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Regeneration of the variance of metric traits by spontaneous mutation in a *Drosophila* population

CARMEN AMADOR†, AURORA GARCÍA-DORADO, DIEGO BERSABÉ AND CARLOS LÓPEZ-FANJUL*
Departamento de Genética, Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain

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Summary

In the C_1 population of *Drosophila melanogaster* of moderate effective size (≈ 500), which was genetically invariant in its origin, we studied the regeneration by spontaneous mutation of the genetic variance for two metric traits [abdominal (AB) and sternopleural (ST) bristle number] and that of the concealed mutation load for viability, together with their temporal stability, using alternative selection models based on mutational parameters estimated in the C_1 genetic background. During generations 381–485 of mutation accumulation (MA), the additive variances of AB and ST approached the levels observed in standing laboratory populations, fluctuating around their expected equilibrium values under neutrality or under relatively weak causal stabilizing selection. This type of selection was required to simultaneously account for the observed additive variance in our population and for those previously reported in natural and laboratory populations, indicating that most mutations affecting bristle traits would only be subjected to weak selective constraints. Although gene action for bristles was essentially additive, transient situations occurred where inbreeding resulted in a depression of the mean and an increase of the additive variance. This was ascribed to the occasional segregation of mutations of large recessive effects. On the other hand, the observed non-lethal inbreeding depression for viability must be explained by the segregation of alleles of considerable and largely recessive deleterious effects, and the corresponding load concealed in the heterozygous condition was found to be temporally stable, as expected from tighter constraints imposed by natural selection.

1. Introduction

Mutation is the primary source of the genetic variability of populations, which will be subsequently shaped by selection and drift. The extent to which spontaneous mutation deteriorates fitness and its component traits, and introduces new variation for metric traits, has received considerable attention in recent years, and a considerable amount of work has been carried out to estimate the corresponding rates and effects of spontaneous mutations in mutation accumulation (MA) experiments (reviewed by García-Dorado *et al*., 1999, 2004; Keightley & Eyre-Walker, 1999; Lynch *et al*., 1999; Caballero & García-Dorado, 2006; Halligan & Keightley, 2009).

For neutral traits under additive genetic control, it has been theoretically shown that an equilibrium genetic variance $V_{A(MD)}=2N_eV_m$ will be attained, where N_e is the effective population size and V_m is the per generation mutational input of variance for the trait considered (Lynch & Hill, 1986). For a number of traits in different species, a typical value around $V_m=10^{-3}\ V_E$ has been obtained, where V_E is the corresponding environmental variance (see review by Houle *et al*., 1996). Therefore, theory implies that the pertinent heritability will tend to an equilibrium value of 1 as N_e increases. As this prediction contradicts the general observation of the heritability of metric traits ranging from 0·05 to 0·65 (Falconer & Mackay, 1996), natural selection must be continuously eroding the

† Present address: Departamento de Mejora Genética y Biotecnología, SGIT-INIA, Carretera de La Coruña km 7, 28040 Madrid, Spain.
* Corresponding author. e-mail: clfanjul@bio.ucm.es

genetic variability incessantly produced by mutation. On the other hand, natural selection is expected to impose strong constraints upon the genetic architecture of fitness and its component traits. Thus, when the equilibrium between mutation, selection and drift is attained (the so-called MSD balance), the amount of additive variance for those traits is expected to be small, but an important load can be concealed in the heterozygous condition due to the segregation of deleterious recessive alleles (Crow, 1993; Charlesworth & Hughes, 2000). Using simple analytical approximations (García-Dorado, 2007), the genetic properties at the MSD balance can be predicted as a function of the effective population size, the rate of deleterious mutation and the corresponding distributions of the homozygous and heterozygous effects. So far, however, the approach to the MSD balance has never been experimentally assessed in the light of mutational parameters estimated in the same genetic background.

In this paper, we present information on the regeneration of the MSD balance for two bristle traits and viability in a population of *Drosophila melanogaster* (C_1) maintained with an effective population size around 500, which was founded from an isogenic stock from which a long-term MA experiment had also been derived. This is the longest running MA experiment in a higher eukaryote, allowing estimation of mutational parameters over a long period, and hence with greater accuracy than ever before (see reviews by García-Dorado *et al*., 1999, 2004). By generation 250, previous results showed that this population was approximately at the MSD balance regarding viability, and indirectly suggested that it was also close to that balance for bristle traits, whose genetic variances were beginning to approach the values observed in segregating populations (García-Dorado *et al*., 2007). In this report, data collected at generations 473–485 were used to assess the temporal stability of such equilibrium, and to inquire further into the nature of both the forces involved and the architecture of the corresponding genetic variance. First, we estimated the inbreeding depression rate for viability and bristle traits, the genetic and environmental variance components of bristle number, and the redistribution of that genetic variance within and among inbred lines, aiming to assay the possible effect of transient segregation of recessive deleterious mutations on the temporal fluctuation of those parameters. Second, we developed alternative models for the pleiotropic side effects on fitness of mutations affecting bristle traits, based on the mutational parameters previously estimated in the long-term MA experiment that was carried out in the C_1 genetic background. Third, neutral and alternative selective models (causal versus apparent stabilizing selection) were used to obtain predictions for the equilibrium genetic variance of bristle traits and for the inbreeding depression rate of viability for a wide range of effective population sizes, and these were compared to our results and to those previously reported for wild and laboratory populations. Thus, present information expands and complements that corresponding to the C_1 population in earlier generations and reinforces the interpretation that was then put forward (García-Dorado *et al*., 2007).

