Maintenance of genetic variation in phenotypic plasticity: the role of environmental variation

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Summary

We study genetic variation in phenotypic plasticity maintained by a balance between mutation and weak stabilizing selection. We consider linear reaction norms allowing for spatial and/or temporal variation in the environments of development and selection. We show that the overall genetic variation maintained does not depend on whether the trait is plastic or not. The genetic variances in height and slope of a linear reaction norm, and their covariance, are predicted to decrease with the variation in the environment. Non-pleiotropic loci influencing either height or slope are expected to decrease the genetic variance in slope relative to that in height. Decrease in the ratio of genetic variance in slope to genetic variance in height with increasing variation in the environment presents a test for the presence of loci that only influence the slope, and not the height. We use data on *Drosophila* to test the theory. In seven of eight pair-wise comparisons genetic variation in reaction norm is higher in a less variable environment than in a more variable environment, which is in accord with the model's predictions.

1. Introduction

Experimental work on phenotypic plasticity has often concentrated on the mean values of different population characteristics such as the mean height or mean slope of a reaction norm (for a survey see Schlichting & Pigliucci, 1998; Pigliucci *et al.*, 1997; Morin *et al.*, 1997; Moreteau *et al.*, 1997). Yet, genetic variation in phenotypic plasticity within and between populations has repeatedly been observed, using split families (Windig, 1994), isofemale lines (David *et al.*, 1994; de Moed *et al.*, 1997) and populations of different geographic origin (van't Land *et al.*, 1999; Morin *et al.*, 1999). Genetic variation in phenotypic plasticity, i.e. in the slope of a reaction norm, will be apparent in the genotype–environment (G∗E) interaction component of variance (Fry, 1992; Scheiner, 1993; de Jong, 1990, 1995; Windig, 1997). The extent of G∗E interaction differs between populations. Both Noach *et al.* (1996) in a *Drosophila melanogaster* population from Tanzania, and Karan *et al.* (1999)) in a *D.*

melanogaster population from southern France, found significant G∗E interaction for wing length. The study of Karan *et al.* (1999) showed lower genetic variation in slope of the reaction norm for wing length, but higher genetic variation for reaction norm height, than was found by Noach *et al.* (1996).

The *D. melanogaster* data point to important questions about the patterns of genetic variation in height and slope that should be expected theoretically and about the role that the variation in environment may play in these patterns. As with experimental work, most theoretical work on phenotypic plasticity has concentrated on the *mean* values of different population characteristics (e.g. mean height or slope of a reaction norm). The corresponding *variances* and *covariances* of these characteristics are usually treated as externally determined fixed parameters. There are only a couple of studies that have incorporated the dynamics of genetic variances of phenotypically plastic traits into a modelling framework. Gavrilets (1986, 1988) studied the maintenance of genetic variation in linear reaction norms by a balance between mutation and stabilizing selection in an environment varying in time. Via & Lande (1987) analysed mutation–selection

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balance in a plastic trait but did not treat environmental variation in any explicit form. Gavrilets & Hastings (1994) considered transient changes in genetic variances of plastic traits brought about by linkage disequilibrium generated by selection. In contrast, there has been extensive theoretical work on different aspects of genetic variation for non-plastic quantitative traits (for reviews see Bürger, 1998; Roff, 1997).

This paper has two general and one specific objective. The first general objective is to emphasize the need for detailed theoretical and experimental studies of genetic variation in phenotypic plasticity. The second one is to relate theoretical studies of phenotypic plasticity with an extensive modelling framework developed for non-plastic traits. The specific objective of this paper is to consider a model for the maintenance of genetic variation in plasticity by a balance of mutation and selection and to relate this model's predictions to experimental data.

2. Model

(i) Description

We consider a diploid population with non-overlapping generations that inhabits a set of 'microhabitats'. Adults leave the microhabitats, form a mating pool and mate at random with respect to both genotype and microhabitats. Zygotes settle in a microhabitat for development. We allow for variation of environmental conditions both in space (i.e. between the microhabitats) and in time (i.e. within a microhabitat). For each individual, we differentiate between its environment of development x (at which a quantitative trait z is developed) and its environment of selection y (at which the individual's viability w is determined). We assume that the distribution of x and y across the system of microhabitats does not change between the generations and has means $\bar{x}$, $\bar{y}$, variances $\mathrm{var}(x)$, $\mathrm{var}(y)$ and covariance $\mathrm{cov}(x, y)$. Without loss of generality the average of x over the whole set of microhabitats can be set to zero: $\bar{x} = 0$.

Here, we consider a model for linear reaction norms:

$$z_x = g_0 + g_1 x + e, \tag{1}$$

where g_0 and g_1 are the genotypic values, and e is an independent stochastic deviation with zero mean and a constant variance E (e.g. Gavrilets, 1986, 1988; de Jong, 1995, 1999; Gavrilets & Scheiner, 1993). We will interpret g_0 and g_1 as the 'height' and the 'slope' of the reaction norm. We will use $\bar{g}_0$, $\bar{g}_1$ for the mean values, G_0, G_1 for the variances, and C for the covariance of the distribution of g_0 and g_1 in the population. We assume that stabilizing selection

operates within each microhabitat. Fitness (viability) is described by a Gaussian fitness function:

$$w = \exp\left\{-\frac{(z-\theta)^2}{2V_{s,y}}\right\}, \tag{2}$$

where the parameter $s_y = 1/(2V_{s,y})$ characterizing the strength of selection and the optimum phenotype $\theta = \theta(y)$ can vary between microhabitats. If the optimum varies, we will occasionally assume a linear function according to $\theta(y) = c_0 + c_1 y$, where c_0 and c_1 represent the optimum height and slope, respectively. We assume that microhabitats contribute to a pool of zygotes that disperse randomly across microhabitats to form the next generation.

