A test of quantitative genetic theory using *Drosophila* – effects of inbreeding and rate of inbreeding on heritabilities and variance components[#]

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Introduction

One consequence of inbreeding and drift relates to changes in the distribution of genetic and environmental variance (López-Fanjul *et al.* 1989; Fernández *et al.* 1995; Fowler and Whitlock 1999; Whitlock and Fowler 1999). Under the assumption of neutrality and purely additive gene action, genetic drift reduces the additive genetic variance within lines (V_A) proportionally to the average coefficient of inbreeding (Falconer and Mackay 1996). Theoretical studies have shown that if non-additive gene-action is present, V_A behaves differently and can even increase in some cases (Robertson 1952; Goodnight 1988; López-Fanjul *et al.* 1999; 2000; 2002). This has also been confirmed experimentally (Bryant *et al.* 1986; Bryant and Meffert 1993; 1995; 1996; Fernández *et al.* 1995; Whitlock and Fowler 1999). In conservation of endangered species and in animal breeding, inbreeding depression and a decrease of the genetic variance constitute threats

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to population survival and to the potential for adaptation and genetic progress. Inbreeding can therefore be interpreted as genetic stress.

The narrow sense heritability (h^2), being a measure of evolutionary potential, is a function of genetic and environmental variance. Apart from being affected by genetic changes, h^2 is sensitive to heterogeneity of environmental conditions. Lerner (1954) hypothesized that inbreeding can lead to an increase in environmental variance (V_E), because inbred individuals are less stable in their development. There is some experimental evidence supporting this hypothesis, primarily for traits closely related to fitness (Whitlock and Fowler 1999). Because the additive genetic and environmental variances can react in a variety of ways under inbreeding, the direction of change in phenotypic variance (V_P) under inbreeding is difficult to predict (Fowler and Whitlock 1999; Whitlock and Fowler 1999). Here we study genetic and environmental parameters for the trait sternopleural bristle number in *Drosophila melanogaster*. The experiment comprised three treatments: fast and slow inbred lines (inbred to the same total level of inbreeding) and control lines. The objective was to test whether the trait sternopleural bristle number behaves according to expectations derived under selective neutrality and additive gene action: no inbreeding depression, and no effect of the rate of inbreeding on heritability, additive genetic, environmental, and phenotypic variances.

Materials and Methods

Experimental design: Ten lines with expected equivalent levels of inbreeding ($F \approx 0.67$) were obtained from a genetic diverse mass population either by 5 generations of full-sib mating (fast rate) or by maintaining a population size of four pairs during 18 generations (slow rate). After reaching the desired level of inbreeding, lines were flushed to sizes of approximately 500 breeding individuals. Ten "non-inbred" control lines, each founded by approximately 500 breeding individuals were also established.

One hundred and four pairs of virgin male and female parents were set up in individual vials per line. Male parents and two male offspring from each mating were sampled from the 104 families within the 10 lines within each treatment.

Sternopleural bristle number on the right side of the flies was counted on male parents and on 2 male offspring in each family. In total, sternopleural bristles were counted on 9360 flies (104 male parents + 208 male offspring from each of 10 lines in each of the 3 treatments).

Statistical analysis: It was assumed that the sampling distribution of data **y** (vector of order *n*), given parameters **β**, **r** , **a** and V_E, is the multivariate normal process

$$\mathbf{y}|\boldsymbol{\beta}, \mathbf{r}, \mathbf{a}, V_E \sim N(\mathbf{X}\boldsymbol{\beta} + \mathbf{W}\mathbf{r} + \mathbf{Z}\mathbf{a}, \mathbf{I}V_E). \quad (1)$$

Here **β** is a vector that contains effects of generation (2 levels), **r** is a vector of line effects (10 levels), **a** is a vector of additive genetic effects of order *q*, **X, W** and **Z** are known incidence matrices associating **β, r** and **a** with **y** and V_E is the variance of the conditional distribution, which, given the model, is interpreted as the environmental variance. The vector **r** is assumed to follow the normal process

$$\mathbf{r}|V_r \sim N(\mathbf{0}, \mathbf{I}V_r) \quad (2),$$

where V_r is the variance between lines and **I** in (1) and (2) is the identity matrix of appropriate order. Additive genetic values are assumed to result from the sum of many independently segregating loci, each with small effect. Therefore, invoking the central limit theorem, the distribution of additive genetic values is

$$\mathbf{a}|\mathbf{A}, V_A \sim N(\mathbf{0}, \mathbf{A}V_A). \quad (3)$$

Above, **A** is the additive genetic relationship matrix (of dimension *q* x *q*) and V_A is the additive genetic variance within lines. The parameters which are the focus of inference are V_r, V_A, and V_E and possible functions thereof, such as the heritability within lines $V_A/(V_A + V_E)$ and the phenotypic variance within lines ($V_P = V_A + V_E$).