2. Materials and methods

(i) *Base population*

The C_1 base population was started from a *D. melanogaster* line isogenic for all chromosomes, obtained by Caballero *et al*. (1991). This population was subsequently maintained during 485 generations with an effective size about 500, estimated at generation 385 by lethal complementation analysis (García-Dorado *et al*., 2007). The isogenic line carried the recessive eye-colour marker *sepia* (*se*) in the third chromosome as an indicator of possible contamination from wild-type flies. Care was taken to avoid contamination between different stocks carrying the *sepia* marker. The heritabilities of abdominal (AB) and sternopleural (ST) bristle number in the original isogenic stock did not significantly differ from zero (Caballero *et al*., 1991; López-Fanjul & Merchante, unpublished data).

(ii) *Culture conditions and traits scored*

Flies were reared in the standard medium formula of this laboratory (Brewer's yeast-agar-sucrose). All cultures described below were incubated in vials (20-mm diameter, 100-mm height, with 15 ml medium added) at 25 ± 1 °C, 45 ± 5% relative humidity, and maintained under continuous lighting. Flies were handled at room temperature under CO_2 anesthesia.

Bristle number was computed as the sum on the 4th and 5th abdominal sternites in males or the 5th and 6th in females (AB), or on the right and left ST plates, which were separately recorded in both instances.

The viability of wild-type chromosomes II sampled from the C_1 population was assayed using a crossing scheme with a Cy/L^2 balancer stock, as described in García-Dorado *et al*. (2007), and it was computed as the natural logarithm of the ratio of wild to Cy/L^2 numbers in the progeny of Cy/wild $\times$ L^2/wild crosses.

(iii) *Experiment 1: redistribution of the phenotypic variance with inbreeding*

At generation 459, 50 males and 50 virgin females were sampled from the C_1 population and they were mated in pairs in individual vials (generation 0). From

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the progeny emerging in each vial (generation 1), 50 inbred lines were initiated by a single brother × sister mating. Synchronously, 50 control lines were also started, each from a single pair mating sampled from the same population. Each line (inbred or control) was kept in a single vial. In the following generation (generation 2), the pertinent bristle trait (AB or ST) was scored in four or two offspring of each sex per inbred ($F=1/4$) or control line ($F=0$), respectively, where F is the inbreeding coefficient.

Assuming additive action within and between loci, the phenotypic variance of a metric trait (V_P) in a large panmictic population can be partitioned into additive genetic (V_A), common environmental (V_{Ec}, due to between family environmental differences) and non-common environmental (V_{Ew}, due to within family environmental differences) causal components of variance. Thus, in populations structured into full-sib families (as our control lines), the observational components of variance within (σ^2_w) and between families (σ^2_b) can be expressed in terms of the causal components as $\sigma^2_w = V_A/2 + V_{Ew}$ and $\sigma^2_b = V_A/2 + V_{Ec}$. If those families are inbred by an amount F (as our inbred lines), the within-line genetic component of variance is reduced by a factor $(1-F)$, so that, assuming that V_{Ec} and V_{Ew} do not change with inbreeding, the overall within-line variance is given by $\sigma^2_{wF} = (1-F)V_A/2 + V_{Ew}$, and the between-line variance by $\sigma^2_{bF} = 2FV_A + (1-F)V_A/2 + V_{Ec}$. For $F=1/4$, $\sigma^2_w - \sigma^2_{wF} = V_A/8$ and $\sigma^2_{bF} - \sigma^2_b = 3V_A/8$, so that the expected increase in the between-line variance of an additive trait is equal to threefold the reduction in the within-line component.

(iv) *Experiment 2: redistribution of the genetic variance with inbreeding*

The following experimental design was run twice, starting at generation 473 or 485. The trait scored was AB or ST bristle number, respectively. From the C_1 population, 60 males and 60 virgin females were sampled and they were mated in pairs in individual vials (generation 0). From the offspring emerging in each of 40 vials (generation 1), 40 inbred lines were established by a single brother × sister mating in each case. In parallel, 20 control lines were also started from the remaining 20 vials, each by a single mating and using a circular mating scheme, i.e., the ith control line was established by mating a male from the ith vial to a virgin female from the $(i+1)$th vial ($i=41$–60). Each line (inbred or control) was kept in a single vial. In the following generation (generation 2), the corresponding bristle trait was scored in 20 male and 20 virgin female offspring per line, and one generation of divergent selection was carried out, using the five individuals of each sex with the highest or the lowest score as parents of the high or the low selected lines, respectively, which were mated in a single vial in each instance. At generation 3, 20 male and 20 female offspring per selected line were scored for the pertinent bristle trait. In each line, the realized heritability h^2_R was estimated as the ratio between the response to divergent selection and the corresponding selection differential, averaged over sexes.

For each trait, the genetic correlation between the number of bristles on different plates (sternites) has been shown to be very close to 1, so that they can be considered as repeated measurements of a trait under the same genetic control (Caballero & López-Fanjul, 1987). Therefore, for each line, we estimated the component of variance due to environmental or developmental causes acting independently on both plates (sternites) in the same individual (special environmental component, V_{Es}) as twice the within-individual error variance for single plate measurements. The additive variance within the ith line was estimated as $V_{Awi} = h^2_{Ri}\ V_{Pwi}$. Average V_A and V_{Es} values were computed over lines, together with their empirical standard errors. For each group of lines, the total phenotypic variance was calculated as $V_P = \sigma^2_w + \sigma^2_b$, and the residual component of the phenotypic variance due to non-additive genetic effects (V_{NA}) and to environmental causes equally affecting both plates in an individual (general environment V_{Eg} variance) was obtained by subtraction ($V_R = V_P - V_A - V_{Es} = V_{NA} + V_{Eg}$).

(v) *Experiment 3: inbreeding depression for viability at chromosome II*

At generation 479, 52 wild chromosomes II were randomly sampled from the C_1 population, using a crossing scheme with a Cy/L^2 balancer stock as in García-Dorado *et al.* (2007), and their viability was synchronously measured by competition to the Cy/L^2 genotype for each of the 52 homozygous genotypes and the 52 panmictic combinations of chromosome pairs generated in a circular scheme (five replicate vials in each case).