(ii) Multi-locus model for diallelic loci

We will study the behaviour of this system using a population genetic model operating in terms of allele frequencies. We assume that there are n diallelic loci with alleles $\mathbf{A}_i$ and $\mathbf{a}_i$ that contribute additively to the trait value. The contribution of allele $\mathbf{A}_i$ is $+0.5\gamma_{i,x}$ whereas that of allele $\mathbf{a}_i$ is $-0.5\gamma_{i,x}$ in environment x. We will assume that locus contributions $\gamma_{i,x}$ change as *linear* functions of the variable x characterizing the environment of development: $\gamma_{i,x} = \alpha_i + \beta_i x$, where α_i and β_i are allelic contributions to the 'height' and 'slope', respectively (cf. de Jong, 1990, 1995, 1999; Gavrilets & Hastings, 1994). Let p_i be the frequency of allele $\mathbf{A}_i$, $q_i = 1 - p_i$. We will assume approximate linkage equilibrium. This assumption implies that selection is weak relative to recombination: $1/s_y = 2V_{s,y} \gg 1$. The population-level characteristics $\bar{g}_0$, $\bar{g}_1$, G_x, G_0, G_1 and C can be represented in terms of allele frequencies and the contributions of individual loci:

$$\bar{g}_0 = \Sigma_i \alpha_i(p_i - q_i), \quad \bar{g}_1 = \Sigma_i \beta_i(p_i - q_i)$$

$$G_0 = \Sigma_i 2\alpha_i^2 p_i q_i, \quad G_1 = \Sigma_i 2\beta_i^2 p_i q_i, \quad C = \Sigma_i 2\alpha_i \beta_i p_i q_i$$

(iii) Allele frequency change

Under approximate linkage equilibrium, the change in allele frequency p_i in one generation as caused by selection and mutation with rate μ_i (assuming equal forward and backward rates) is

$$\Delta p_i = \frac{p_i q_i}{2\bar{w}} \frac{\partial \bar{w}}{\partial p_i} + \mu_i(q_i - p_i), \tag{3}$$

where $\bar{w}$ is the mean fitness of the population (Wright, 1935; Barton, 1986). With quadratic stabilizing selection and hard selection, the mean fitness of the population can be written as

$$\bar{w} = E\{1 - s_y((c_0 - \bar{g}_0) - (c_1 y - \bar{g}_1 x))^2$$
$$- s_y(G_0 + 2xC + x^2 G_1)\}. \tag{4a}$$

With Gaussian stabilizing selection and soft selection, the logarithm of the mean fitness of the population can be written as

$$\ln \bar{w} = E\{-s_y((c_0 - \bar{g}_0) - (c_1 y - \bar{g}_1 x))^2$$
$$- s_y(G_0 + 2xC + x^2 G_1)\}, \quad (4b)$$

where $E\{\ldots\}$ denotes the mathematical expectation over the set of microhabitats. Hard or soft selection does not influence our results.

(iv) *Selection on linear reaction norm at equal selection strength in all environments*

Assume first that the selection intensity $s = 1/(2V_s)$ is the same in all environments. A straightforward computation shows that in our model the allele frequencies dynamics are given by the following equation:

$$\Delta p_i = -\frac{1}{2V_s} p_i q_i [(\alpha_i^2 + \beta_i^2 \operatorname{var}(x))(q_i - p_i) + 2\alpha_i(\bar{g}_0 - \theta)$$
$$+ 2\beta_i^2(\bar{g}_0 \operatorname{var}(x) - \operatorname{cov}(x, \theta))] + \mu_i(q_i - p_i). \quad (5)$$

Let the deviations of the mean height and mean slope from $\bar{\theta}$ and $\operatorname{cov}(\theta, x)/\operatorname{var}(x)$, respectively, be much greater than the corresponding contributions of the individual loci: $\bar{g}_0 - \bar{\theta} \gg \alpha_i$ and $\bar{g}_1 - \operatorname{cov}(x, \theta)/\operatorname{var} x \gg \beta_i$. This corresponds to initial stages of the dynamics away from equilibrium. In this case, the first and last terms in the right-hand side of (5) can be neglected relative to the second and third terms. The changes in the mean height and slope of the reaction norm induced by changes in allele frequencies are $\Delta \bar{g}_0 = \Sigma 2\alpha_i \cdot \Delta p_i$ and $\Delta \bar{g}_1 = \Sigma 2\beta_i \cdot \Delta p_i$. Using matrix notation, these equations can be represented as

$$\begin{bmatrix} \Delta \bar{g}_0 \\ \Delta \bar{g}_1 \end{bmatrix} = \begin{bmatrix} G_0 & C \operatorname{var}(x) \\ C & G_1 \operatorname{var}(x) \end{bmatrix} \begin{bmatrix} \bar{g}_0 - \bar{\theta} \\ \bar{g}_1 - \dfrac{\operatorname{cov}(x, \theta)}{\operatorname{var}(x)} \end{bmatrix}. \quad (6)$$

