

Lerner's theory on the genetic relationship between heterozygosity, genomic co-adaptation, and developmental instability revisited

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ABSTRACT

Question: What is the genetic relationship between heterozygosity and developmental instability?

Methods: Using a new method for the quantification of developmental instability, we compare homozygous strains of *Drosophila mercatorum* (either parthenogenetic or highly inbred) and of their F1 progeny for the analysis of a symmetrical meristic trait (sternopleural bristle number). The method also implies the choice of samples in which the left–right correlation is close to zero.

Conclusions: In the parthenogenetic strain, developmental instability was seven times greater than in the F1 progeny. In the highly inbred strain, developmental instability was only 2.6 times greater than in the F1 progeny. The greater developmental instability in the parthenogenetic strain may be accounted for by the immediate fixation of a large number of deleterious alleles. In the inbred strain, on the other hand, progressive inbreeding is likely to have purged the genome of some of its genetic load and its most deleterious variants. The greater developmental instability in the parthenogenetic and inbred strains implies a non-linear relationship between heritability (h^2) and additive genetic variance (σ_a^2) (which are both related to evolutionary potential), which is discussed in an evolutionary context. A linear relationship between the σ_a^2 of a trait and its h^2 is expected if developmental instability is constant and independent of the σ_a^2 of that trait. If the relationship between the σ_a^2 of a trait and its developmental instability is negative, an increased σ_a^2 , which is in the numerator of the h^2 equation, will imply a reduced phenotypic variability (σ_p^2), which is in the denominator of the heritability equation. Consequently, the concomitant increase in σ_a^2 and reduction in σ_p^2 both contribute to an increase in h^2 and therefore an increase in evolutionary potential.

Keywords: admixture analysis, developmental instability, environmental variability, homeostasis, Lerner's conjecture.

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INTRODUCTION

The genetic basis of developmental instability has been debated for more than 50 years among developmental and evolutionary biologists and is still only partly understood. Developmental instability is the product of developmental noise or stress, which affects an individual's capacity to buffer the processes that otherwise result in the development of a specific phenotype (Zakharov, 1981; Palmer, 1996). The two mechanisms commonly proposed to explain developmental instability have a genome-wide basis (Clarke, 1998):

1. The genomic co-adaptation theory predicts that more balanced co-adapted gene complexes will show higher developmental stability (Lerner, 1954; see Markow, 1995, for a review). It was Lerner's view that the components of co-adaptation are: (a) co-adapted homozygosity, (b) co-adapted heterozygosity in complex polygenic systems, and (c) co-adapted interactions of alleles at loci in homologous and non-homologous chromosomes (i.e. co-adapted inter-locus interactions). On the intra-chromosomal level, co-adaptation may be of two kinds, *relational* or *internal*. The relational kind refers to the optimal combinations between homologous loci in the diploid state. Heterozygosity at a locus maintained by natural selection (i.e. over-dominance) is a form of relational co-adaptation. The internal kind relates to the accumulation of alleles along a chromosome segment or at neighbouring loci.

2. The heterozygosity theory predicts that levels of heterozygosity at loci coding for functional proteins will be inversely correlated with levels of developmental instability. Lerner suggested that multiple heterozygosity in complex multi-genic systems provides a mechanism for maintaining potential plasticity, genetic variability, and for promoting canalization (Lerner, 1954).