(vi) *Mutational models*

In order to compute theoretical predictions for the equilibrium genetic properties of bristle traits and viability, two different mutational models were used for the deleterious mutational effects (**T** and **S**, described below and graphically represented in Fig. 1). They were based on the mutational parameters estimated in a long-term MA experiment started from the same isogenic line that was used as the C_1 base population. One of those models was denoted **T**, as it implied that the genome 'tolerated' an important proportion of mutations causing no relevant fitness reduction, so that the deleterious mutation rate was relatively

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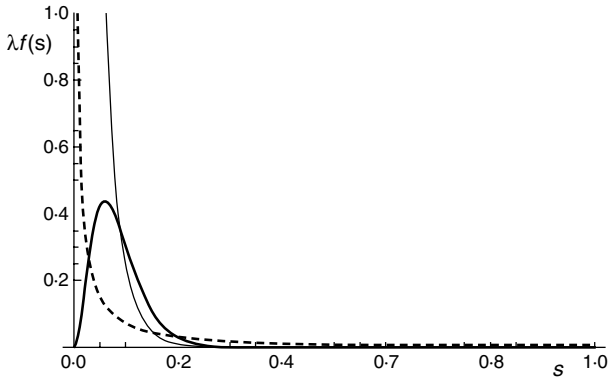

Fig. 1. Models of deleterious mutation: density functions for deleterious effects $f(s)$, multiplied by the corresponding deleterious mutation rate λ, under different mutational models defined by their respective values of the rate (λ) and average homozygous effect of deleterious mutation $E(s)$ for viability (**T**: tolerant mutation model, $\lambda = 0{\cdot}044$, $E(s) = 0{\cdot}085$, shape parameter $\alpha = 3{\cdot}35$, thick solid line; **T**′: tolerant mutation model, $\lambda = 0{\cdot}1$, $E(s) = 0{\cdot}05$, shape parameter $\alpha = 0{\cdot}1$, thick dashed line; **S**: sensitive mutation model, $\lambda = 0{\cdot}5$, $E(s) = 0{\cdot}017$, shape parameter $\alpha = 0{\cdot}5$, thin solid line). The area below a curve for any s interval gives the rate of mutation per gamete and generation for the deleterious effects within the interval.

small (García-Dorado, 2003). In this case, we used the rate ($\lambda = 0{\cdot}044$) and the average homozygous effect of deleterious mutations for viability ($E(s) = 0{\cdot}085$) estimated in the MA experiment at generation 255 (Chavarrías *et al.*, 2001), and assumed that mutational deleterious effects s were gamma-distributed with a shape parameter ($\alpha = 3{\cdot}35$) calculated by Minimum-Distance techniques (García-Dorado & Marín, 1998), so that practically all deleterious effects ranged from 0·001 to 0·25, but a few were mild ($\lambda = 0{\cdot}011$ for $0{\cdot}001 < s < 0{\cdot}05$). Alternatively, we considered a model denoted **S** based on the assumption that fitness was very 'sensitive' to mutations, which usually had relevant deleterious effects, implying a large deleterious mutation rate $\lambda = 0{\cdot}5$. In this instance, we assumed a leptokurtic gamma distribution, with the scale parameter ($\alpha = 0{\cdot}5$) required to account for the same genomic mutational input variance as in the **T** model, giving $E(s) = 0{\cdot}017$. This implied a high rate of mild deleterious mutation ($\lambda = 0{\cdot}36$ for $0{\cdot}001 < s < 0{\cdot}05$). Although those two models cover a wide parametric space, we have also used a **T**′ additional model ($\lambda = 0{\cdot}1$, $\alpha = 0{\cdot}1$ and $E(s) = 0{\cdot}05$). This parameter combination accounted for a rate of deleterious mutation with effect $0{\cdot}001 < s < 0{\cdot}25$, roughly similar to that included in the **T** model, but allowed for a rate $\lambda = 0{\cdot}006$ of mutations with $s > 0{\cdot}25$, which were likely to have been removed by natural selection in the MA experiment. It also allowed for an additional rate ($\lambda = 0{\cdot}056$) of deleterious mutations with tiny effects ($s < 0{\cdot}001$), which will pass undetected in MA experiments. Under the **T**′ model, the expected rate of viability decline from MA in brother–sister lines (0·0021, computed from diffusion theory as in García-Dorado, 2003) was in rough agreement with that previously found in our MA experiment (0·0015, averaging estimates obtained at generations 104–105, 210 and 255; Fernández & López-Fanjul, 1996; García-Dorado *et al.*, 1998; Chavarrías *et al.*, 2001; Caballero *et al.*, 2002), while the rate expected under the **S** model was about four times larger (0·008). For the three models considered, the degree of dominance h of deleterious mutations (the fraction of the homozygous deleterious effect that is expressed in the heterozygous condition) was sampled from a uniform distribution defined between 0 and $\exp[-7s]$ (García-Dorado, 2003), giving $E(h)$ equal to 0·29, 0·45 and 0.43 for the **T**, **S** and **T**′ models, respectively ($h = 0$, 1/2 or 1 for complete recessive, additive, or complete dominance action, respectively).

For bristle traits, we assumed additive mutational effects a (defined as the difference between the values of the homozygous genotypes for the wild and the mutant alleles at each locus), which were positive or negative with the same probability. We used the Minimum-Distance distributions of mutational effects obtained in the MA experiment for AB ($\lambda = 0{\cdot}008$ and gamma-distributed homozygous effects with $\alpha = 4{\cdot}58$ and $E(a) = 0{\cdot}791$ bristles) or ST ($\lambda = 0{\cdot}043$ and gamma-distributed homozygous effects with $\alpha = 0{\cdot}13$ and $E(a) = 0{\cdot}115$ bristles) (García-Dorado & Marín, 1998).

