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 *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 × generation interaction variance were obtained. Mutational heritabilities of morphological traits ranged from $2 \times 10^{-1}$ to $2 \times 10^{-3}$ 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 × environment interaction.

1. Introduction

For quantitative traits, the amount of variation produced each generation by mutation (mutational variance, $\sigma_m^2$) is a fundamental parameter of the models analysing the maintenance of genetic variability in populations and the response to selection. To compare $\sigma_m^2$ values, dimensionless quantities are obtained by scaling, either with the environmental variance of the trait $\sigma_e^2$ (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: $5 \times 10^{-3}$; range: $10^{-4}$–$10^{-2}$). Many of those $\sigma_m^2$ 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 $\sigma_m^2$ 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 $\sigma_m^2$ reported by Mackay et al. (1995). To do that, we compared estimates of $\sigma_m^2$ 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...
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 & Lopez-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 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.

(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 maintained 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(v), 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(τ)²), generation × line interaction (σ(g×τ)²) and within lines. The mutational variance σ(τ)² was estimated by σ(τ)²/2F, where F is the forward cumulative inbreeding coefficient of the lines at generation τ. The interaction component of variance (σ(g×τ)²) can be ascribed to mutational effects varying between consecutive generations (σ(g×τ)², a type of genotype × environment interaction) and/or to environmentally caused between-vial differences (σ(ec)², common environmental variance). Thus, σ(τ)² = σ(τ)⁴ + σ(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

<table>
<thead>
<tr>
<th>Trait</th>
<th>Early evaluations</th>
<th>Late evaluations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generation</td>
<td>No. of lines</td>
</tr>
<tr>
<td>AB</td>
<td>93–94; 99–100</td>
<td>154*; 171*</td>
</tr>
<tr>
<td>ST</td>
<td>119–120–122</td>
<td>170*</td>
</tr>
<tr>
<td>WL</td>
<td>93–94; 96–97</td>
<td>153*; 172*</td>
</tr>
<tr>
<td>V</td>
<td>104–106</td>
<td>176*</td>
</tr>
</tbody>
</table>

* Monedero et al. (1997).
* 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 $\sigma_{m}^2$ 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: $0.63 \times 10^{-3}$, generations 60–62 and 65–67; López & López-Fanjul, 1993) or a different laboratory (ST: $0.76 \times 10^{-3}$, WL: $2.0 \times 10^{-3}$; 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 ($0.9934 \pm 0.00046$). 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 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–environment interaction resulting in fluctuating parameter values. Table 3 gives the line × generation interaction variance component $\sigma_{l	imes g}^2$.

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

<table>
<thead>
<tr>
<th>Generation</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1}$</td>
<td>AB</td>
</tr>
<tr>
<td>$t_{4}$</td>
<td>10</td>
</tr>
<tr>
<td>$t_{5}$</td>
<td>36</td>
</tr>
<tr>
<td>$t_{6}$</td>
<td>(36)</td>
</tr>
<tr>
<td>$t_{7}$</td>
<td>31</td>
</tr>
<tr>
<td>$t_{8}$</td>
<td>(31)</td>
</tr>
</tbody>
</table>

$\sigma_{l	imes g}^2$ is the interaction variance component which reflects the between-line variation in fluctuating parameter values. The interaction variable is defined as a measure of the extent of the interaction between the lines and the environment. The interaction variable is computed as the product of the between-line variance and the environment variance. The interaction variable is used to measure the extent of the interaction between the lines and the environment. The interaction variable is computed as the product of the between-line variance and the environment variance. The interaction variable is used to measure the extent of the interaction between the lines and the environment.

Table 2. ANOVA $\sigma_{m}^2$ ($\times 10^3$) estimates for different traits at specified generations (see text for explanation)

<table>
<thead>
<tr>
<th>Generation</th>
<th>Trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1}$</td>
<td>AB</td>
</tr>
<tr>
<td>$t_{2}$</td>
<td>0.49 ± 0.11</td>
</tr>
<tr>
<td>$t_{3}$</td>
<td>0.48 ± 0.11</td>
</tr>
<tr>
<td>$t_{4}$</td>
<td>0.42 ± 0.10</td>
</tr>
</tbody>
</table>

$\sigma_{m}^2$ is the variance component which reflects the overall variation in fluctuating parameter values. The variance component is computed as the product of the between-line variance and the environment variance. The variance component is used to measure the extent of the overall variation in fluctuating parameter values. The variance component is computed as the product of the between-line variance and the environment variance. The variance component is used to measure the extent of the overall variation in fluctuating parameter values.
for different evaluations and traits, expressed as a proportion of the corresponding between-line component of variance ($\sigma^2_{g}$). Estimates range from 7% to 179%, the largest corresponding to WL. Nevertheless, these values just provide upper bounds for the genotype × environment component of variance $\sigma^2_{g\times e}$ (that due to mutational effects varying across consecutive generations). For AB and WL, estimates of $\sigma^2_{g\times e}$ could be obtained and are also given in Table 3, as proportion of $\sigma^2_{l}$. Comparisons of $\sigma^2_{g\times e}$ and $\sigma^2_{g}$ indicates that, in practice, all $\sigma^2_{g\times e}$ can be attributed to genotype × environment interaction. In these conditions, $h^2_{m}$ 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 $\sigma^2_{g\times e}$ was detected for ST. In this case, $\sigma^2_{g\times e}$ 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 × environment interaction. This suggests that the environmental factors responsible for genotype × 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 $\sigma^2_{m}$ of four quantitative traits were compared. For AB, WL and V, no temporal decrease in $\sigma^2_{m}$ was detected, and a significant reduction could be established only for ST. For morphological traits, temporal homogeneity of $\sigma^2_{m}$ 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, $\sigma^2_{m}$ 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 $\sigma_e$). 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 $h^2_{m}$ for ST can be attributed to changes in the expression of mutations of large effect on this trait, i.e. to genotype × environment interaction affecting mutations of large effect, those making a greater contribution to $h^2_{m}$.

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 × environment interaction as an additional explanation of the results. In the present experiment, genotype × 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 × environment interaction was reported, revealing a high degree of
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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