Investigations of the genetic basis of developmental instability, using several approaches, have yielded inconclusive results for several reasons. A commonly used approach has been to compare highly inbred parental strains with their hybrid, assuming homozygosity in the inbreds and higher heterozygosity in the hybrid. However, both the genomic co-adaptation theory and the heterozygosity theory lead to similar predictions of decreased developmental instability in F1 hybrids compared with their parental strains (Andersen *et al.*, 2002). A problem associated with the hybrid approach is that the parental strains are often not totally homozygous, since during the process of inbreeding there is potentially strong selection for heterozygotes. This leads to problems concerning dominant and epistatic interactions. Furthermore, the inverse relationship between heterozygosity (or genetic variability, σ_g^2) and developmental instability may only become apparent in certain ecological and/or population contexts, possibly because it is concealed by various exogenic or endogenic factors (for reviews, see Britten, 1996; Vøllestad *et al.*, 1999; Pertoldi *et al.*, 2006a). Finally, the lack of common agreement about the association between heterozygosity and developmental instability could partially be due to problems associated with the two estimators of developmental instability. The first, phenotypic variability (σ_p^2), which has been used in several studies (Livshits and Kobylansky, 1985; Imasheva *et al.*, 1997; Pertoldi *et al.*, 2001a), can be considered an ideal estimator of developmental instability, but only if σ_p^2 is estimated among genetically identical individuals ($\sigma_g^2 = 0$) that are reared under identical environmental conditions ($\sigma_e^2 = 0$). These conditions are of course very difficult to achieve empirically (Pertoldi *et al.*, 2001a; Kristensen *et al.*, 2003; Andersen *et al.*, 2006). The second most common estimator of developmental instability

is fluctuating asymmetry (small deviations from symmetry in bilateral characters). This measure has the advantage that dissimilarity in expression of a given character at the individual level on the left (L) and right (R) side cannot be explained by either σ_g^2 or σ_e^2 (Parsons, 1992). The estimation of developmental instability by means of fluctuating asymmetry at the population level, however, has the problems associated with among-individual heterogeneity in the degree of developmental instability (Whitlock, 1998). Among-individual heterogeneity in developmental instability can be due to the presence of σ_e^2 within the population sample and/or to different susceptibilities to stress of the genotypes comprising the population under study (Kristensen *et al.*, 2003).

As a consequence of the difficulty of investigating the genetic basis of developmental instability, the negative relationship between developmental instability and heterozygosity hypothesized by Lerner (1954), has largely been accepted and promoted in subsequent reviews (see Mitton, 1993; Britten, 1996). However, many studies have failed to support the existence of this relationship (see Vøllestad *et al.*, 1999, for a review). These latter 'negative' results have not been fairly represented by authors of reviews of developmental instability. Hence, the debate about the association between developmental instability and heterozygosity is still open.

The aim of this study was to investigate the genetic relationship between heterozygosity and developmental instability. Two novel aspects made the study suitable to address this question. First, compared with previous studies in which two highly inbred strains were used as the parental generation, we used one highly inbred strain of sexually reproducing *Drosophila mercatorum* and one parthenogenetic strain of the same species in which all individuals were identical and completely homozygous, due to their pronuclear duplication mode of reproduction (Templeton *et al.*, 1976). The second innovation consisted in taking into account the potential bias produced by the presence of σ_e^2 , using admixture analysis and the methods suggested by Pertoldi *et al.* (2006b). These methods allowed us to take into account the experimental errors due to unpredictable environmental components (for applications, see Kristensen *et al.*, 2003, 2005; Røgilds *et al.*, 2005; Pertoldi *et al.*, 2005, 2006a). Obtaining a precise and reliable estimate of developmental instability for the two parental strains and the F1 generation allowed us to demonstrate which effect heterozygosity has on developmental instability.

MATERIAL AND METHODS

Theoretical background

When two parental strains consist of totally homozygous and identical individuals ($\sigma_g^2 = 0$), the F1 hybrid strain is also expected to be composed of completely identical individuals ($\sigma_g^2 = 0$), but with heterozygosity at the loci where different alleles are present in the two parental strains. Thus, σ_p^2 in the F1 strain will be equal to the sum of developmental instability (DI) and σ_e^2 .

$$\sigma_p^2 = \text{DI} + \sigma_e^2$$

This equation is simplified since two potential sources of error are ignored: measurement error and the sampling error of the variances. However, in this investigation the sample size was high enough to minimize the error associated with the sampling variance and no measurement error was introduced, as we used a meristic trait (sternopleural bristles, where no error is made by counting bristles). Thus, we can consider the equation reliable.