When predictions were obtained assuming deleterious pleiotropic side effects on fitness for the mutations affecting bristle traits, these effects were sampled from the **S** or **T** models. Although these models refer to viability, relevant deleterious fitness effects are likely to be of the same order as those for viability, although the fitness mutation rate should be larger (Ávila & García-Dorado, 2002). Under the **S** model, all mutations affecting AB or ST had deleterious effects sampled from the corresponding distribution. For the **T** model, however, only a fraction ϕ of the mutations affecting the trait had deleterious side effects, and we show results for those ϕ values that allowed a better prediction of the additive variance of the trait in the C_1 population ($\phi = 0{\cdot}25$), or of the average additive variance reported for natural populations ($\phi = 0{\cdot}98$). We assayed different values of the Pearson's correlation coefficient ρ between a and s in the subset of mutations affecting both the morphological trait and fitness, from $\rho = 0$ to its maximum possible value ($\rho < \sqrt{(\alpha_1/\alpha_2)}$, where α_1 and α_2 are the smallest and largest α values of the two gamma-distributed variables, respectively).

(vii) *Equilibrium predictions*

The expected equilibrium value of the additive variance for bristles depends on the type and magnitude

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Table 1. *Mean* ($\bar{X}$), *within-line* (σ^2_w), *between-line* (σ^2_b) *and within-family* (*non-common*) *environmental* (V_{Ew}) *components of the phenotypic variance* ($\pm$*S. E.*), *for the control and inbred lines in experiment 1* (*generation 459, AB* = *abdominal and ST* = *sternopleural bristle number*)

	AB		ST	
	Control	Inbred	Control	Inbred
$\bar{X} \pm$ S. E.	31·54 ± 0·18	30·50 ± 0·16[a]	31·45 ± 0·20	31·78 ± 0·15
$\sigma^2_w \pm$ S. E.	5·93 ± 1·02	8·30 ± 0·83[b]	7·48 ± 1·62	7·13 ± 0·53
$\sigma^2_b \pm$ S. E.	1·04 ± 0·73	2·56 ± 0·74	0·80 ± 0·83	2·47 ± 0·63
$V_{Ew} \pm$ S. E.	4·33 ± 0·49	4·48 ± 0·40	3·78 ± 0·34	4·38 ± 0·29

[a] Significantly smaller than the control value ($P < 10^{-5}$).
[b] Significantly larger than the control value ($P < 0{\cdot}02$).

of the evolutionary forces that create and maintain that variance, namely mutation, selection and drift. Approximate predictions for different models were obtained as follows where, for simplicity, additive gene action within and between loci was assumed.

Under the neutral model, the expected additive variance at the mutation-drift (MD) balance is

$$V_{A(MD)} = 2N_e V_m,$$

where $V_m = \lambda\ E(a^2)/2$, E stands for expectation and λ is the gametic mutation rate for the pertinent trait (Lynch & Hill, 1986).

If the bristle trait is not causally related to fitness, but mutations with equal homozygous effects a on the trait have pleiotropic deleterious side effects (s,h), the expected additive variance at the MSD balance is $V_{A(MSD)} = (a/2)^2 \sum H$, where $\sum H$ is the expected heterozygosity added up over loci, and it can be readily approximated using the Short-Cut approach (García-Dorado, 2007) as

$$V_{A(MSD)} = \frac{\lambda a^2/2}{(1/2N_e) + hs + Ks(1-2h)},$$

where K is the proportion of deleterious copies undergoing selection in the homozygous condition at the MSD balance, which can be approximated as

$$K \approx \frac{1}{4N_e hs + \sqrt{2\pi N_e s} + 2}.$$

The expected genetic variance at the MSD balance for a trait under causal stabilizing selection described by the fitness function $W(G) = \mathrm{Exp}[-G^2/2V_s]$, where V_s is an inverse measure of the strength of selection and G is the genotypic value of the trait, can be predicted using the Stochastic House of Cards approximation (Bürger *et al.*, 1998) as

$$V_{A(SHC)} = \frac{4\lambda V_s}{1 + (4V_s/a^2 N_e)}.$$

Finally, if deleterious mutations with effects (s,h) occur at rate λ, the fitness inbreeding depression rate δ at the MSD balance can be predicted using the approximate expression (García-Dorado, 2007):

$$\delta = \frac{\lambda s(1-2h)}{(1/2N_e) + hs + Ks(1-2h)}.$$

All these predictions were averaged over a large number of mutations (10^4–10^6) with mutational effects and degrees of dominance randomly sampled from the distributions corresponding to the mutational models specified above for bristle traits and fitness.

3. Results

(i) *Bristle traits*

The average bristle number for AB or ST, the within- and between-line phenotypic variance components, and the within-family (non-common) environmental variance, are shown in Table 1 for the inbred and control lines in experiment 1.

A significant inbreeding depression was detected for AB, indicating the presence of directional dominance for this trait. This was accompanied by a significant increase of the within-line component of the phenotypic variance with inbreeding, as expected from the segregation at low frequencies of (partially) recessive alleles reducing the expression of the trait (Robertson, 1952; López-Fanjul *et al.*, 2002).

For ST, however, no inbreeding depression was observed, the within-line variance decreased with inbreeding although not significantly, and the increase in the between-line variance after inbreeding (1·67 ± 1·04) did not statistically depart from its additive expectation (three times the decrease in within-line variance, i.e. 1·05 ± 5·11). These results suggest an additive genetic basis for ST in the C_1 population.

For both traits, the within-family environmental component of variance showed some increase with inbreeding, albeit not significant.