Equation (6) shows that the population evolves towards a state with

$$\bar{g}_0 = \bar{\theta}, \quad (7a)$$

$$\bar{g}_1 = \frac{\operatorname{cov}(x, \theta)}{\operatorname{var}(x)} = r \sqrt{\frac{\operatorname{var}(\theta)}{\operatorname{var}(x)}}, \quad (7b)$$

where r is the correlation of x and θ. If the optimal reaction norm is linear, i.e. $\theta(y) = c_0 + c_1 y$, mean reaction norm height $\bar{g}_0$ evolves to optimal reaction norm height c_0, and mean reaction norm slope $\bar{g}_1$ evolves to $\bar{g}_1 = c_1 \operatorname{cov}(x, y)/\operatorname{var}(x)$ (de Jong, 1999). That is, the mean height of the reaction norms is expected to match the phenotypic optimum, whereas the mean slope deviates from the optimal slope. The evolved mean slope equals the optimum slope times

the regression of the environment of selection, y, on the environment of development, x. In an alternative expression, the evolved mean slope is proportional to the correlation between the environment of development, x, and the optimum phenotype and the squared root of the ratio of variances in the phenotypic optima and the environment of development. These conclusions correspond to those based on simple quantitative genetic models that assumed bivariate normality of the distribution of g_0 and g_1 in the population (Gavrilets, 1988; Gavrilets & Scheiner, 1993; de Jong, 1999).

(v) *Genetic variance under mutation–selection balance*

If there is no mutation ($\mu_i = 0$), the population will eventually reach a state with genetic variation maintained in no more than a single locus. (This can be proven using Result 3 in Zhivotovsky & Gavrilets (1992).) Obviously, mutation will maintain genetic variation in all n loci. Assuming that the population has evolved to a state where both (7a) and (7b) are true, allele frequencies do not change ($\Delta p_i = 0$) if $p_i = q_i = 1/2$ or

$$p_i q_i = \frac{2\mu_i V_s}{\alpha_i^2 + \beta_i^2 \operatorname{var}(x)}. \quad (8)$$

Polymorphic equilibria with some p_i at one-half should be unstable unless mutation rates are very high. Thus, for small mutation rates the allele frequencies at the mutation–selection balance equilibrium are defined by (8).

Using (8), the equilibrium genetic variances and covariance at mutation–selection balance are:

$$G_0^* = 4V_s \sum \mu_i \frac{\alpha_i^2}{\alpha_i^2 + \beta_i^2 \operatorname{var}(x)}, \quad (9a)$$

$$G_1^* = 4V_s \sum \mu_i \frac{\beta_i^2}{\alpha_i^2 + \beta_i^2 \operatorname{var}(x)}, \quad (9b)$$

$$C = 4V_s \sum \mu_i \frac{\alpha_i \beta_i}{\alpha_i^2 + \beta_i^2 \operatorname{var}(x)}. \quad (9c)$$

Here, the covariance C between g_0 and g_1 arises only because of the loci that have pleiotropic effects on both the height and slope of the reaction norm. In principle, linkage disequilibrium, which is neglected here, could contribute to covariance C. Equations (9) are similar to those obtained earlier assuming bivariate normality of the distribution of g_0 and g_1 in the population (Gavrilets, 1988).

The total genotypic variance of a plastic trait over the whole set of microhabitats is

$$G_{\text{tot}} = G_0 + G_1 \operatorname{var}(x) + \bar{g}_1^2 \operatorname{var}(x),$$

with $G = G_0 + G_1 \operatorname{var}(x)$ due to genetic variation and $\bar{g}_1^2 \operatorname{var}(x)$ due to plasticity even if no genetic variation is present (de Jong, 1990; Scheiner, 1993). Note that covariance C does not enter these equations because of our scaling assumption that $\bar{x} = 0$. For a non-plastic trait (that is if $\beta_i = 0$ for all i), g_1, G_1 and C all equal zero, resulting in $G_{tot} = G = G_0$; at a balance of mutation and stabilizing selection

$$G_{tot} = G^* = G_0^* = 4\mu V_s, \tag{10}$$

where $\mu = \Sigma_i \, \mu_i$ is the rate of mutation per gamete. This is the standard rare-allele approximation for the variance maintained by mutation in non-plastic traits (reviewed in Bürger, 1998). For a plastic trait and equal selection intensity in all environments, the genetic variance is

$$G^* = G_0^* + G_1^* \operatorname{var}(x) = 4\mu V_s, \tag{11}$$

which is the same as given by (10). Equations (9) and (11) show as expected that increasing the mutation rate and/or decreasing the strength of stabilizing selection increases the total genotypic variance G_{tot} the genetic variance G^* and genetic variation in reaction norms characterized by G_0, G_1 and C. Increasing the variation in the environment of development ($\operatorname{var}(x)$) decreases the evolved genetic variances in the height and slope of reaction norms (and their covariance). The variation in the environment of selection ($\operatorname{var}(\theta) = c_1 \operatorname{var}(y)$) does not affect the equilibrium values G_0^*, G_1^* and C^*.

(vi) Linear reaction norm at unequal selection strength in all environments

Assuming that the strength of stabilizing selection varies between microhabitats (Zhivotovsky et al., 1996) modifies the formulae derived above but does not result in any qualitatively new effects. It is convenient to re-scale the environmental variable x so that the mean value of the product of the selection strength s_y and x over the whole set of microhabitats is zero: $E\{s_y x\} = 0$. (This is an analogue of the assumption $\bar{x} = 0$ made above for analysing the case of constant s_y.)