The equation also shows that the presence of σ_e^2 , if undetected, may lead to an erroneous interpretation of σ_p^2 as an estimator of developmental instability. If positive, σ_e^2 will increase σ_p^2 and therefore overestimate developmental instability. Pertoldi *et al.* (2001b) suggested that the presence of σ_e^2 in a monoclonal population can be detected by calculating the covariance between the right and the left sides of individuals ($\text{cov}(\mathbf{R}, \mathbf{L})$). Here, both the totally homozygote parental strains and their F1 hybrid generation are composed of genetically identical individuals equivalent to a monoclonal population. If $\text{cov}(\mathbf{R}, \mathbf{L})$ is observed in these types of strains, the only reason can be the presence of σ_e^2 (see Pertoldi *et al.*, 2001b, for details of the method).

$\text{Cov}(\mathbf{R}, \mathbf{L})$ is equivalent to $r(\sigma_R)(\sigma_L)$, where (σ_R) and (σ_L) are the standard deviations of the right and left side respectively. For bilateral traits, σ_p^2 of the right side is equivalent to σ_p^2 of the left side multiplied by the squared value of the correlation between the two sides (r^2).

The proportion of variation in one trait that is independent of the variation in the other trait is equivalent to $(1 - r^2)$ and can be used to estimate developmental instability. Hence:

$$\text{DI} = \sigma_p^2(1 - r^2)$$

This equation is correct if we assume that developmental instability is constant across different environments. This can be verified by checking the homoscedasticity of the regression between the right and the left sides. Another assumption is that σ_p^2 does not vary with the size of the traits. Both assumptions are typically violated. The first assumption is often violated because it requires that there is no interaction between developmental instability and σ_e^2 (developmental instability is insensitive to different environments). The second assumption is often violated because of the scaling effect of $\bar{\mu}$ with σ^2 . The effects of the violation of the first assumption can be reduced by minimizing σ_e^2 ($\sigma_e^2 = 0$), and the effects of the violation of the second assumption can be reduced by log-transforming the estimates of developmental instability. Quantification of σ_e^2 can thus be used to detect and discard samples where σ_e^2 has seriously affected the σ_p^2 estimates. If σ_g^2 is present, σ_p^2 is also affected by the genetic components, which are σ_g^2 and the covariance produced by σ_g^2 , i.e. $\text{cov}(\sigma_g^2)$, where $\text{cov}(\sigma_g^2)$ is the covariance due to the presence of σ_g^2 among individuals (see Pertoldi *et al.*, 2003):

$$\sigma_p^2 = \text{DI} + \text{cov}(\sigma_e^2) + \sigma_g^2 + \text{cov}(\sigma_g^2)$$

where $\text{cov}(\sigma_e^2)$ is the covariance due to σ_e^2 . Although in this study we can exclude the presence of $\text{cov}(\sigma_g^2)$ in the parthenogenetic strain, we cannot exclude the possibility that the inbred strain is not completely homozygous ($\sigma_g^2 \neq 0$), which would imply also that the F1 hybrid is not composed of genetically identical individuals. In this case, σ_p^2 in the F1 strain will also be affected by σ_g^2 , making the comparison of developmental instability between the inbred and the F1 strain difficult. However, this problem will not affect our conclusions when comparing the parthenogenetic and the F1 strains. In fact, if a significantly smaller $\text{DI} = \sigma_p^2(1 - r^2)$ is observed in the F1 than in the parthenogenetic parental strain, we will be able to confirm that a negative correlation between σ_g^2 and developmental instability must exist even if σ_g^2 is different from 0.

In fact, the presence of σ_g^2 will increase σ_p^2 and consequently the DI estimates of the F1 strain, and if there is a significant reduction in developmental instability in the F1 strain compared with the parthenogenetic strain, we can affirm that we have tested the eventual differences between these two strains in a conservative way.

To take into account the potential bias due to the presence of σ_e^2 , all the vials and the pooled samples for which r^2 between the right and left side for the number of sternopleural

bristles is higher than 5% are excluded. Thus, the effect of the violations will be minimized, and the 5% threshold is small enough to permit unequivocal conclusions to be drawn from the experiment.