Parameter estimates for AB and ST in experiment 2 are presented in Table 2. In this instance, a small but

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Table 2. *Mean* ($\bar{X}$), *within-line phenotypic* (σ^2_w), *within-line additive genetic* (V_{Aw}), *between-line* (σ^2_b), *and special environmental* (V_{Es}) *components of variance, and heritability* (h^2) ($\pm$ *S.E.*), *for the control and inbred lines in experiment 2* (*AB = abdominal and ST = sternopleural bristle number at generations 473 and 485, respectively*)

	AB		ST	
	Control	Inbred	Control	Inbred
$\bar{X} \pm$ S. E.	30·27 ± 0·15	30·17 ± 0·10	32·97 ± 0·08	32·65 ± 0·06[a]
$\sigma^2_w \pm$ S. E.	15·79 ± 1·30	12·84 ± 0·36	5·14 ± 0·12	4·53 ± 0·04
$\sigma^2_b \pm$ S. E.	1·37 ± 0·52	2·35 ± 0·52	0·21 ± 0·11	0·65 ± 0·15
$V_{Es} \pm$ S. E.	5·81 ± 0·39	5·96 ± 0·24	2·44 ± 0·14	2·86 ± 0·11[b]
$V_{Aw} \pm$ S. E.	3·41 ± 0·60	2·10 ± 0·54	0·54 ± 0·19	1·10 ± 0·14[b]
$h^2 \pm$ S. E.	0·23 ± 0·04	0·18 ± 0·05	0·11 ± 0·04	0·24 ± 0·03[b]

[a] Significantly smaller than the control value ($P < 10^{-3}$).
[b] Significantly larger than the control value ($P < 0·01$).

Table 3. *Inbreeding depression at* F = *0·25 for bristle traits* (*AB = abdominal and ST = sternopleural bristle number*) *or that caused by homozygosis of non-lethal chromosomes II for log-viability* (V_{II}) *at different generations* (*exact* t *number given within brackets when necessary*), *together with the proportion of lethal chromosomes*

Approximate generation	250	374–385	459	473–485
AB			1·05 ± 0·24[a]	0·10 ± 0·18 ($t = 473$)
ST			−0·33 ± 0·25	0·32 ± 0·10[a] ($t = 485$)
V_{II}	0·090 ± 0·037[b]	0·091 ± 0·060 ($t = 374$)		0·091 ± 0·050[b] ($t = 479$)
% of lethal chromosomes		11·2 ± 1·3 ($t = 385$)		5·8 ± 3·2 ($t = 479$)

[a] Significantly greater than zero ($P < 0·001$).
[b] Significantly greater than zero ($P < 0·05$).

significant inbreeding depression was detected for ST, and the corresponding within-line additive variance and heritability significantly increased with inbreeding. Those results could be ascribed to the segregation at low frequencies of (partially) recessive alleles affecting this trait.

On the contrary, no inbreeding depression was detected for AB, and the reduction of the within-line additive component of variance (1·31 ± 1·35) was compatible with its additive expectation (0·82 ± 0·15, computed as one-quarter of the within-line additive variance in the panmictic population). In addition, the heritability of the control lines was larger than that of the inbred ones, although the difference was not significant.

For both traits, the between-line variance increased with inbreeding, as expected for any type of gene action. However, σ^2_b estimates were smaller than those for V_{Aw} ones, which is against the theoretical expectation (i.e. $\sigma^2_b = V_A/2 + V_D/4 + V_{Ec}$ should be equal to or larger than $V_{Aw} = V_A/2$). This could be due to some environmental factor producing a downward bias in the σ^2_b estimates, such as within-vial competition inflating the within-line variance, and would preclude adequate comparisons to their additive expectations. In parallel, the estimates of the within-line variance for AB differed substantially between experiments, which could in principle be ascribed to generation environmental effects on variance. As in experiment 1, the special environmental component of the phenotypic variance increased with inbreeding for both traits and, in this occasion, significantly for ST.

Summarizing, the results illustrated drift-induced fluctuations of the frequencies of (partially) recessive alleles of large negative effect on bristle number, so that those affecting AB were present at relatively low frequencies at generation 459 but practically disappeared 14 generations later, and others affecting ST, which passed undetected at that generation, attained substantial frequencies 26 generations later.

(ii) *Viability*

Inbreeding depression caused by one generation of brother × sister mating for the two bristle traits or by homozygosity of chromosomes II for log-viability, as well as the proportion of lethal chromosomes II in the C_1 population, estimated in different generations, are given in Table 3. The remarkable temporal stability of the inbreeding depression for viability, implying a constant load concealed in the heterozygous condition, contrasted with the erratic changes of the depression

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Table 4. *Additive genetic* (V_A)*, special environment* (V_{ES}) *and residual* (V_R) *components of the phenotypic variance* (V_P) *of abdominal* (*AB*) *and sternopleural* (*ST*) *bristle number in laboratory* (*LAB*) *and natural* (*NAT*) *populations, and in the* C_1 *population at different generations* t

	LAB[a]	NAT[c]	$t=381$[d]	$t=430$[e]	$t=431$[d]	$t=459$[e]	$t=463$[e]	$t=473$ (AB) or 485 (ST)[e]
AB:								
V_A	4·50	8·36±0·68	2·10±0·41	3·70±0·36				6·82±1·20
V_{ES}	4·23		4·73±0·30	6·55±0·42	5·05±0·47	4·33±0·49	5·63±0·47	5·81±0·39
V_R	1·47		1·21±0·51	1·16±0·55				4·53±1·26
V_P	10·20	25·30±4·95	8·04±0·53	11·41±1·18	9·19±1·59	6·97±1·25	7·39±0·65	17·16±1·40
ST:								
V_A	1·77	2·83±0·26	1·34±0·47					1·08±0·39
V_{ES}	1·50[b]		3·32±0·22		3·59±0·38	3·78±0·34	3·74±0·35	2·44±0·14
V_R	0·69		0·31±0·51					1·83±0·41
V_P	3·96	4·74±0·38	4·97±0·33		4·16±0·50	8·28±1·82	7·82±0·72	5·35±0·16

[a] Average values for three (AB) or eight (ST) laboratory populations (Clayton *et al*., 1957; Sheridan *et al*., 1968; López-Fanjul & Hill, 1973; Madalena & Robertson, 1975; Salgado, 1984; López-Fanjul *et al*., 1989).
[b] Madalena & Robertson (1975).
[c] Average values for four (AB) or 12 (ST) natural populations (Salgado, 1984; Coyne & Beecham, 1987; López-Fanjul & Ruano, 1987; Ichinose *et al*., 1992; Rodríguez-Ramilo *et al*., 2006).
[d] García-Dorado *et al*. (2007). Estimates recalculated to include the between-vial environmental component of variance, as in the present experiment.
[e] Present experiment.

observed for both bristle traits. Although the proportion of lethal chromosomes was somewhat smaller in the last evaluation, the difference did not reach significance.