The prior distribution assigned to **β**, V_r, V_A, and V_E is the improper uniform prior

$$p(\boldsymbol{\beta}, V_r, V_A, V_E) = p(\boldsymbol{\beta})\, p(V_r)\, p(V_A)\, p(V_E) \propto \text{constant}. \quad (4)$$

Then the joint posterior density of all unknown quantities is

$$p(\boldsymbol{\beta}, \mathbf{a}, V_r, V_A, V_E|\mathbf{y}) \propto p(r|V_r)\, p(\mathbf{a}|V_A)\, p(\mathbf{y}|\boldsymbol{\beta}, \mathbf{a}, V_E). \quad (5)$$

This model was fitted using a Gibbs sampler. All the fully conditional posterior distributions are of standard form; that is, normal for (**β**,**a**) and scaled inverted chi-squares for the variance components. Details of the algorithm can be found, for example, in Sorensen and Gianola (2002).

Differences between treatments were studied via the Monte Carlo estimates of the posterior probabilities: $(X_{\text{control}} - X_{\text{slow}} > 0|\text{y})$, $(X_{\text{control}} - X_{\text{fast}} > 0|\text{y})$, and $(X_{\text{slow}} - X_{\text{fast}} > 0|\text{y})$, for $X = h^2$, V_A, V_E and V_P.

Results

The means for sternopleural bristle number over lines within treatments were 9.80 for control, 9.75 for slow inbred and 9.98 for fast inbred. The statistical test (not shown) indicates that inbreeding did not affect mean sternopleural bristle number.

Heritabilities within treatments are shown in table 1. The inference from this analysis is that heritabilities differ in all three contrasts tested (figure 1a). Heritability was higher in the control than in inbred lines, and higher in slow than in fast inbred lines.

Additive genetic variances within lines are shown in table 1. The inference from the analysis is that additive genetic variances differs in all three contrasts tested (figure 1b), showing the same pattern as the heritabilities. The expected additive genetic variance in both inbred treatments, based on additive gene action and selective neutrality, is 0.25. The estimated additive genetic variance (estimated posterior mean) is 0.18 and 0.29 within the fast and slow inbred treatments, respectively (table 1). The posterior probability that the additive genetic variance in the fast inbred treatment is smaller than 0.25, is 88%. On the other hand, the posterior probability that V_A in the slow inbred treatment is smaller than 0.25, is 25%. This indicates a larger departure from expectation in the fast than in the slow inbred treatment.

Environmental variances within treatments are shown in table 1. The inference from the analysis is that V_E differs in all three contrasts tested (figure 1c).

Phenotypic variances of the three treatments are shown in table 1. The inference from the analysis is that the variance was higher in the control than in the inbred treatments, and that there is no difference between phenotypic variance in the two inbred treatments (figure 1d).

Table 1. Monte Carlo estimates of marginal posterior means for the three treatments (control, slow inbred, fast inbred) of heritability h^2, additive genetic variance V_A environmental variance V_E, and phenotypic variance V_P.

Treatment	h^2	V_A	V_E	V_P
Control	0.42	0.75	1.03	1.78
Slow inbred	0.20	0.29	1.13	1.42
Fast inbred	0.12	0.18	1.25	1.43

Discussion and conclusions

The overall objective of this study was to test whether inbreeding and the rate of inbreeding impacts on heritabilities and variance components for sternopleural bristle number according to expectations based on selective neutrality and additive gene action. This does not seem to be the case. This may be explained invoking the interaction between selection operating on genes of large effect, and genetic drift. As for many other quantitative traits, natural selection appears to favour individuals with sternopleural bristle number in the middle of the phenotypic range. Further, stabilizing selection has been shown to operate on sternopleural bristle number and on other correlated traits in *D. melanogaster* (López-Fanjul and Hill 1973; Gibson and Bradley 1974). The presence of alleles of large effect affecting sternopleural bristle number has been documented by Dilda and Mackay (2002). In the fast inbred treatment, selection has relatively less opportunity to operate than random drift, thereby resulting in more or less random fixation of alleles conferring very small or very large number of bristles. This results in a relatively small variance within lines. On the other hand, selection is likely to be more effective in the slow inbred lines. This favors the heterozygote genotype which restrains the fixation process leading to a relatively larger variance within lines.

