Genet. Res., Camb. (1994), **64**, *pp.* 115–125 *With 2 text-figures*

Selection on reaction norms, genetic correlations and constraints

PETER H. VAN TIENDEREN*[1,2] AND HANS P. KOELEWIJN[3]

[1] *Netherlands Institute of Ecology, Centre for Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands*
[2] *Department of Genetics, Agricultural University, Wageningen*
[3] *Netherlands Institute of Ecology, Centre for Limnology, Nieuwersluis*

Summary

Two approaches to the evolution of phenotypic plasticity in heterogeneous environments have recently been put forward. The first focuses on selection on the character expression within each environment; plasticity is seen as a by-product of local selection in various habitats. The second approach focuses on selection on the parameters of the response function of genotypes, and selection is thought to change the frequencies of 'plasticity' genes that affect the function. This paper discusses the relationship between the two approaches, with emphasis on applications. A method is described that allows switching from one approach to the other. It is argued that character state and reaction norm approaches, while to a large extent interchangeable, usually differ in the response function chosen. This choice, however, may strongly affect the biological interpretation. The methods outlined in this paper permit one to look at the data from different perspectives in order to avoid this danger.

1. Introduction

The world of phenotypic plasticity appears to be in a schismatic phase. One view focuses on selection on the character expression within each environment (Via & Lande, 1985; Van Tienderen, 1991; Gomulkiewicz & Kirkpatrick, 1992). Phenotypic selection on a focal trait may result in a selection response within a particular environment, which in turn may affect the expression of the trait in other environments by means of correlated responses (Via & Lande, 1985). The evolution of plasticity is thus seen as a by-product of local selection in various habitats (Via, 1993*a*). The other view focuses on selection on the parameters of the reaction norms of genotypes (De Jong, 1990*a*, *b*; Gavrilets & Scheiner, 1993*a*, *b*), for instance the parameters of a polynomial function. Selection is thought to change the frequencies of genes ('plasticity' genes) that affect the shape of the function. Apparent differences between these, here called character state- and reaction norm approaches, and their underlying assumptions on the mechanisms for plasticity have been discussed at length (Via, 1993*a*, *b*; Schlichting & Pigliucci, 1993; Scheiner, 1993*a*). Indeed, they may lead to very different descriptions of the selection process (Stearns, 1992) and adherents to the different views sometimes seem to speak different languages.

Falconer (1952) noted that a trait expressed in two environments could be seen as two characters that are genetically correlated. Discrete habitats may arise naturally, such as different host species, or alternatively, they may form a subset of environments from a potentially continuous range. In either case, data are represented as character values for each environment. This character state approach was expanded by Via & Lande (1985, 1987) to model evolution in several habitats. They concluded that the additive genetic covariances across environments strongly affect the outcome of selection: optimal values for a quantitative trait cannot evolve if the matrix of genetic (co)variances **G** is singular. For a case with only two environments this occurs if there is no genetic variation in one or both environments, or if breeding values in the two environments are perfectly correlated (i.e. plus or minus one). Within this framework all constraints on the outcome of selection are reflected in **G** (Via, 1987). These conclusions may be particular to the assumed underlying genetic details (see discussion). However, it seems plausible that adverse genetic covariances across environments may retard the evolution of adaptive reactions. Gomulkiewicz & Kirkpatrick (1992) generalized this approach for an arbitrary (and potentially infinite)

* Corresponding author. E-mail: TIENDEREN©NIOO.NL Fax: +31.8306.23227.

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

number of environments by using genetic covariance functions and selection gradient functions.

In the reaction norm approach, a genotype is described by its reaction to an, often continuously varying, environmental factor (Woltereck, 1909). Theoretical models mostly use polynomial functions (De Jong, 1990*a*, *b*; Gavrilets & Scheiner, 1993*a*, *b*), but other functions can be employed also. Gavrilets & Scheiner (1993*b*) concluded that genetic correlations between the parameters of the function may strongly affect heritabilities within a given environment, and hence responses to selection. Again, constraints may be reflected as singularity of the matrix of genetic (co)variances, now of the parameters of the function.

This paper focuses on the following issues. (i) To what extent are character state and reaction norm approaches equivalent? (ii) Can we infer the selective pressures on the parameters of a reaction norm from data on selection within environments, and *vice versa*? (iii) What is the relationship between the genetic (co)variances in the two approaches and the underlying biological constraints?