(iii) *Equilibrium predictions for the genetic variance of bristle traits*

Table 4 summarizes estimates of variance components for AB and ST reported in the literature for natural and laboratory populations, together with those corresponding to the C_1 population at different times. For AB, the additive variance at generation 473 ($V_A=6·82$) was almost twice that obtained at generation 430. Furthermore, the upper-bound estimate of the additive variance at generation 473 ($V_P-V_{ES}=11·35$) was well above all previous values (average $V_P-V_{ES}=3·34$), and V_P was more than twice the average of previous estimates. This indicated that the environmental conditions of the last evaluation were atypical, and suggested that the corresponding estimate for the additive variance can be considered as an outlier. Excluding this estimate, the average V_A for generations 381 and 430 was 2·9, i.e. 64% of the average value reported for laboratory populations. For ST, the average V_A (generations 381 and 485) was 1·21, i.e. 68% of the average value reported for laboratory populations. Summarizing, after 381–485 generations of MA, our population, originally devoid of any genetic variation, had recovered a substantial amount of additive variance for bristles, which approached that reported for laboratory populations. It should also be noted that the average V_{Es} value for C_1 was somewhat larger than that commonly observed in laboratory populations, which may be indicative of inbreeding depression for developmental homeostasis in the original isogenic line. On the other hand, laboratory populations had on the average about half the additive variance of natural populations, which should have much larger effective population sizes.

To compute predictions for the additive variance of bristles under different assumptions, we used the mutational parameters of bristle traits and viability given in the Materials and Methods section. These predictions are given in Fig. 2 as a function of the effective population size (up to 10^6), together with the average estimate for C_1 (see above) and that reported for natural populations. As shown in the figure, the additive variance of the C_1 population, for both traits, was of the order of those predicted for the neutral model or for the two selective models. The N_e value for the whole *D. melanogaster* species has been estimated to be of the order of 10^6 (Charlesworth, 2009), and many natural populations only showed minor differentiation for a sample of 117 enzyme and protein loci (Singh & Rhomberg, 1987), implying effective sizes ranging from 10^3 to 10^6. Therefore, in Fig. 2, the average additive variance estimated for natural populations was assigned to effective population sizes within that interval. Of course, predictions for $V_{A(MD)}$ increase linearly with N_e (exponentially with its logarithm), giving expected variances that are too big, even for moderately large populations.

Predictions for $V_{A(MSD)}$ were first computed under the **T** model, which implied a relatively low rate of deleterious mutations, most of them of considerable

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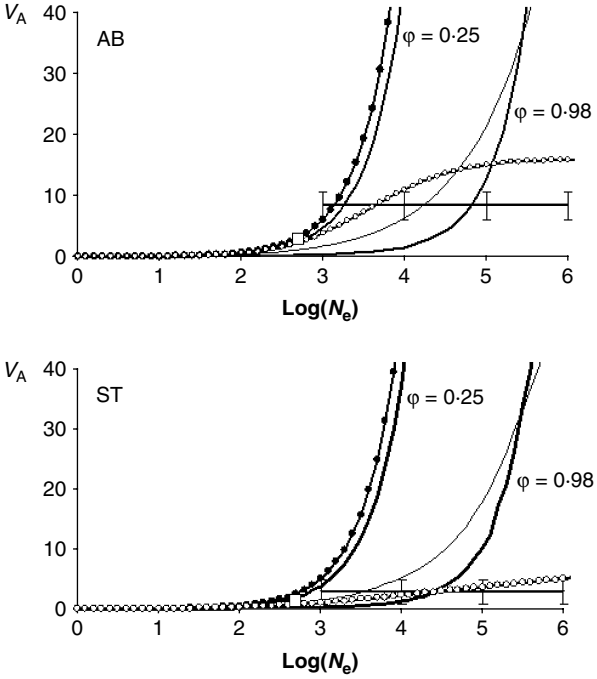

Fig. 2. Equilibrium additive variance versus effective population size: equilibrium predictions of the additive variance (V_A) for AB (upper panel) and ST (lower panel) plotted against the decimal logarithm of the effective population size, together with average estimates for the C_1 population (empty square) and for natural populations (horizontal line, with vertical bars covering two standard deviations of the distribution of available estimates above and below the average). MD balance (solid line with black dots), SHC balance (solid line with empty dots), MSD balance for the **T** mutational model with different fractions of the mutations affecting the trait having deleterious side effects ($\phi = 0{\cdot}25$, $\phi = 0{\cdot}98$, thick solid lines), or for the **S** mutational model ($\phi = 1$, thin solid line).