The analogues of (7) are

$$\bar{g}_0 = E\{s_y \theta\}/E\{s_y\}, \quad \bar{g}_1 = \operatorname{cov}(s_y x, \theta)/\operatorname{cov}(s_y x, x),$$

and the corresponding analogue of (8) is

$$p_i q_i = \frac{\mu_i}{E\{s_y\}} \frac{1}{\alpha_i^2 + \beta_i^2 \operatorname{cov}(s_y x, x)/E\{s_y\}}. \tag{12}$$

For a plastic trait and unequal selection intensity over environments, the genetic variance under mutation–selection balance is

$$G_0^* E\{s_y\} + G_1^* \operatorname{cov}(s_y x, x) = 2\mu. \tag{13}$$

Equation (13) is analogous to (11). Greater variation in the environment x reduces the genetic variance in height and slope.

3. Numerical examples

(i) Alternative equilibria

Above, we considered the genetic variance maintained in linear reaction norms assuming that the mean height and slope have evolved towards the optimum values. For a non-plastic trait, it is known that at a mutation–selection balance, mean genotypic values can slightly deviate from the optimum (Barton, 1986; Hastings, 1990). At such a non-optimum equilibrium, the genetic variance can be several times higher than when the mean genotypic value is exactly at the optimum. We will demonstrate this property of the mutation–selection balance in the case of selection on a phenotypically plastic trait by iterating (7) numerically.

In all simulations, the number of diallelic loci is $n = 100$, selection strength is given by $2V_s = 20$, mutation rate per locus per generation is $\mu_i = 10^{-5}$, and genomic mutation rate $\mu = 10^{-3}$. The expected genetic variance is therefore $G^* = G_0^* + G_1^* \operatorname{var}(x) = 4\mu V_s = 0.04$. The optimum phenotype is given by $\theta(y) = c_0 + c_1 y$, with optimum height $c_0 = 10$ and optimum slope $c_1 = 1$. The genotypic values for the heterozygote $A_i a_i$ are $g_{0,Aa,i} = z_0/n$ for the height and $g_{1,Aa,i} = z_1/n$ for the slope. The genotypic values for homozygote $A_i A_i$ are $g_{0,AA,i} = z_0/n + \alpha_i$ and $g_{1,AA,i} = z_1/n + \beta_i$. Here, $\alpha_i = z_0/n + az_0/n \ (U_{a,i} - 0.5)$ and $\beta_i = z_1/n + az_1/n \ (U_{\beta,i} - 0.5)$, where $U_{\alpha,i}$ and $U_{\beta,i}$ are independently drawn from a uniform distribution $(0, 1)$, and the variable a is used to scale the random effect. The genotypic values for homozygote $a_i a_i$ are $g_{0,aa,i} = z_0/n - \alpha_i$ and $g_{1,aa,i} = z_1/n - \beta_i$. Note that the expected genotypic values for genotype $A_i A_i$ are $E\{g_{0,AA,i}\} = 2z_0/n$ for height and $E\{g_{1,AA,i}\} = 2z_1/n_i$ for slope. The range of the mean genotypic value in the population is $0 \leqslant \bar{g}_0 \leqslant 2z_0$ for height, and $0 \leqslant \bar{g}_1 \leqslant 2z_1$ for slope. The correlation over loci between allelic values α_i and β_i is zero, but the genetic covariance C between height and slope is not. Initially, each allele frequency is at $p_i = 1$ or $p_i = 0$. The iterations were stopped when the change in G_0 was less than 10^{-7} per generation.

Mean reaction norm height, mean reaction norm slope, mean fitness and genotypic variance depend on the number of loci starting at $p_i = 1$ (Fig. 1). For this set of simulations, $\operatorname{var}(x) = 1$ and $\operatorname{cov}(x, y) = 0.5$. At $z_0 = c_0 = 10$ and $z_1 = c_1 = 1$, the optimum reaction norm height c_0 and optimum slope c_1 are both reached with 50 loci starting at $p = 1$. At $z_0 = 8$ and $z_1 = 1$, the optimum height is reached with 63 loci starting at $p = 1$ while the equilibrium slope $\bar{g}_1 = c_1 \operatorname{cov}(x, y)/\operatorname{var}(x) = 0.5$ is reached with 50 loci starting at $p = 1$.
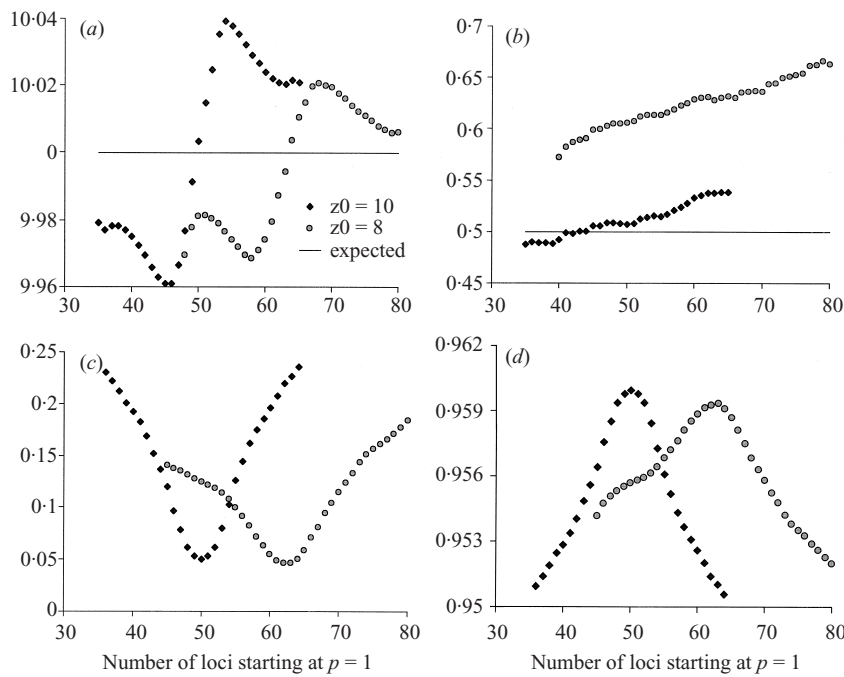
Fig. 1. Mutation–selection balance equilibrium for a phenotypically plastic trait. Mean reaction norm height, mean reaction norm slope, mean fitness and genotypic variance depend upon the number of loci starting at $p_i = 1$. Selection on reaction norm height overrules selection on slope as the allelic values for height are much larger. Alternative equilibria for mean genotypic value occur. Variation in the environment equals $\text{var}(x) = 1$. Allelic values are $\alpha_i = z_0/n \cdot (0.5 + U_{\alpha,i})$ and $\beta_i = z_1/n \cdot (0.5 + U_{\beta,i})$ (the parameter $a = 1.0$). Averages of five runs. (A) Mean height $\bar{g}_0$; (B) mean slope $\bar{g}_1$; (C) genetic variance $G^* = G_0^* + G_1^* \text{var}(x)$; (D) mean fitness.