Besides the covariance, there is another potential bias that can affect the DI estimate. This bias, which we call 'environmental heterogeneity', is due to the fact that there is heterogeneity of developmental instability among the individuals comprising the population. Environmental heterogeneity can be due to the presence of σ_e^2 , associated with a change in the mean size of the trait ($\bar{\mu}$), which means that the individuals comprising the population are in a heterogeneous environment and that both $\bar{\mu}$ and the individual's developmental instability are heterogeneous. This kind of heterogeneity can be detected as it will produce a covariance between the right and left sides of the trait measured. There is, however, another kind of environmental heterogeneity that is undetectable following the above-mentioned procedure, as it will not produce any covariance between the right and left sides. If environmental heterogeneity does not alter $\bar{\mu}$, but only their developmental instability (individual phenotypes are considered as random variables with a fixed expectation: the population $\bar{\mu}$ and different σ^2), then the σ_p^2 estimate will be equal to the mean of the individual's developmental instability:

$$\sigma_p^2 = \Sigma \text{ DI}$$

Also, the vials and the pooled samples that are affected by the bias due to DI heterogeneity will be removed if environmental heterogeneity is detected by means of an admixture analysis using the software EMMIX (for details of the analysis, see McLachlan and Krishnan, 1997; McLachlan and Peel, 1998; for an application of the admixture analysis, see Pertoldi, 2006b). The main purpose of this program is to fit a mixture model of multivariate normal or t -distributions to a user supplied data set. This is done via the EM algorithm. An admixture analysis is therefore conducted for every single vial and for the pooled sample. The fitting of normal or t -component mixture models to multivariate data, using maximum likelihood via the EM algorithm, is a common procedure. The use of the EM algorithm to fit this t mixture model is called EMMIX and is described in McLachlan and Krishnan (1997) and McLachlan and Peel (1998). Additionally, the algorithm used for this purpose does not require any specification of the above-mentioned parameters and clusters automatically each individual into its most probable group, and the *a posteriori* probability of an individual to belong to the cluster in which it has been assigned is estimated. The option of unrestricted component covariance matrices for the data set has been chosen. The level of significance (P -value) is produced by the optional bootstrap analysis. By sequentially testing n , $n + 1$, $n + 2$, etc., and stopping when the step becomes insignificant, the number of components can be assessed.

This kind of analysis can therefore be used to ascertain the eventual presence of environmental heterogeneity in those vials where r^2 is found to be below the threshold value of 5%. Vials that exceed this value are discarded. Therefore, by removing all the vials where σ_e^2 and σ_g^2 have a large effect on the DI estimates, we were able to obtain reliable estimates of developmental instability.

Experimental design

We used two different strains of *Drosophila mercatorum*: a sexually reproducing strain of Brazilian origin, which has been inbred by consecutive brother–sister mating for more

than 20 generations, and a parthenogenetic strain, Iv-23-olm, isolated from the sexual population in Hawaii in 1990 (Kramer and Templeton, 2001). In this experiment, we made the hybrid F1 generation by mixing a randomly chosen sexually reproducing male from the inbred strain with a parthenogenetic female. The flies were kept at 25°C in vials containing instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA) and added live yeast. They were allowed to mate and lay eggs for 3 days. The number of offspring produced was low enough to minimize any effects of crowding and competition between the larvae. Only females were used for the investigation, as females alone comprise the parthenogenetic strain.

We only used progeny from mothers that were 4–8 days old to minimize any maternal effects, as developmental instability in the progeny has been shown to increase with maternal age in *Drosophila* (Faurby *et al.*, 2005; Røgilds *et al.*, 2005). Upon hatching, flies were transferred to smaller vials and stored at –20°C. Then, 33 females from each of the vials had their sternopleural bristles counted on both sides. Females from the parental strains (sexual, inbred, and parthenogenetic) were bred and sampled under similar conditions.

Statistical analyses

The vials in which the squared correlation (r^2) between the number of sternopleural bristles on the right and left sides exceeded 5% were excluded from further analyses. Vials for which r^2 was below the threshold value of 5% were submitted to an admixture analysis in which the mean number of sternopleural bristles counted on the right side was tested for environmental heterogeneity; if environmental heterogeneity was found, the vials were excluded from further analyses.

