm 0.02

^a Scaled by the environmental variance ($h_m^2 = \sigma_m^2/\sigma_e^2$: AB, ST and WL) or by the square of the mean ($CV_m^2 = (\sigma_m/\bar{x})^2$: V).

^b t_1 = generations 93–94 (AB and WL), 104–106 (V) and 119–120–122 (ST).

^c t_2 = generations 96–97 (WL) and 99–100 (AB).

^d t_3 = generations 169–170 (WL), 203–204 (AB and ST) and 208–209 (V).

for different evaluations and traits, expressed as a proportion of the corresponding between-line component of variance (σ_1^2). Estimates range from 7% to 179%, the largest corresponding to WL. Nevertheless, these values just provide upper bounds for the genotype \times environment component of variance $\sigma_{\text{g} \times \text{t}}^2$ (that due to mutational effects varying across consecutive generations). For AB and WL, estimates of $\sigma_{\text{g} \times \text{t}}^2$ could be obtained and are also given in Table 3, as proportion of σ_1^2 . Comparisons of $\sigma_{1 \times \text{t}}^2$ and $\sigma_{\text{g} \times \text{t}}^2$ indicates that, in practice, all $\sigma_{1 \times \text{t}}^2$ can be attributed to genotype \times environment interaction. In these conditions, h_m^2 for mutational effects expressed at single generations should be much larger than those corresponding to the average mutational effects over generations (about twice or threefold for WL and about 30% larger for AB; for details see García-Dorado & Marín, 1998). On the other hand, no $\sigma_{\text{g} \times \text{t}}^2$ was detected for ST. In this case, $\sigma_{1 \times \text{t}}^2$ can be entirely attributed to common environmental factors. Excluding lines of large effect from the analysis, the covariance between the ST means of the lines at the early and late evaluations (0.03) was 56% of the early between-line variance. This proportion indicates the extent to which mutations with moderate effect on ST have the same expression at evaluations separated by a long period of time. Thus, when environments from generations long apart were considered, mutational effects for ST also showed important genotype \times environment interaction. This suggests that the environmental factors responsible for genotype \times environment interaction may differ for each trait.

4. Discussion

Using data from the same set of inbred lines, early (generation 93–122) and late (generation 169–209) estimates of the σ_m^2 of four quantitative traits were compared. For AB, WL and V, no temporal decrease in σ_m^2 was detected, and a significant reduction could be established only for ST. For morphological traits, temporal homogeneity of σ_m^2 values could also be extended to the mutation accumulation period previous to that considered in this paper ($t < 67$), although estimates for ST and WL were obtained in a different laboratory.

There are four potential, not mutually exclusive, explanations for the discrepant behaviour of ST. On the one hand, estimates of the mutational input of variance may decline with time, due to: (1) deleterious effects on fitness (direct or pleiotropic) of the mutations affecting ST, so that natural selection will reduce the rate of mutation accumulation; and (2) diminishing epistatic effects on ST, implying that individual mutational effects decrease as mutations accumulate. On the other hand, σ_m^2 estimates obtained at different times are likely to fluctuate, due to: (3)

genotype–environment interaction of the mutations involved; and (4) sampling error.

In the early evaluation, seven lines had a relatively large effect on ST (of about σ_e). By the late evaluation, three of those lines were lost (a rate of loss similar to that of the remaining lines) and the effect of the other four, although of the same sign, did not reach significance. Excluding those lines from the analyses, much smaller and temporally unchanged mutational heritabilities were obtained (generation 119–122: $(0.22 \pm 0.05) \times 10^{-3}$; generation 203–204: $(0.23 \pm 0.08) \times 10^{-3}$). By the time of the early evaluation, direct natural selection on the trait had not prevented fixation of mutations of large effect on ST. Thus, it seems unlikely that the loss of effect observed in the late evaluation could have been caused by selection favouring the fixation of new mutations of opposite sign in the pertinent lines. Furthermore, losses of lines with large effect on ST (37%) were similar to total losses (32%) and, therefore, they do not seem related to line effects on ST. In parallel, significant pleiotropic effects on fitness were not detected for individual mutations of moderate effect on ST ($a < \sigma_e/2$; Santiago *et al.*, 1992). On the whole, the results suggest that the observed temporal fluctuation in h_m^2 for ST can be attributed to changes in the expression of mutations of large effect on this trait, i.e. to genotype \times environment interaction affecting mutations of large effect, those making a greater contribution to h_m^2 .