Selection on height overrules selection on slope as the allelic contributions to g_0 are about 10 times larger than those to g_1 (Fig. 1A). As a consequence, the evolved slope is drawn away from its equilibrium value of $\bar{g}_1 = c_1 \text{cov}(x,y)/\text{var}(x) = 0.5$, towards a value given by the number of loci starting at $p_i = 1$ for which the optimum g_0 is reached at $z_0 = 8$ (Fig. 1B). Here, pleiotropy is acting as a constraint.

Alternative equilibria are found for $\bar{g}_0$ when a larger or a smaller number of loci than $n = 50$ (for $z_0 = 10$) or $n = 63$ (for $z_0 = 8$) start at $p_i = 1$. The alternative equilibria are very near to the optimum equilibrium: the difference in height is less than one allelic value (Fig. 1A, B). The lowest genetic variances G_0^*, G_1^* and $G^* = G_0^* + G_1^* \text{var}(x)$ (Fig. 1C) and the highest mean fitness (Fig. 1D) are found when the evolved value of $\bar{g}_0$ coincides with the optimum value. At the alternative equilibria, a lower mean fitness and higher genetic variances, in height and in slope, are found. The increase in genetic variance at the alternative equilibria can be up to 7 times the predicted equilibrium genetic variance, which is similar to the situation found by Barton (1986) for a non-plastic trait. The alternative equilibria are more prominent with no random effects in the genotypic values, that is, at $a = 0$. With higher random effects, e.g. with $a > 2.0$, the alternative equilibria fade out, and the genetic variance settles at a level somewhat lower than predicted.

Alternative equilibria are not found when the genotypic values for the homozygote $\mathbf{A}_i \mathbf{A}_i$ are $g_{0,AA,i} = z_0/n + \alpha_i$ and $g_{1,AA,i} = z_1/n + \beta_i$, with $\alpha_i = z_0/n (U_{\alpha,i} - 0.5)$ and $\beta_i = z_1/n (U_{\beta,i} - 0.5)$, that is, when the expected genotypic values for both homozygotes equal the genotypic value of the heterozygote. If so, the genetic variance is always somewhat lower than expected from (11).

(ii) *Varying levels of pleiotropy and environmental variation*

Not all loci need be pleiotropic, influencing both height and slope. Let us assume that n_0 loci only control the height of the reaction norm ($\alpha_i \neq 0$, $\beta_i = 0$), n_1 loci only control the slope ($\alpha_i = 0$, $\beta_i \neq 0$), while n_p loci are pleiotropic ($\alpha_i \neq 0$, $\beta_i \neq 0$). The genotypic variances for reaction norm height and slope become:

$$G_0^* = 4V_s \sum_{n_0} \mu_i + 4V_s \sum_{n_p} \mu_i \frac{\alpha_i^2}{\alpha_i^2 + \beta_i^2 \text{var}(x)},$$

$$G_1^* = 4V_s \frac{1}{\text{var}(x)} \sum_{n_1} \mu_i + 4V_s \sum_{n_p} \mu_i \frac{\beta_i^2}{\alpha_i^2 + \beta_i^2 \text{var}(x)}.$$