A number of conclusions can be drawn from this study on sternopleural bristle numbers in *D. melanogaster*. 1: On average, inbreeding reduces the heritability and additive genetic variance within lines and thereby the potential for within line evolution and genetic gain. 2: In disagreement with the postulate of selective neutrality and additive gene action fast inbreeding reduces the additive genetic variance and the heritability within lines more than slow inbreeding, in lines inbred to the same absolute level of inbreeding. 3: The phenotypic variance within lines decreases with inbreeding but is not affected by the rate of inbreeding. 4: Environmental sensitivity increases with inbreeding and is higher in fast compared to slow inbred lines. 5:

Inbreeding depression was not observed for sternopleural bristle number, so the trait is not likely affected by directional dominance.

References

Bryant, E.H., & Meffert, L.M. 1993. The effect of serial founder-flush cycles on quantitative genetic variation in the housefly. *Heredity* 70: 122-129.

Bryant, E.H. & Meffert, L.M. 1995. An analysis of selectional response in relation to a population bottleneck. *Evolution* 49: 626-634.

Bryant, E.H. & Meffert, L.M. 1996. Non-additive genetic structuring of morphometric variation in relation to a population bottleneck. *Heredity* 77: 168-176.

Bryant, E.H., McCommas, S.A. & Combs, L.M. 1986. The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. *Genetics* 114: 1191-1211.

Dilda, C.L. & Mackay, T.F.C. 2002. The genetic architecture of *Drosophila* sensory bristle number. *Genetics* 162: 1655-1674.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to quantitative genetics*. Longman, Harlow.

Fernández, A., Toro, M.A. & López-Fanjul, C. 1995. The effect of inbreeding on the redistribution of genetic variance of fecundity and viability in *Tribolium castaneum*. *Heredity* 75: 376-381.

Fowler, K. & Whitlock, M.C. 1999. The distribution of phenotypic variance with inbreeding. *Evolution* 53: 1143-1156.

Gibson, J.B. & Bradley, B.P. 1974. Stabilizing selection in constant and fluctuating environments. *Heredity* 33: 293-302.

Goodnight, C.J. 1988. Epistasis and the effect of founder events on the additive genetic variance. *Evolution* 42: 441-454.

Lerner, I.M. 1954. *Genetic homeostasis*. Oliver and Boyd, Edinburgh.

López-Fanjul, C & Hill, W.G. 1973. Genetic differences between populations of *Drosophila melanogaster* for a quantitative trait. 2. wild and laboratory populations. *Genet. Res.* 22: 69-78.

López-Fanjul, C., Guerra, J. & García, A. 1989. Changes in the distribution of the genetic variance of a quantitative trait in small populations of *Drosophila melanogaster*. *Genet. Sel. Evol.* 21: 159-168.

López-Fanjul, C., Fernández, A. & Toro, M.A. 1999. The role of epistasis in the increase in the additive genetic variance after population bottlenecks. *Genet. Res.* 73: 45-59.

López-Fanjul, C., Fernández, A. & Toro, M.A. 2000. Epistasis and the conversion of non-additive genetic variance at population bottlenecks. *Theor. Popul. Biol.* 58: 49-59.

López-Fanjul, C., Fernández, A. & Toro, M.A. 2002. The effect of epistasis on the excess of the additive and nonadditive variances after population bottlenecks. *Evolution* 56: 865-876.

Robertson, A. 1952. The effect of inbreeding on the variation due to recessive genes. *Genetics* 37: 189-207.

Sorensen, D. and Gianola, D. 2002. *Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics*. Springer-Verlag, New York.

Whitlock, M.C. & Fowler, K. 1999. The changes in genetic and environmental variance with inbreeding in *Drosophila melanogaster*. *Genetics* 152: 345-353.