Individual samples from three vials were subsequently pooled. These pooled samples were tested again to verify that they still fulfilled the criteria of $r^2 < 5\%$ and no environmental heterogeneity present. Pooled samples were used for the estimation of σ_p^2 and the mean ($\bar{\mu}$) of the number of sternopleural bristles on the two sides of individuals. This procedure was performed for the females of the two parental strains and the females of the F1 generation. Differences among $\bar{\mu}$ in the three strains were tested with a one-way analysis of variance (ANOVA). The pairwise comparisons were performed with a Scheffé test (Zar, 1999).

To take into account the scaling effect of σ^2 with $\bar{\mu}$ (Taylor, 1961), which was significantly different in the three strains (see below), the DI estimates were log-transformed and the logDI of the right and left sides were averaged. The average logDI estimates were compared between groups with a resampling F -test (1000 permutations) according to Lewontin (1966). The maximum estimates of the errors associated with the estimation of developmental instability (due to the r^2) were estimated by multiplying r^2 with σ_p^2 :

$$\text{max-error(DI)} = r^2 \sigma_p^2$$

The max-error(DI) was added to the DI estimates and the F -test between the average logDI was repeated. All statistical analyses were performed using the softwares PAST (<http://folk.uio.no/ohammer/>) and STATVIEW (<http://www.abacus.com/Abacus/HOME>).

RESULTS

It was necessary to use six vials of the sexually reproducing inbred strain as three of the vials showed an r^2 above 5%; the remaining three vials were pooled and r^2 was found to be below 5% and environmental heterogeneity was not observed.

Five vials of the hybrid F1 strain were used, despite the fact that none of the vials showed r^2 above 5%. The reason for this was that the admixture analysis detected the presence of environmental heterogeneity and individuated two subgroups among the first three vials. One subgroup was composed of the sum of the first two vials (with a certain $\bar{\mu}$ and σ^2) and a second subgroup comprised the third vial that showed a lower $\bar{\mu}$ and approximately equal σ^2 to the first subgroup. Therefore, the third vial was discarded and replaced with the samples from a fourth vial. Again environmental heterogeneity was detected as with the third vial. Once the fifth vial was pooled with the first two, we obtained a pooled group with an r^2 below 5% and no environmental heterogeneity.

For the parthenogenetic strain, the first three vials analysed were used as no r^2 above 5% or environmental heterogeneity was observed within the single vials or when pooling the three vials.

Hence, a r^2 value above 5% was noted three times within the vials when considered singularly, and environmental heterogeneity was observed twice when pooling three vials together (data available from the first author on request).

The one-way ANOVA revealed highly significant ($P < 0.001$) differences among the mean number of sternopleural bristles in the parthenogenetic, sexually reproducing, and F1 strains. The Scheffé test indicated that the pairwise comparisons between these three strains were all significant ($P < 0.05$), with the parthenogenetic strain having the highest $\bar{\mu}$ followed by the F1 and sexual strains. The $\bar{\mu}$ of the F1 strain was much closer to that of the parthenogenetic strain than the inbred strain (see Table 1).

The F -tests that tested for significant differences in the mean of the logDI values between the three strains were all highly significant ($P < 0.001$) for all the comparisons both with and without the addition of the max-error(DI), showing that the F1 strain had significantly less developmental instability than the sexually reproducing and the parthenogenetic strains. The sexually reproducing strain showed significantly less developmental instability than the parthenogenetic strain (see Table 1).

DISCUSSION

In this study we have shown a heterotic effect produced by crossing a highly inbred strain with a parthenogenetic strain and we have provided evidence for a reduced developmental instability with increased heterozygosity. Possible sources of bias and/or errors or statistical artifacts, associated with the estimation of developmental instability, have been taken into account. The differences in developmental instability between the F1 hybrids and the two parental strains are quite marked, with the inbred strain having more than twice the developmental instability of the F1 strain; the parthenogenetic strain had nearly seven times more developmental instability than the F1 strain.