For those loci that only influence slope, the variance in the environment $\text{var}(x)$ functions as a component of the selection strength. In Fig. 2, the effect of the
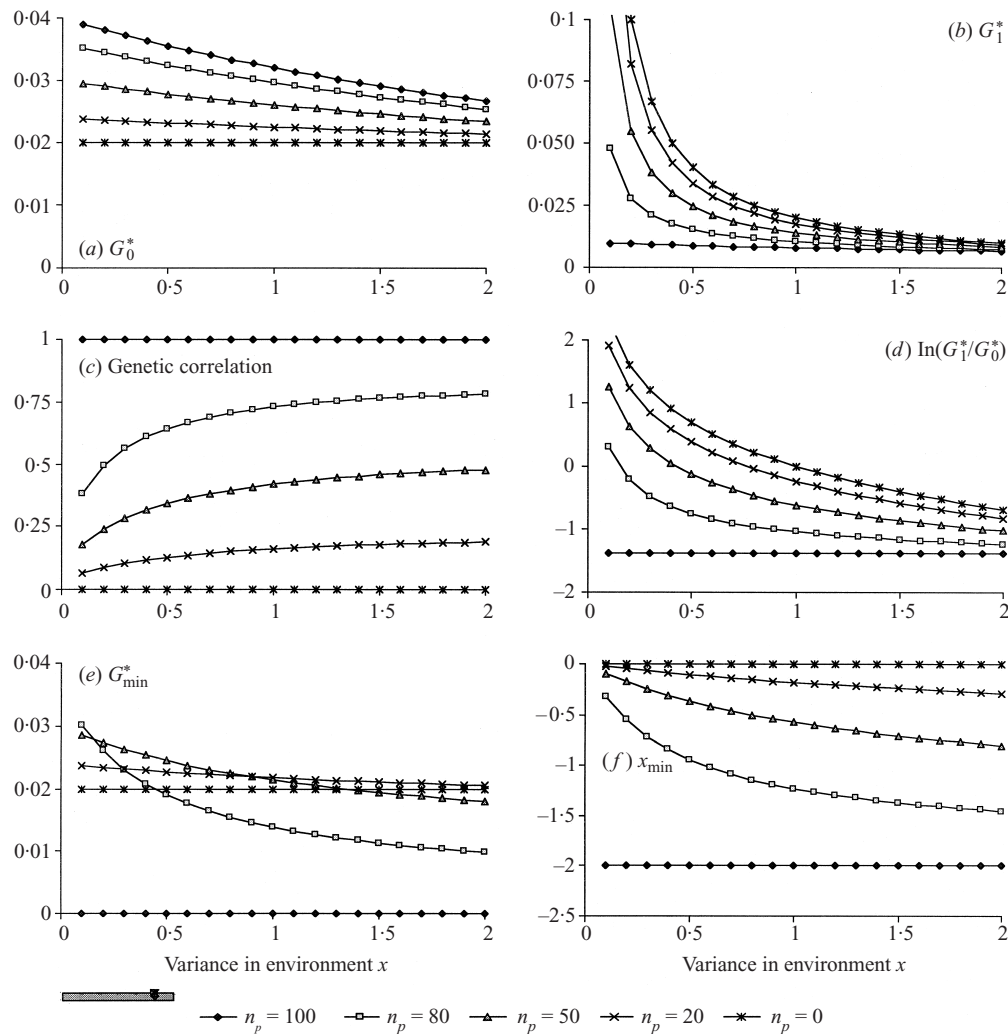
Fig. 2. The influence of variation in the environment and pleiotropy on genetic variances under a mutation–selection balance. Loci might be pleiotropic (n_p loci), or coding for height ($n_0 = (100 - n_p)/2$ loci) or slope ($n_1 = (100 - n_p)/2$ loci); the number of pleiotropic loci n_p is indicated by different symbols. Allelic values are identical over loci, at $\alpha_i = 0.1$ for height and at $\beta_i = 0.05$ for slope. (A) Equilibrium genetic variance in height; (B) equilibrium genetic variance in slope; (C) equilibrium genetic correlation; (D) ratio of equilibrium genetic variances in height and slope; (E) minimum genetic variance; (F) environment of minimum genetic variance.

variation in the environment of development on the genetic variances in height G_0^* and slope G_1^*, the logarithm of the ratio G_1^*/G_0^*, and the genetic correlation between height and slope, $C^*/\sqrt{G_0^* G_1^*}$, are given for different numbers of pleiotropic loci with equal allelic values. The genetic variances in height and slope decrease with the environmental variation (Fig. 2A, B). If some loci influencing only the slope are present, the genetic variance in slope decreases faster with the environmental variance than the genetic variance in height (Fig. 2C). The decrease in the logarithm of the ratio G_1^* to G_0^* is observed only if there are non-pleiotropic loci. The genetic correlation between height and slope too depends on the number of pleiotropic loci (Fig. 2D).

Genetic variation among linear reaction norms causes the genotypic variance in the phenotypically plastic trait to be a quadratic function of the

environment (de Jong, 1990). The minimum of the genetic variance in the trait equals $G_{min} = G_0^* - C^{*2}/G_1^*$, and is found at $x_{min} = -C^*/G_1^*$. When all loci are pleiotropic and have equal allelic values, $G_{min} = 0$ and $x_{min} = -\alpha/\beta$. When some loci are not pleiotropic but only code for reaction norm height or reaction norm slope, G_{min} and x_{min} depend upon the variation in the environment. The position of the minimum variance, x_{min}, is at an environmental value x higher than $\bar{x} = 0$ if the covariance C is negative, but lower if the covariance C is positive. Examples of G_{min} and x_{min} are plotted in Fig. 2E and F.