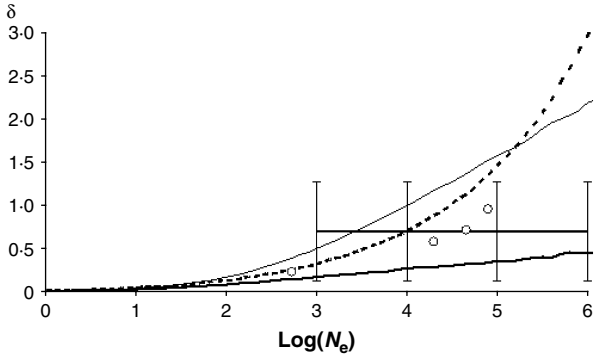

Fig. 3. Viability inbreeding depression rate versus effective population size: Equilibrium predictions for the non-lethal inbreeding depression rate (δ) for viability (**T** model: thick solid line: **T′** model: thick dashed line; **S** model: thin solid line) plotted against the decimal logarithm of the effective population size. The empty dots give the inbreeding depression estimates adjusted for the whole genome for our C_1 population and for three natural populations for which the effective population size can be obtained from lethal-complementation analysis, while the horizontal line (with vertical bars covering two standard deviations) gives the average of several available estimates from natural populations (see text for references).

effect, so that the probability ϕ of a mutation belonging to this group was required for calculations. Results were obtained for different values of the correlation ρ between s and a in the subset of mutations with deleterious side effects. However, correlations larger than zero did not modify our general conclusions, and only results for $\rho = 0$ are given. When ϕ was fitted to roughly account for the average additive variance observed in the C_1 population ($\phi = 0{\cdot}25$), the predicted $V_{A(MSD)}$ for the effective size of natural populations was extremely large. On the other hand, when ϕ was big enough to account for the additive variance of natural populations ($\phi = 0{\cdot}98$), the prediction of $V_{A(MSD)}$ for $N_e = 500$ was much smaller than our estimates.

Alternatively, we used the **S** model to obtain MSD predictions, assuming that all mutations affecting bristles had some deleterious side effect ($\phi = 1$), and results are given for $\rho = 0$. In this situation, the additive variance predicted for $N_e = 500$ was somewhat smaller than our C_1 estimates, but it became extremely big for large N_e values.

Finally, Fig. 2 also shows approximate predictions for the variance $V_{A(SHC)}$ expected in a finite population where the trait is under causal stabilizing selection, so that deleterious effects of genes contributing to the trait's variance were exclusively due to fitness impairment caused by extreme values of the trait. In this situation, we have fitted the strength of selection ($1/V_s$) to account for the additive variance of our C_1 population, which implied $V_s = 100$ or 20 for AB or ST, respectively. For AB, this value was close to that reported for the same genetic background in a laboratory experiment (García-Dorado & González, 1996; $V_s = 116$) but, unfortunately, no V_s estimate for ST was available. That procedure set an upper bound for the additive variance as N_e increased, which was in qualitative agreement with the range of the estimates reported for natural populations.

(iv) *Equilibrium predictions for viability depression*

In contrast with the temporal fluctuation of the genetic properties of bristle traits observed in the C_1 population, the non-lethal inbreeding depression rate for log-viability was consistently constant over generations.

Inbreeding depression rates predicted for viability are given in Fig. 3, together with the average estimates obtained for the C_1 population and for a set of natural populations (average non-lethal inbreeding depression rate for viability around 0·7, ranging from 0·27 to 1·08; Temin *et al.*, 1969; Mukai & Yamaguchi, 1974; Mukai & Nagano, 1983; Kusakabe & Mukai, 1984; Kusakabe *et al.*, 2000; Rosa *et al.*, 2005). Predictions

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from model **T**′ were quite close to the C_1 value, while the estimate for natural populations laid between the predictions from models **T** and **T**′.

4. Discussion

Previous experiments showed that the C_1 population had reached a MSD balance for viability after 250 generations, and suggested that the genetic variances for bristle traits were also close to their equilibrium values (Ávila *et al*., 2006; García-Dorado *et al*., 2007). Present results confirmed the former conclusion regarding viability and the corresponding implication for bristle traits. Moreover, they provided further insight both on the pattern of natural selection acting upon those traits and on the corresponding architecture of their equilibrium genetic variance.

After 473–485 generations of MA, population C_1 had attained a level of additive variance for bristle number which was approaching that typically observed in standing laboratory populations, which were usually maintained with similar effective sizes ($N_e \sim 500$, Malpica & Briscoe, 1981; López-Fanjul & Torroja, 1982). Note that the genetic variances of these laboratory populations are likely to be somewhat above their MSD balance values for the corresponding effective population sizes, as the approach to this balance should be slow. In these populations, most genetic variance for AB and ST has been usually considered to be additive, as the repeatability between the number of bristles in different abdominal segments or sternopleural plates was only slightly higher than the corresponding heritability, with only about 15% residual variance being left for non-additive gene action together with general environmental effects (see Table 4). In addition, the change in mean and additive variance after inbreeding, which had been previously assayed in a single occasion for each trait, conformed to the neutral additive expectations, i.e. no inbreeding depression was detected and the additive variance decreased proportionally to the inbreeding coefficient (López-Fanjul *et al*., 1989; Kristensen *et al*., 2005). However, the genetic variance for bristle traits in population C_1 only fitted the above pattern at some generations (for ST at generation 473 and for AB at generation 485) but, at other moments, inbreeding induced depression and increased the additive variance (for AB at generation 473 and for ST at generation 485), as expected from the segregation of non-additive alleles affecting those traits (Robertson, 1952; López-Fanjul *et al*., 2002). Information on 29 putative individual mutations affecting AB or ST has been obtained in the MA experiment derived from the original isogenic stock which was also the base of the C_1 population (Santiago *et al*., 1992; López & López-Fanjul, 1993). Out of these 29 mutations, 24 showed effects of either sign on the metric traits which were smaller than 0·5 phenotypic standard deviations, their type of gene action was predominantly additive, and behaved quasi-neutrally. However, five mutations had effects, also of either sign, that were larger than one phenotypic standard deviation, and all of them were totally or partially recessive and deleterious. Thus, the results obtained for AB at generation 473 and for ST at generation 485 could be reasonably ascribed to the transient segregation at moderate frequency of a recessive deleterious mutation with a large negative effect on the pertinent trait. This suggests that, in populations of relatively small effective population size ($N_e \approx 500$), the genetic architecture of typically additive metric traits can often be affected by the occasional segregation of low-frequency recessives that contribute little genetic variance in the panmictic population but induce an excess in additive variance and a considerable inbreeding depression after bottlenecks.