2. The relationship between character states and reaction norms

For phenotypically plastic trait, the character state in a particular environment i, z_i, can be written as a function of a set of environmental variables for that environment, $\mathbf{x}_i$, and a set of reaction norm parameters specific for each genotype, $\mathbf{g}$ (Appendix 1). For example, a reaction norm could be written as $z_i = g_0 + g_1 \times food_i + g_2 \times temp_i + g_3 \times temp_i^2$. In this case, each genotype is characterized by four parameters, the intercept g_0, a term for the linear effect of food level g_1, and linear and quadratic terms for temperature, g_2 and g_3. Each environment is characterized by three parameters, the actual food level, the ambient temperature and its square. For convenience, a leading one is added to the environmental vector $\mathbf{x}_i = (1\ food_i\ temp_i\ temp_i^2)$, so that we can write $z_i = \mathbf{x}_i\mathbf{g}$ (Appendix 1). We examine the relationship between the two approaches for soft and hard selection, assuming a coarse-grained, spatially heterogeneous environment, characters that have multivariate Gaussian distributions of genotypic and environmental values, and functions that can be written as $\mathbf{z} = \mathbf{X}\mathbf{g}$ (Appendix 1), i.e. $\mathbf{z}$ is a linear function of $\mathbf{g}$. De Jong (1994*b*) presented a rigorous analysis of the model, using a multi-locus population genetic approach, and assuming hard selection. This derivation was based on reaction norms that were written as a Taylor series around the average value for a particular environmental variable. Earlier papers (e.g. Gavrilets, 1986; De Jong, 1990*a*; Gavrilets & Scheiner, 1993*a*, *b*) often used a particular subset of possible linear transformations. Given a linear transformation between $\mathbf{g}$ and $\mathbf{z}$, a mathematical relationship between the character state and reaction norm approaches exists in genetic covariance matrices, selection gradients and selection responses, independent of the underlying genetics (De Jong, 1994*b*; Appendix).

The additive genetic covariance matrix of character states $\mathbf{G}_z$ and the matrix of function parameters $\mathbf{G}_g$ are related through a simple transformation of scale: $\mathbf{G}_z = \mathbf{X}\mathbf{G}_g\mathbf{X}^t$ (eqn 1.2). The matrices can be transformed into one another, and they contain the same biological information. Constraints in one matrix are also present in the matrix of the alternative representation. However, differences in the order of the matrix, the scaling and units of the matrix elements, the correlations among matrix elements, and even the decomposition in principal components might mistakenly be interpreted as differences in underlying biology.

In the character state approach, the selection gradient $\boldsymbol{\beta}_z$ describes the selective forces within each environment; the contributions of the different environments to the next generation depend on the relative frequencies of environments and the mode of selection (hard or soft) (Via & Lande, 1985). Low-productive environments have a lower impact because they produce fewer propagules and therefore hardly affect the trait in the focal or in other environments (or, such habitats produce less 'by-product', Via, 1993*a*). The selection gradient for function parameters, $\boldsymbol{\beta}_g$, depends both on selection within each environment and the contributions of the environments to the next generation. For example, selection on the slope of the reaction norm depends on the selective forces in all the habitats, weighted by their relative importance. Low-productive environments contribute little and therefore have minor effects on reaction norms at the level of the global population, whereas high-productive environments tend to pull harder at the reaction norm parameters. The two selection gradients are related by the equality $\boldsymbol{\beta}_g = \mathbf{X}^t\mathbf{Q}\boldsymbol{\beta}_z$ (eqn 1.3), with $\mathbf{Q}$ a diagonal matrix of frequencies of the environment. Again, there is no fundamental difference between the approaches.

Finally, the changes due to selection in the two approaches are also related by a simple equality: $\Delta\bar{\mathbf{z}} = \mathbf{X}\Delta\bar{\mathbf{g}}$ (eqn 1.4).