4. Comparison with data

Two studies might provide enough detail on the environment and on phenotypic plasticity to attempt to compare the model with data. Barker & Krebs

Table 1. *Genetic variance and covariance in height and slope of isofemale lines. The genetic correlation, r_G, between height and slope is tested for difference from zero*

Species	Locality	Sex	$T = 0$	G_0	C	G_1	r_G	P	Significance
D. aldrichi	Dixalea		$21 \cdot 6^c$	33·188	$-1 \cdot 705$	0·220	$-0 \cdot 631$	0·050	NS
D. aldrichi	Oxford Downs		$22 \cdot 1^c$	45·729	$-4 \cdot 050$	0·632	$-0 \cdot 753$	0·012	*
D. buzzatii	Dixalea		$21 \cdot 6^c$	21·511	$-1 \cdot 404$	0·501	$-0 \cdot 428$	0·217	NS
D. buzzatii	Oxford Downs		$22 \cdot 1^c$	22·119	$-2 \cdot 338$	0·670	$-0 \cdot 607$	0·063	NS
D. melanogaster	France	F	20^d	16·890	$-0 \cdot 908$	0·207	$-0 \cdot 486$	0·016	*
D. melanogaster	France	M	20^d	10·872	$-0 \cdot 731$	0·273	$-0 \cdot 424$	0·039	*
D. melanogaster	Tanzania	F	$22 \cdot 7^c$	19·854	$-1 \cdot 216$	0·322	$-0 \cdot 481$	0·017	*
D. melanogaster	Tanzania	M	$22 \cdot 7^c$	13·622	$-0 \cdot 438$	0·123	$-0 \cdot 338$	0·106	NS

Drosophila aldrichi[a] and *D. buzzatii*[a]: trait = ln(wing length/thorax length).
D. melanogaster[b]: trait = wing length.
* $P < 0.05$.
[a] Data from Barker & Krebs (1995).
[b] Data from Noach *et al.* (1996).
[c] Yearly-average temperature.
[d] Indication for growing season.

Table 2. *Relative size of genetic variance in height and slope, minimum in genetic variance over temperatures and temperature of minimum genetic variance. Genetic variances are computed over isofemale lines*

Species	Locality	Sex	$T = 0$	T_{min}	G_{min}	$\ln(G_1/G_0)$
D. aldrichi	Dixalea		$21 \cdot 6^c$	29·4	19·47	$-5 \cdot 02$
D. aldrichi	Oxford Downs		$22 \cdot 1^c$	28·5	19·78	$-4 \cdot 28$
D. buzzatii	Dixalea		$21 \cdot 6^c$	24·4	17·58	$-3 \cdot 76$
D. buzzatii	Oxford Downs		$22 \cdot 1^c$	25·6	13·96	$-3 \cdot 50$
D. melanogaster	France	F	20^d	24·4	12·91	$-4 \cdot 40$
D. melanogaster	France	M	20^d	22·7	8·91	$-3 \cdot 68$
D. melanogaster	Tanzania	F	$22 \cdot 7^c$	26·5	15·26	$-4 \cdot 12$
D. melanogaster	Tanzania	M	$22 \cdot 7^c$	26·3	12·06	$-4 \cdot 71$

[a, b, c, d] As Table 1.

(1995) studied isofemale lines from two Australian populations of the cactus drosophilas *Drosophila buzzatii* and *D. aldrichi*. They showed the logarithm of the wing/thorax ratio (ln(W/T)) to be linear over the temperature range 18–31 °C. Noach *et al.* (1996) compared phenotypic plasticity in isofemale lines from two populations of *Drosophila melanogaster*. They showed that wing length of *D. melanogaster* is linear with temperature from 17·5 °C to 27·5 °C; overall wing length is greater and of more negative slope with temperature in the French population than in the Tanzanian population. Genotype by environment interaction over isofemale lines was statistically significant in *D. buzzatii* and *D. aldrichi*, and in the Tanzanian population of *D. melanogaster*. Here, Barker & Krebs' (1995) data on ln(W/T) and Noach *et al.*'s (1996) data on wing length are re-analysed; results are presented in Tables 1 and 2.

The data presented in Table 1 show that the between-isofemale-line genetic correlations between height and slope are negative at the yearly-average temperature taken as $T = 0$. In both *D. buzzatii* and *D. aldrichi*, the genetic variation in height and in slope of the regression of ln(W/T) on temperature is lower in the locality Dixalea than in the locality Oxford Downs, in agreement with a higher variability of temperature in Dixalea. The ratio G_1/G_0 is higher in *D. buzzatii* than in *D. aldrichi*, and more negative at the locality Dixalea than at Oxford Downs; again, this might be in agreement with a higher variability of temperature in Dixalea. The temperature of minimum variance is higher in *D. aldrichi* than in *D. buzzatii*, but does not differ between localities for the two cactus drosophilas. The data might indicate that in *D. buzzatii* and *D. aldrichi* some loci affect reaction norm slope but not reaction norm height. In *D. melanogaster*, genetic variation for slope and height in females and for height in males is lower in France than in Tanzania, in agreement with a higher variability of temperature in France. However, the genetic variation for slope is lower in Tanzanian males than in French males. The ratio G_1/G_0 does not show any pattern.

The temperature of minimum genetic variance is higher in the *D. melanogaster* isofemale lines from Tanzania than in those from France (Table 2).

Overall, in seven of eight pair-wise comparisons genetic variation in reaction norms is higher in the less variable environment, which is in accord with the model's predictions.