We have obtained equilibrium predictions for the additive variance of both bristle traits under different mutational and selective models, and we have found that pleiotropic deleterious side effects, although occasionally important, were unable to account for the relationship between the equilibrium additive variance and the effective population size arising from the joint consideration of the values observed in the C_1, laboratory and natural populations. Thus, causal stabilizing selection was required to set an upper bound to the increase in additive variance with increasing effective population sizes. For AB, the strength of stabilizing selection accounting for the additive variance estimated in C_1 implied a bound that was moderately higher than the average value observed for natural populations, as would be expected if the strength of stabilizing selection was higher in nature than in laboratory conditions. Notwithstanding, it should be borne in mind that the SHC equation only gives a rough approximation, due to the restrictive assumptions involved (Turelli, 1984). For ST, the stabilizing selection required to account for the additive variance of C_1 ($V_s = 20$) was more intense than in the case of AB ($V_s = 100$), but this could be attributed to the highly leptokurtic distribution estimated for the mutational effects on that trait (García-Dorado & Marín, 1998), accounting for occasional mutations with very large effects (several standard deviations). In any case, the predictions of $V_{A(SHC)}$ were within the range observed for laboratory and natural populations for their corresponding effective population sizes.

We have also obtained equilibrium predictions for the non-lethal viability inbreeding depression rate under three mutational models, using parameters based on specific experimental estimates. Notwithstanding, those models should not be taken as precise alternative descriptions of viability mutations, but as a means to explore the role of spontaneous deleterious

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mutation in the determination of the genetic properties of populations. Thus, they could be qualitatively described as: (1) a 'tolerant' **T** model implying a low rate of deleterious mutation of mainly moderate to severe effect, as inferred from an MA experiment carried out in the C_1 genetic background; (2) a 'sensitive' **S** model implying an important rate of mildly deleterious mutations, as originally suggested by the classical Mukai studies (Mukai *et al*., 1972); and (3) a **T**-like model (**T**′) assuming that an important proportion of the severely deleterious mutations had been removed by natural selection from the MA lines.

The **T**′ model gave the best fit to the non-lethal inbreeding depression rate observed in C_1, although model **T** also provided close approximations, and the values reported for natural populations laid between the **T** and **T**′ predictions. Taking into account that important sampling errors are usually involved in the estimation of effective population sizes, the results did not allow a formal rejection of the **S** model, for which the equilibrium inbreeding depression was determined by a high rate of mildly deleterious mutations. This model, however, implied a rate of viability decline for our MA lines that was about four times the observed value ($\sim$0·008 versus $\sim$0·0015, see Materials and Methods section). On the contrary, for models **T**′ and **T**, the inbreeding depression rate was mainly accounted for by a relatively small rate of largely recessive mutations with moderate to severe deleterious effects. This is of particular relevance, as the efficiency of the purge of inbreeding depression in small populations should be much more efficient in the **T**′ scenario than in the **S** scenario, due to the larger deviation of the genotypic fitness values from their additive expectations obtained in the former model (García-Dorado, 2008). Furthermore, model **T**′ was also the one giving more accurate predictions for the modest rate of mutational viability decline previously found in our MA experiment, as well as for the accelerated increase of the inbreeding depression rate with increasing effective population sizes shown in Fig. 3.

Due to the assumption of a gamma distribution of effects, the T′ model also incorporated a considerable rate of mutations with tiny deleterious effects ($\lambda \approx 0{\cdot}056$ and $s < 0{\cdot}001$). In fact, the frequency of this class was likely to be even larger, as direct estimates of the proportion of sites in the genome that are subjected to selection implied deleterious mutation rates that were usually higher than those revealed by the quantitative analysis of MA experiments (Halligan & Keightley, 2009). For our MA lines, a large deleterious mutation rate has been inferred by applying the above proportion of selected sites to the differences found between the sequences of different lines ($\lambda = 0{\cdot}6$, Haag-Liautard *et al*., 2007). As this λ value was not associated with an estimate of the distribution of deleterious effects, it was consistent both with our MA results and with those from the **T**′ mutational model, as far as most deleterious mutations have tiny effects. Of course, if lethal mutations were considered (which were excluded from our estimates and predictions), the distribution of deleterious mutational effects would become bimodal and could not be fitted by a gamma distribution. In any case, the tiny deleterious mutation class would make a negligible contribution to the rate of mean decline during the MA experiment and to the inbreeding depression rate and the genetic variance at the MSD balance for natural or laboratory populations.

On the whole, in agreement with Zhang & Hill (2002), we concluded that natural selection exclusively based on deleterious side effects cannot account for the empirical observations as, due to the occasional occurrence of quasi-neutral mutations affecting the trait, this selection model failed to set an upper bound to the equilibrium additive variance with increasing N_e. Thus, the main factor constraining the genetic variance of bristle traits in large populations should be weak causal stabilizing selection. In this situation, recessive mutations of relatively large effect could occasionally drift and cause inbreeding depression and an excess in the additive variance after bottlenecks. This should be common in populations of moderate size, as it is the case of laboratory populations, where the additive variance observed was close to both the MD and the SHC predictions. For fitness component traits, however, the temporal stability of the viability inbreeding depression rate implied that its value was subjected to strong selective constraints, even for the effective sizes of laboratory populations. The non-lethal inbreeding depression rate can be mainly ascribed to largely recessive mutations with moderate to severe deleterious effects, which were likely to occur at a somewhat larger rate than that previously estimated from MA experiments carried out in the same genetic background.

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