Although these equalities are independent of the actual genetic background of the traits, they may not always contain the relevant information for the selection process. For a deterministic, Gaussian quantitative genetic model and weak selection (Lande, 1979; Via & Lande, 1985; Gavrilets & Scheiner, 1993*b*) the relations are indeed relevant, and connected through familiar selection equations for coarse-grained environments. The selection equation in the character state approach, $\Delta\bar{\mathbf{z}} = \mathbf{G}_z\mathbf{Q}\,\boldsymbol{\beta}_z$ (eqn 1.5) and in the reaction norm approach, $\Delta\bar{\mathbf{g}} = \mathbf{G}_g\boldsymbol{\beta}_g$ (eqn 1.6) are in fact mathematically equivalent. De Jong (1994*b*) arrived at this conclusion using a multi-locus selection

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

model. Other genetic models, for instance, non-Gaussian distributions of breeding values with non-zero higher moments or models with epistatic gene action may require a more extensive set of parameters to derive expected responses to selection (Barton & Turelli, 1987; Turelli & Barton, 1990). This applies both to character state and reaction norm models.

Changes in the parameters of the reaction norm can also be written as a by-product (Via, 1993*a*) of selection within environments ($\Delta\bar{\mathbf{g}} = \mathbf{G}_g\mathbf{X}^t\mathbf{Q}\boldsymbol{\beta}_z$), but the reverse is mathematically equally possible. But again these are representations of the same biology. A choice for one representation above the other is a matter of preferences rather than underlying biology. Not all possible reaction norm functions can be written in the form here discussed, i.e. as a linear transformation from **g** to **z**. For example, the metabolic rate of a poikilothermic organism might be an exponential function of temperature; such a function cannot be inverted which makes it impossible to switch freely between approaches. However, many functions may be transformed to, or approximated by a function linear in **g**.

3. Data analysis

We focus on data for a number of families of genetically related individuals in a set of (natural or experimental) environments. Such split-brood designs allow estimation of genetic variances within environments, and genetic covariances across environments. Variation in the parameters of a reaction norm function cannot be calculated directly from variation in the mean values for each family in all environments (Gavrilets & Scheiner, 1993*b*). This can easily be understood. Imagine, for instance, a situation without genetic variation in slopes. Sib-group means will vary due to individual variation among the members of a sib groups, and consequently the calculated slopes of different sib groups will not be the same. The higher the standard error of the sib group mean per environment, the higher the variance among sib group slopes; therefore, variation based on sib groups means overestimates the true genetic component of variance among sib-groups. Gavrilets & Scheiner (1993*b*) present a correction factor for the intercept and slope of linear reaction norms. Appendix 2 shows how the genetic covariances of parameters for arbitrary reaction norm functions with transformation matrix **X** can be derived.

Such an analysis proceeds in three steps. Firstly, components of variance and covariance are estimated from the data, typically by equating observed and expected mean squares from analyses of variance (Falconer, 1989; Fry, 1992). Secondly, these observable components are converted to genetic variation within environments, and covariation between pairs of environments, by taking into account the relatedness of the individuals. For instance, the variance component for full- and half sibs families are estimates of, respectively, one-half and one-quarter of the additive genetic variance (Falconer, 1989). These estimates may be contaminated by non-additive variation, e.g. full-sib estimates include maternal effects and dominance variance. Several breeding designs have been developed to minimize the bias in the estimated additive component of genetic (co)variation (Falconer, 1989). The variances and covariances together comprise the 'raw' genetic covariance matrix $\mathbf{G}_z$. Techniques are available that convert 'impossible' covariance matrices (e.g. containing negative variances, or covariances that result in correlations outside the range $\{-1, +1\}$) to proper (i.e. positive definite) matrices (Hill & Thompson, 1978; Hayes & Hill, 1981). In an optional third step, this covariance matrix can be converted to genetic variation in the parameters of a particular reaction norm function (Appendix 2). Genetic variation in function parameters can be estimated from the genetic covariance matrix across environments using equation 2.4. In this third step, one has to choose a particular environmental factor, and a particular function for the norm of reaction. For planned experiments the function may simply be a polynomial expansion of the treatment levels applied. For data from natural environment, where many environmental factors may covary, this choice may not be so obvious.

Having selected a particular model for the data, for instance a polynomial function, it is possible to recalculate expected variances within- and covariance across environments under the assumption that the model is valid (Appendix 2). These values may differ from the initial 'raw' matrix; ideally, the model adequately describes the pattern in the data, whereas the initial matrix contains pattern plus noise. If so, the character state covariance matrix calculated for a particular model may be a better representation of the real genetic covariance matrix than the raw matrix (Kirkpatrick, Lofsvold & Bulmer, 1990). Furthermore, if intermediate environments exist, it is possible to intrapolate for values for which there are no actual measurements. Gomulkiewicz & Kirkpatrick (1992) used orthogonal polynomials after spline intrapolation, but essentially their approach is the similar: genetic covariances are estimated from a particular statistical method that separates pattern from noise.