5. Discussion

Our model makes several points about genetic variation maintained by mutation–selection balance and the influence of environmental variation on genetic variation in height and slope of reaction norms. At mutation–selection balance, the total genotypic variance, G_{tot}, of a plastic trait is always larger than that of a non-plastic trait. However, the genetic variances, $G^* = G_0^* + G_1^* \text{var}(x)$ of a plastic trait and a non-plastic trait are the same. This result is in agreement with general results on the maintenance of genetic variation under mutation–selection balance in pleiotropic traits. Under the house-of-cards approximation, pleiotropy reduces the variance in a trait below that predicted by a single-character analysis (Turelli, 1988). Tanaka (1996) proved that if mutations have pleiotropic effects on many characters under stabilizing selection, the weighted sum of the genetic variances of these characters always equals 2μ (his equation 15). The weighting is by the intensities of selection s_t on each trait: i.e. $\sum_t s_i G_i = 2\mu$ for t traits; for one trait, $t = 1$, which gives the classical result (reviewed in Bürger, 1998). With selection on a plastic trait, the weighting is by the effective selection intensities $E\{s_y\}$ on the reaction norm height and $\text{cov}(s_y x, x)$ on the reaction norm slope (13). Genetic load equals 2μ, whatever the number of pleiotropic traits or the distribution of genetic variances over pleiotropic traits (Tanaka, 1998). Stabilizing selection on a phenotypically plastic trait is a special case of this general result.

Phenotypic plasticity influences the allocation of the total genetic variance. This applies to the character state model too; in the character state model, the total genetic variation over all environments under mutation–selection balance becomes $E\{s_x G_x\} = 2\mu$. The genetic variance per environment in the character state model does not vary as the inverse of the selection intensity in that environment, as would be expected for a single trait. The observed genetic variance per environment depends on selection over all environments.

Our model of a phenotypically plastic trait under mutation–selection balance is related to a multi-locus diallelic model for a single non-plastic trait introduced by Wright (1935) and further analysed by Barton (1986). Barton (1986) discovered the existence of alternative equilibria with very similar mean trait values but with substantially different genotypic variances. Alternative equilibria exist for a phenotypically plastic trait as well (Fig. 1 *A*). At the alternative equilibria the genetic variances in height and slope markedly increase, even though the mean values for both height and slope do not deviate from their optima by more than one allelic effect. Alternative equilibria exist only if the allelic contributions for the $\mathbf{A}_i$ alleles have expected values of the same sign; that is, if all $\mathbf{A}_i$ alleles increase (or decrease) trait value. Random allelic values, without similar contributions of the $\mathbf{A}_i$ alleles to the trait, do not lead to alternative equilibria. The genetic variance found in iterations using random allelic values in height and slope is lower than expected (data not shown). Random allelic values lead to some noise in the ratio of G_0^* and G_1^*, but the ratio remains independent of the environment. The ratio of G_0^* and G_1^* depends upon the environment when some loci contribute only to the genetic variation in slope. Therefore, the ratio of G_0^* and G_1^* presents a potential test for the presence of pleiotropy in all loci coding for height and slope.

Under mutation–selection balance, pleiotropy proved to lead to a constraint between reaction norm height and slope. The optimal value for slope and the optimal value for height could not always be simultaneously reached for some initial conditions. The outcome of selection depended upon the size of the allelic values; higher allelic values for a trait (height or slope) led to more effective selection and carried the other trait along. The evolved genetic correlation does not equal 1. The deviation from the optimum slope is not observed in iterations if all loci are started at random allele frequencies. Migration between populations will randomize allele frequencies and restore selection towards optimum genotypic values for both height and slope.

The genetic variation in the height and slope of a linear reaction norm are not independent if height and slope are pleiotropically determined by the same loci. Genetic variance in height, G_0^*, and slope, G_1^*, both decrease with increasing variation in the environment of development, $\text{var}(x)$, but do not depend on variation in the environments of selection, $\text{var}(\theta)$. The studies of Barker & Krebs (1995) and Noach *et al.* (1996) report genetic variation in linear reaction norms in *Drosophila* species, and meteorological data about the populations' environments. Necessary for evaluating the influence of variation in the environment on the genetic variances in height and slope are the mean and variance of the environmental variable (here, temperature) influencing plasticity. In the analysis of the data presented in Tables 1 and 2, the average annual temperature was set as $\bar{x} = 0$. Overall, in seven of eight pair-wise comparisons genetic variation in height or slope was higher in the less variable environment;

this is in accord with the model's predictions. However, the meteorological annual average temperature need not be the relevant average temperature for *Drosophila* development. This leads to some uncertainties in the analysis, as the estimates of the genetic variances are sensitive to the choice of the mean temperature. Variation in the environment is gauged from the annual temperature range, the only relevant statistic available. No error margins on the estimates of the genetic variances are available. More data on the relevant environment would be necessary for a more reliable comparison of the model with data on genetic variances. Moreover, the influence of variation in the environment on genetic variances at the environmental mean might be compromised if selection intensity is not equal in each environment. Unfortunately, data on selection intensities over environments are lacking in the *Drosophila* studies.

Some patterns in genetic variation of height and slope cannot be found when only the variance of the relevant environmental variable (for instance temperature) changes. Karan *et al.* (1999) found larger genetic variation in height and smaller genetic variation in slope in their French population of *Drosophila melanogaster* than Noach *et al.* (1996) found in their Tanzanian population. The genetic variance in height and slope have to be inversely related, however, if we compare two populations with the same variation in the environment, the same selection intensity and the same mutation rate but potentially differing in allelic values for g_0 and g_1. Then, it follows from (11) that larger G_0^* has to imply a smaller G^*. Replication over populations and better environmental data are necessary to interpret patterns of genetic variation in phenotypic plasticity.

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