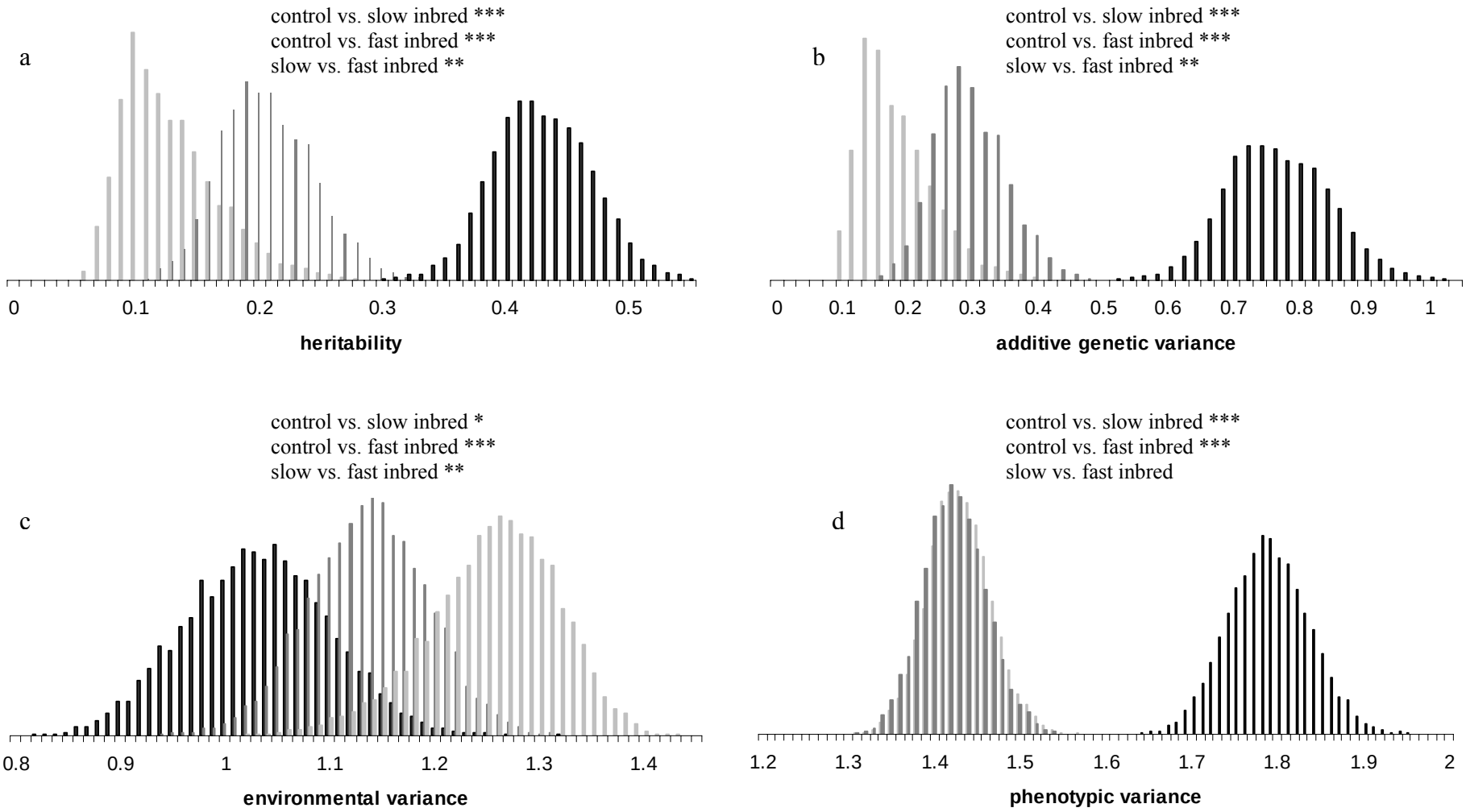

Figure 1. Histograms of Markov Chain Monte Carlo samples from the marginal posterior distributions of a) h^2, b) V_A, c) V_E and d) V_P in control (black bars), slowly inbred (dark grey bars), and fast inbred (light grey bars) lines. The significance level for each contrast tested (more formally, the posterior probability that the contrast has a mean of zero) is indicated above the histograms (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).