4. Selection on developmental time in *Daphnia*

We use data from an experiment with *Daphnia galeata* (Koelewijn, unpubl.) to illustrate the relationship between character state and reaction norm models. Newborn offspring from females that were reared under uniform conditions were individually grown in test tubes under three temperatures, 10, 15 and 20 °C. Different food levels were used, but here we analyse only the data for the intermediate food level, with a culture solution of 40000 cells/ml of a mixture of

Table 1. *Analyses of variance for age at maturity (ln-transformed) of* Daphnia galeata *at three temperature levels, i.e. 10, 15 and 20 °C**

Model		F-ratio's			
		Temperature (D.F.)	Clone (D.F.)	Interaction temperature by clone (D.F.)	Unexplained vs. Within groups (D.F.)
A.	Main effects	1895*** (2)	3·0*** (31)	—	8·3*** (62)
B.	Full-factorial	5186*** (2)	8·2*** (31)	8·3*** (62)	—
C.	Equidistant linear norms	2707*** (1)	2·2** (31)	—	12·3*** (63)
D.	Linear norms	3726*** (1)	3·0*** (31)	4·1*** (31)	13·1*** (32)
E.	Equidistant quadratic norms	93·2*** (+1)†	3·0*** (31)	—	8·3*** (62)
F.	Quadratic norms	5186*** (2)	8·2*** (31)	8·3*** (62)	—

* Six models were fitted. The within group mean square was 0·00546 (D.F. = 189). Pooled unexplained and within-group mean square was used as a denominator of the F-ratio's for Temperature, Clone and Interaction effects. Significance levels: **: $P < 0{\cdot}01$, ***: $P < 0{\cdot}001$.

† F-ratio refers to addition of quadratic term to a linear model as in (C).

Scenedusmus obliquus and *Chlamydomonas globosa* (in a 1:3 ratio) added to filtered lake water, and renewed three times a week. We analysed the age at maturity (after log transformation) of 32 groups of newborns (i.e. asexual offspring of the same mother), with on average 2·8 replicates in each environment.

Different statistical models were fitted, proceeding from simple models (e.g. parallel responses of all genotypes) to the most elaborate model, i.e. the full-factorial model with in this case three parameters for each genotype (Tables 1, 2). In general, simple models will leave pattern in the genotype by environment table unexplained (underfitting), whereas complex models explain pattern plus noise (overfitting). Clearly, an intermediate solution that explains pattern but avoids overfitting is desirable. Normally, one would not present the results of all these models, but here we aim to explain their relationship, the constraints in the different models, and the computation of genetic covariance matrices.

The character state approach would typically involve testing genotypic differences by factorial analyses of variance, with temperature as a fixed-treatment factor. A simple main effect model without interaction (i.e. with reaction norms that are parallel between all pairs of environments, Fig. 1*a*), did not fit the data sufficiently well (Table 1, model A). Clones that developed faster at one temperature regime were not necessarily faster at other temperatures. Indeed, the two-way factorial ANOVA revealed a significant genotype by environment interaction (Table 1, model B). Such fully parametrized models that include the genotype by environment term leave no variation between clones unexplained (Fig. 1*b*).

The reaction norm approach could start with a simple model with equal slopes for all genotypes, but now with temperature as a covariate for the age at maturity rather than a factorial treatment. The equal slopes model left significant variation unexplained (Fig. 1*c*, Table 1, model C), as could be expected from the significance of the genotype by environment interaction in the factorial design. Addition of heterogeneity of slopes (Fig. 1*d*) improved the fit: variation in slopes was significant. This model explains part of the genotype by environment interaction, but the unexplained variance was still significant (Table 1, model D) and the linear model unsatisfactory. Finally, two different quadratic models were fitted: functions where clones only differed in height (Fig. 1*e*) and functions with differences in height and curvature (Fig. 1*f*). The former model is analogous to the main effects model (B), the latter to the full-factorial model (A) since only three environments were present. We concluded that the quadratic model F was statistically significantly better than the linear and equidistant quadratic model (Table 1, model F).