The differences in developmental instability between the inbred and the parthenogenetic strain could be due to the fact that mildly deleterious alleles have been purged during the process of inbreeding in the inbred strain, reducing the developmental instability of the population, whereas the parthenogenetic strain has had no possibility of getting rid of

Table 1. Number of individuals (n), mean (μ) and variance (σ^2) of sternopleural bristles counted on the right (R) and left (L) side of individuals

Strain	n	Side	μ	σ^2	r^2	max-error (DI) = $r^2 \sigma^2$	$(1 - r^2)$	DI = $\sigma^2(1 - r^2)$	(logDI(R) + logDI(L))/2
F1	99	R	18.111	1.222	0.029	0.035	0.971	1.187	0.039
	99	L	18.020	1.040	0.029	0.030	0.971	1.01	
sexual	99	R	14.838	1.178	0.013	0.015	0.987	1.163	0.102
	99	L	14.949	1.395	0.013	0.018	0.987	1.377	
parthenogenetic	99	R	18.990	1.847	0.006	0.011	0.994	1.836	0.275
	99	L	19.434	1.942	0.006	0.012	0.994	1.930	

Note: The squared correlation (r^2) between the sternopleural bristles on the right and on the left side is equivalent to the proportion of the variation on the left side that is explained by the proportion of the variation on the right side and vice versa. $(1 - r^2)$ is the proportion of variation on one side that is independent of the variation on the other side. The max-error of developmental instability (DI) is equivalent to $r^2 \sigma^2$. The developmental instability of one side $DI = \sigma^2(1 - r^2)$ is equivalent to the variance of that side, multiplied by the proportion of variation that is independent of the variation on the other side $(1 - r^2)$. The mean of logDI on the right and left side (logDI(R) + logDI(L))/2 is the scaled DI and mean DI estimates respectively, which take into account the scaling effect of the mean with the variance. The scaled estimates of DI have been tested among the F1, the sexual, and the parthenogenetic strains by means of an F -test according to Lewontin (1966). The differences in DI were all highly significant ($P < 0.001$).

mildly deleterious alleles. It might be worthwhile making more crosses with several independent inbred strains inbred at different rates to determine the effect of different strains and levels of dominant interactions on developmental instability.

Additionally, it is possible that the inbred strain could have some heterozygosity left. This, however, will not affect our conclusion about the observed lower developmental instability in the F1 hybrids compared with the parental inbred strain. In fact, if some residual σ_g^2 was left in the inbred strain, it would have increased the r^2 term due to increased $\text{cov}(\sigma_g^2)$. Such an effect would have been removed when estimating the unbiased estimate of developmental instability.

Finally, the scaling effect of the mean with the variance has been taken into account by log-transforming the DI estimates. This effect would have been less relevant when comparing the unscaled DI estimates of the F1 and parthenogenetic strains, which have more or less the same $\bar{\mu}$, but might have constituted a problem when comparing the DI estimates of these two strains with the inbred strain, which has a relatively smaller $\bar{\mu}$. Our results reveal how many factors can impact on DI estimates and can probably also explain the contrasting results of meta-analyses performed to test for associations between developmental instability and σ_g^2 (for reviews, see Britten, 1996; Völlestad *et al.*, 1999).

The methodological difficulties mentioned above help to explain the inconsistency of results on associations between developmental instability and the level of inbreeding (or, more generally, genetic stress). The effect of inbreeding on variance components has also been studied (Fowler and Whitlock, 1999; Kristensen *et al.*, 2005). Theoretical predictions based on an additive model state that the additive part of σ_g^2 decreases within populations proportional to the inbreeding coefficient and to the amount of genetic drift, and for neutral traits like sternopleural bristles this is confirmed by empirical research (Clayton *et al.*, 1957).