After 100 generations of mutation accumulation, a decelerated increase of the between-line variance for AB and ST has been reported by Mackay *et al.* (1995). This was explained in terms of deleterious effects on fitness of bristle mutations and/or duplicate epistasis between those mutations. Nevertheless, after 180 generations of mutation accumulation, the average competitive fitness of the lines was only about 30% below that of their corresponding F1 crosses, giving a per generation rate of decline of 0.0017 (Nuzhdin *et al.*, 1995). Moreover, evidence for epistatic effects on bristle number is very scarce and, when found, those effects were small relative to the additive variance of the base population (Shrimpton & Robertson, 1988). On the other hand, the rank order of the Mackay *et al.* (1995) lines changed constantly throughout the entire experiment and common environmental effects were also found, suggesting genotype \times environment interaction as an additional explanation of the results. In the present experiment, genotype \times environment interaction can be wholly ascribed to random uncontrolled environmental agents, which may result in temporally fluctuating parameter values. The same set of mutation accumulation lines used in this experiment was evaluated for V in the standard and in three stressful media and a strong genotype \times environment interaction was reported, revealing a high degree of

specificity of the mutations involved (Fernández & López-Fanjul, 1997). Notwithstanding, mutational heritability and coefficient of variation estimates did not increase with intensified environmental harshness.

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References

- Caballero, A., Toro, M. A. & López-Fanjul, C. (1991). The response to artificial selection from new mutations in *Drosophila melanogaster*. *Genetics* **128**, 89–102.
- Fernández, J. & López-Fanjul, C. (1996). Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. *Genetics* **143**, 829–837.
- Fernández, J. & López-Fanjul, C. (1997). Spontaneous mutational genotype–environment interaction for fitness-related traits in *Drosophila melanogaster*. *Evolution* **51**, 856–864.
- García-Dorado, A. (1997). The rate and effects distribution of validity mutation in *Drosophila*: minimum distance estimation. *Evolution* **51**, 1130–1139.
- García-Dorado, A. & Marín, J. M. (1998). Minimum distance estimation of mutational parameters for quantitative traits. *Biometrics* **54**, 214–230.
- Houle, D., Morikawa, B. & Lynch, M. (1996). Comparing mutational variabilities. *Genetics* **143**, 1467–1483.
- Keightley, P. D. (1996). Nature of deleterious mutation load in *Drosophila*. *Genetics* **144**, 1993–1999.
- López, M. A. & López-Fanjul, C. (1993). Spontaneous mutation for a quantitative trait in *Drosophila melanogaster*. II. Distribution of mutant effects on the trait and fitness. *Genetical Research* **61**, 117–126.
- Lynch, M. & Hill, W. G. (1986). Phenotypic evolution by neutral mutation. *Evolution* **40**, 915–935.
- Mackay, T. F. C., Lyman, R. F. & Hill, W. G. (1995). Polygenic mutation in *Drosophila melanogaster*: non-linear divergence among unselected strains. *Genetics* **139**, 849–859.
- Monedero, J. L., Chavarrias, D. & López-Fanjul, C. (1997). The lack of mutational variance for fluctuating asymmetry in *Drosophila melanogaster*. *Proceedings of the Royal Society of London, Series B* **264**, 233–237.
- Mukai, T. (1969). The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interaction of spontaneous mutant polygenes controlling viability. *Genetics* **61**, 749–761.
- Nuzhdin, S. V., Fry, J. D. & Mackay, T. F. C. (1995). Polygenic mutation in *Drosophila melanogaster*: the causal relationship of bristle number to fitness. *Genetics* **139**, 861–872.
- 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.
- Shrimpton, A. E. & Robertson, A. (1988). The isolation of polygenic factors controlling bristle score in *Drosophila melanogaster*. II. Distribution of third chromosome bristle effects within chromosome sections. *Genetics* **118**, 445–459.