How are these models related to genetic covariance matrices of character states and function parameters? Variance components were estimated from nested ANOVA's for all three environments separately, and covariance components from the three pairwise factorial ANOVA's (Fry, 1992). Since families contained genetically identical individuals, the (co)variance components due to variation among families were equated to genetic components; this resulted in the 'raw' covariance matrix $\mathbf{G}_z$ (Table 3*a*). These genetic components may be contaminated with non-additive genetic variation, and maternal effects

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

Table 2. *Specifications of reaction norm models corresponding to Fig. 1*

Model		p	X	c
A.	Main effects	1	$\begin{pmatrix}1\\1\\1\end{pmatrix}$	$\begin{pmatrix}z_1\\z_2\\z_3\end{pmatrix}$
B.	Full-factorial	3	$\begin{pmatrix}1 & 1 & 0\\1 & 0 & 1\\1 & -1 & -1\end{pmatrix}$	—
C.	Equidistant linear norms	1	$\begin{pmatrix}1\\1\\1\end{pmatrix}$	$\begin{pmatrix}bt_1\\bt_2\\bt_3\end{pmatrix}$
D.	Linear norms	2	$\begin{pmatrix}1 & t_1\\1 & t_2\\1 & t_3\end{pmatrix}$	—
E.	Equidistant quadratic norms	1	$\begin{pmatrix}1\\1\\1\end{pmatrix}$	$\begin{pmatrix}b_1t_1+b_2t_1^2\\b_1t_2+b_2t_2^2\\b_1t_3+b_2t_3^2\end{pmatrix}$
F.	Quadratic norms	3	$\begin{pmatrix}1 & t_1 & t_1^2\\1 & t_2 & t_2^2\\1 & t_3 & t_3^2\end{pmatrix}$	—

* Temperature levels were $t_1 = 10$, $t_2 = 15$ and $t_3 = 20$. The general model formulation is $\mathbf{z} = \mathbf{c} + \mathbf{Xg} + \boldsymbol{\varepsilon}$. Column '**p**': number of parameters that vary in the population. Column '**X**': transformation matrix from **g** to **z**. Column '**c**': model term that is the same for all genotypes, i.e. the assumed mean trend under the different models. For instance, b is the mean decrease in age at maturity with temperature (Fig. 1*c*).

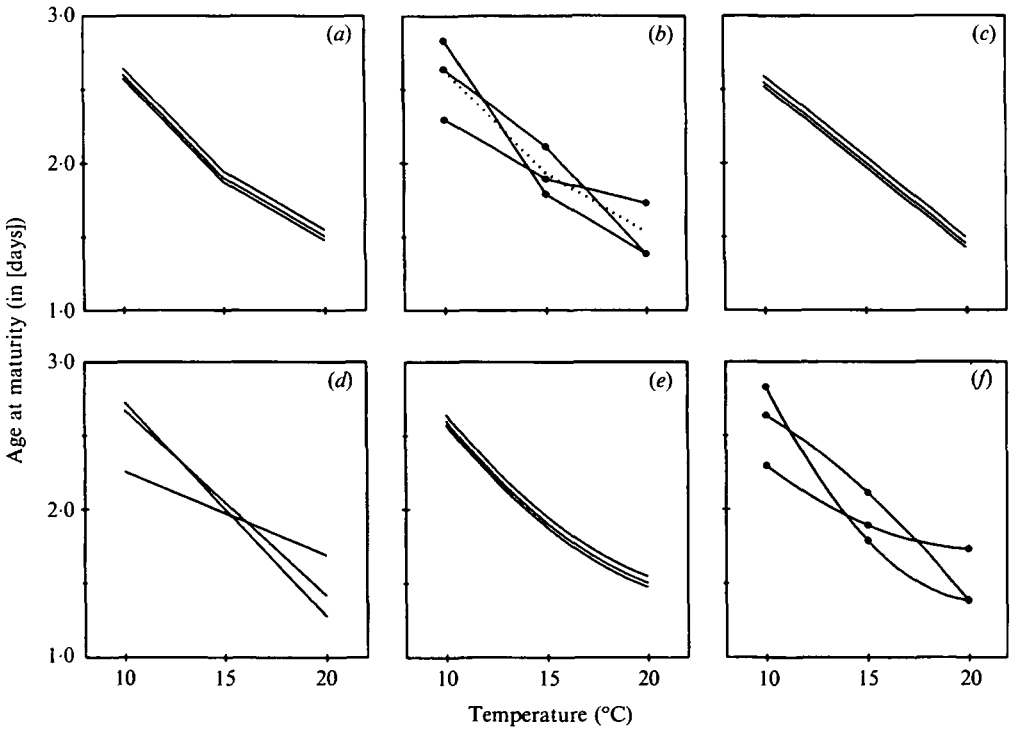

Fig. 1. Reaction norms of three clones in an experiment with *Daphnia galeata* at three temperature levels. (*a*) Main effects model, (*b*) full-factorial model (dashed line is mean reaction norm), (*c*) equidistant linear norms, (*d*) linear norms, (*e*) equidistant quadratic norms, (*f*) quadratic norms.