In the present study, we used a meristic trait (sternopleural bristles). Meristic characters may be thought of as threshold traits (Falconer and Mackay, 1996). Such characters have an underlying continuity with thresholds, which impose discontinuity on their visible expression. The underlying continuous variable, termed the 'liability', is both genetic and environmental in origin and could in theory be measured and studied as a metric character. In the absence of σ_g^2 and σ_e^2 among individuals, variance in liability (like morphometric variance) depends only on the precision of the underlying developmental processes, whereas variances in meristic counts (unlike morphometric variance) also depend on the position of the mean liability between thresholds of liability [i.e. the position of the mean count between whole values (Swain, 1987)]. Even if the meristic traits have the above-mentioned peculiarities, the results of this investigation can be generalized also for metric traits, as sternopleural bristles are a polygenic character (Mackay and Lyman, 2005), as are most morphometric characters. Furthermore, the possible biases due to selective forces do not constitute a problem. Forces of selection are distributed over a large number of loci, rendering the selective forces on specific loci sufficiently overwhelmed by random genetic drift (Lynch, 1996). Lastly, even if there are dominance interactions for some of the loci controlling the trait, the average effect of all the loci that are controlling the trait should be approximately additive.

The presence of dominance interactions confirmed by the deviation of $\bar{\mu}$ of the F1 strain from the expected average value of the $\bar{\mu}$ of the two parental populations could suggest some associations between the degree of dominance of the genes and the level of developmental instability.

Implications

The implications of the results of this investigation are multiple, as they can contribute to the open debate about the correlation between σ_g^2 and σ_p^2 . This is important as the potential for an evolutionary response in quantitative traits remains of central importance for quantitative genetic analysis. Another unresolved matter that could benefit from the clear-cut results of this investigation is the controversy between ecologists and geneticists on the importance of genetic variability for the persistence of populations. The ‘central dogma of conservation genetics’ is that genetic variability is beneficial; hence preservation is a primary concern. This perception of the advantages of σ_a^2 in a population stems from considering short- and long-term adaptability and evolution in a changing environment. Evolutionary biologists often assume that increasing genetic variance always enhances the probability of population survival. However, this is not generally true. For example, in a constant environment σ_a^2 in a quantitative character creates a segregational load in each generation due to stabilizing selection against individuals that deviate from the optimum phenotype (Lande and Shannon, 1996).

Our findings will allow us to affirm that genetic variability places an additional premium on many populations in rapidly changing environments. In fact, the main implication of this investigation is that the relationship between the σ_a^2 of a trait and its heritability (h^2) (which are both related to the evolutionary potential) is not linear, as an increased σ_a^2 , which is in the numerator of the h^2 equation, will imply a reduced σ_p^2 , which is in the denominator of the h^2 equation. Consequently, the concomitant increase in σ_a^2 and reduction in σ_p^2 both contribute to an increase in h^2 .

When discussing the ‘central dogma of conservation genetics’, therefore, we ought also to consider this additional premium due to the association between σ_a^2 and σ_p^2 , which should be taken into account when making a cost–benefit analysis of σ_a^2 in changing environments.

Developmental instability has been interpreted as a bet-hedging strategy in an unpredictable environment (Simmons and Johnston, 1997). Increased developmental instability together with increased stress could also be considered in an evolutionary context as a mechanism of defence for populations in fluctuating environments. However, when the magnitude of genetic variation is insufficient to create a diversity of phenotypes that can be exposed to selection, developmental instability, by producing stochastic variation, will enrich the evolutionary potential of a population.

Thus threatened populations with a very low effective population size and consequently very low genetic variability may be more able to adapt to changing environments than is usually assumed. The same developmental instability and plasticity that can increase the short-term ability to respond to environmental changes can also reduce the ability for long-term changes, as argued by Sultan (1996) and Pertoldi *et al.* (2005). Selection can only work on differences in phenotypes and developmental instability can reduce the correlation between genotype and phenotype.

Efforts should be made in the future to quantify the extent of the negative relationship between σ_a^2 and σ_p^2 under different environmental conditions. Furthermore, different strains with different degrees of genetic differentiation should be used for the cross experiments, as the negative relationship between σ_a^2 and σ_p^2 observed in this study is based on only a single replicated cross. The use of different strains will allow us to test the generality of the relationship and whether it is always negative, bearing in mind that extreme outcrossing may also decrease offspring fitness. Crosses between distantly related populations, F1 and/or F2

generations are in fact sometimes less fit than the average of the original parental strains (Wu and Palopoli, 1994).

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