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

Table 3. *Genetic covariance matrix for age at maturity (ln-transformed) of* Daphnia galeata *at three different temperatures*

(*a*) Raw matrix estimated from data, $\mathbf{G}_z$

Temperature	10	15	20
10	0·0152		
15	0·0028	0·0099	
20	−0·0038	0·0011	0·0150

(*b*) Genetic covariance matrix for parameters of the linear model (model D), with $\mathbf{G}_g = \mathbf{U}\mathbf{G}_z\mathbf{U}^t$,

$$\mathbf{X} = \begin{pmatrix} 1 & 10 \\ 1 & 15 \\ 1 & 20 \end{pmatrix}, \quad \mathbf{U} = \begin{pmatrix} 1\frac{5}{6} & \frac{1}{3} & -1\frac{1}{6} \\ -\frac{1}{10} & 0 & \frac{1}{10} \end{pmatrix}$$

	Intercept	Slope
Intercept	0·0915	
Slope	−0·0057	0·00038

(*c*) Genetic covariances of characters across environments, assuming linear norms (model D) $\mathbf{G}_z = \mathbf{X}\mathbf{G}_g\mathbf{X}^t$, $\mathbf{X}$ as above.

Temperature	10	15	20
10	0·0146		
15	0·0048	0·0045	
20	−0·0050	0·0042	0·0133

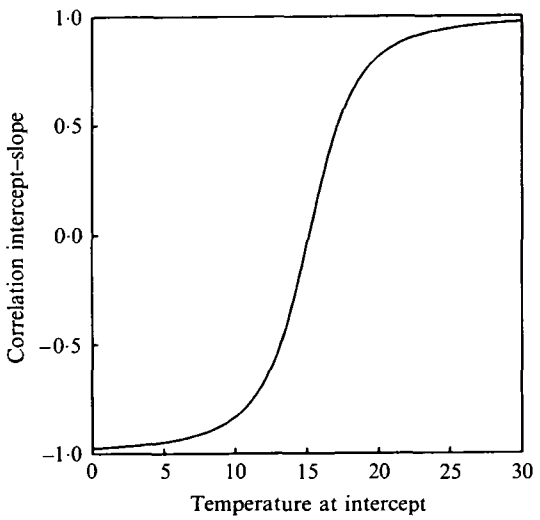

Fig. 2. Relationship between the correlation between intercept and slope in a model with linear reaction norms and the position of the intercept. Data on age at maturity (ln-transformed) of *Daphnia galeata* at three temperature levels, 10, 15 and 20 °C.

(although all mothers were reared under uniform conditions). The results should be interpreted with caution, and more elaborate breeding designs are required to remove these possible biases. The estimated covariances were not very high (Table 3*a*), and genetic correlations across sites computed from these estimates were close to zero; this was expected given the significant $g \times e$ interaction in the factorial model. The amount of genetic variation was lowest at the intermediate temperature.

The full-factorial (Table 2, model B) and quadratic model (Table 2, model F) differ in their $\mathbf{G}_g$-matrices, because a different parametrization is used, whereas the ANOVA results for the observed environments are identical (Table 1). Both models give a complete fit to the data, and consequently, the genetic covariance matrix under these models (using eqn 2.5) is identical to the raw matrix (Table 3*a*). A quadratic parametrization may be preferable if one also wants to intrapolate between the actual temperatures in the experiment (cf. Fig. 1*b*, *f*). The matrices of reaction norm parameters for models A, C and E were identical, since the models only differ in the assumed average effect of the environment (Table 2); only one parameter is estimated, the genetic variance due to deviations from the mean value per environment (Fig. 1*a*) or due to deviations from the overall linear or quadratic trend (Fig. 1*c*, *e*, respectively). The absence of genotype by environment interaction in these models implies that all genetic variances within environments are the same, and all correlations across environments plus one. The latter would strongly affect the selection response. Potential differences among environments in variances and covariances cannot be accommodated by such models.

A linear model with unequal slopes is different (Fig. 1*d*, Table 3). The genetic correlation between intercept and slope is $r = -0{\cdot}97$. However, this value depends strongly on the scaling of the environment. Taking the intermediate temperature as reference point, and using the differences from this point $(-5, 0, +5)$ instead of the actual temperatures (10, 15, 20) gives a correlation $r = -0{\cdot}05$ between intercept and slope, despite the identical spacing of the data. The genetic variance in slope remains unaffected by such changes in point of reference. The correlation between intercept and slope (and also the variance in intercept values) reflects the scaling of the environment rather than possible biological constraints (Fig. 2). This artifact may explain the wide range of correlations summarized by Scheiner (1993*b*). One could cautiously use the mean environment under natural conditions (if known, and if constant in time) as point of reference (De Jong, 1994*b*). For a least-squares linear regression equation, the estimated trait value in the mean environment equals the mean trait over all environments, so that the correlation can be interpreted as the genetic correlation between the mean trait value and the plasticity of the reaction norm. For quadratic reaction norms (Fig. 1*f*), the dependency of parameters on the scaling of the environmental variable is even more complex. Yet, only the quadratic model represented the pattern in the data accurately. The interpretation of matrices for function parameters is therefore not straightforward, and in general they contribute little in identifying underlying constraints.

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

For our *Daphnia*'s, all models suggest that genetic correlations across environments are low and that selection may change the reaction of developmental time on temperature. This does not imply that selection responses are expected to be faster than in a situation with high correlations across environments. For instance, selection for faster development time in several environments may initially be enhanced if developmental time is positively correlated across environments, i.e. if some clones have a consistent faster development than others under all circumstances. Fine-tuning of the reaction norm, however, is possibly easier in the absence of strong correlations. In general, the biological importance of constraints does not depend on the genetic covariance structure *per se*, but on its combination with actual selection regimes. Hypothetical constraints are the expected bedfellows of hypothetical selective forces (Antonovics & Van Tienderen, 1991).

5. Discussion

Character state models were originally developed for discrete environments, reaction norm models for continuous environments. However, the distinction between continuous and discrete environments may be dim: organisms may distinguish discrete hosts species or food sources by their quantities of particular substances, and similarly continuously varying environments may be perceived as discrete (e.g. the presence of a substance may be observed rather than its concentration). The distribution and nature of the environmental factor is not necessarily a useful guideline for a choice for one approach or another. Furthermore, reaction norm models can also be used for discrete environments (Gavrilets & Scheiner, 1993*b*), character state models for an arbitrary number of environments (Gomulkiewicz & Kirkpatrick, 1992). Fortunately, character state and reaction norm models are mathematically largely interchangeable.

The models may differ in the representation of the response. Reaction norm models typically employ polynomial functions, character state models do not use explicit function (i.e. use a full-factorial model). Simple polynomials or Taylor expansions around the mean environment (De Jong, 1994*b*) are related, but their different parametrizations may lead to different **G**-matrices and selection coefficients for reaction norm parameters. Finding an appropriate model may increase the reliability of estimated genetic covariance matrices if the model succeeds in separating pattern from noise (Kirkpatrick & Heckman, 1989) and simplify the biological interpretation of the data. However, there are three potential complications. Firstly, we may end up with several candidate models. Although some models will be statistically better than others, several alternative models may be indistinguishable without additional experiments, whereas they may differ in purported constraints on the selection process. Secondly, the choice for a particular model has the danger that artificial constraints are introduced and the statistical power of the data analyses may be too low to detect complexities in reaction norms (Kirkpatrick & Lofsvold, 1992). Obviously, such limitations are quite different from the biological constraints we look for. And lastly and perhaps most importantly, the choice for a particular model may make comparisons with other, equally reasonable approaches difficult, and may also lead to a different biological interpretation. The methods outlined in this paper permit a switch between the various models, which allows one to look at the data from different perspectives in order to minimize this risk.

Deduction of constraints directly from genetic covariance matrices is not straightforward. Some specific constraints may be easy to detect in the reaction norm approach, others in the character state approach. For instance, suppose that two environments out of a broader range do not differ in the clue that triggers the plastic response. Genotypic values in the two environments would then be the same, and in the character state approach this would show up as a correlation across environments close to one. In a polynomial model this would show up as singularity in the **G**-matrix, that may not be easy to decipher. Alternatively, suppose that reaction norms are linear over the range of environments studied. This would show up fairly directly in a polynomial approach, but could be quite hidden in the genetic covariance matrix of the character state approach. Looking at the problem from different angles may therefore be helpful.

Gomulkiewicz & Kirkpatrick (1992) suggested an approach to assess the consequences of genetic constraints by graphing the expected response to selection under different genetic covariance patterns. A weighted mean reaction norm can be calculated for the parental population, using relative fitness as a weighting factor. The difference between the mean reaction norm of all parents, and this weighted mean reflects the strength and direction of selection (Endler, 1986). Furthermore, the predicted mean reaction norm after one generation of selection can be deduced from the selective forces and the genetic covariance structure. By comparing these two norms graphically, it becomes apparent how much, and in what directions the predicted response deviates from the selective forces.

Genetic covariances across environments, or among function parameters, may not tell the complete story. Firstly, constraints may exist without singularity of **G** or strong correlations, depending on the underlying genetic architecture (Houle, 1991; Van Noordwijk & De Jong, 1986; De Jong & Van Noordwijk, 1992), or on mutational effects on the covariance due to pleiotropy (Clark, 1987; Charlesworth, 1990). For instance, mutations may cause variation in a particular

trait, which would suggest that further selection is possible; however, selection may be offset by mutations that are biased in the opposite direction (Houle, 1991). These theoretical studies concerned the correlation among different traits within a single environment, but similar arguments could be developed for the correlations across environments. Secondly, the covariance structure may change in time. For instance, Schlichting & Pigliucci (1993) emphasized the importance of epistasis and suggest that their effects may only show up as long term effect on selection responses. The speed and direction in which genetic covariance matrices change may depend on the underlying genetic and developmental system. Constancy of the **G**-matrix would imply that if evolution is unconstrained now (**G** not singular), it will never become constrained, and if there are constraints now, evolution will be constrained forever. It seems that an essential element in evolution is missing in this view. Lenski's studies on virus resistance in *E. coli* clearly demonstrated that genetic covariances change during the course of evolution. Negative side-effects of novel resistance genes (and hence a negative correlation between performance with and without the virus) were eliminated during 400 generations of selection (Lenski, 1988*a*, *b*). It seems wise to investigate the importance of constraints by other methods than genetic covariances and correlations, e.g. by evaluating of possible costs of plasticity (Van Tienderen, 1991), by artificial selection experiments (summarized in Scheiner, 1993*b*), or by studying the functional and developmental background of the traits (Maynard Smith *et al.* 1985). Even then, radical changes in underlying architecture of plastic reactions may come unexpected.

We would like to thank S. Via, R. Gomulkiewicz, S. Scheiner, C. Schlichting and G. de Jong, who all contributed in developing the ideas presented in this paper, and S. Gavrilets and F. van Eeuwijk for comments on an earlier version of the manuscript.

Appendix 1

Assume n discrete environments, c_i the relative frequency of a particular environment i, and reaction norms that are fully described by $m+1$ parameters. Furthermore, say

$$\mathbf{z} = (z_1 z_2 \dots z_n)^t$$

is a column vector of breeding values for environment $1 \dots n$ (t denoting matrix transposition),

$$\mathbf{g} = (g_0 g_1 \dots g_m)^t$$

is a column vector of $m+1$ coefficients of the reaction norm function, and

$$\mathbf{x}_i = (1 x_{i,1} x_{i,2} \dots x_{i,m})$$

is a row vector of $m+1$ values: a leading 1, and m values that characterize a particular environment i.

The breeding value in environment i can thus be written as

$$z_i = g_0 + x_{i,1} g_1 \dots + x_{i,m} g_m = \mathbf{x}_i \mathbf{g}.$$

The entries of $\mathbf{x}_i$ characterize the different environments. They may consist of values for different environmental factors (e.g. temperature, light, humidity, etc.) and/or their polynomial expansion. The vector of breeding values in n environments, with a function of $m+1$ coefficients becomes:

$$\mathbf{z} = \mathbf{X}\mathbf{g} \qquad \text{(A1.1}a\text{)}$$

with $\mathbf{X}$ the transformation matrix from $\mathbf{g}$ to $\mathbf{z}$, and each row i equal to the vector $\mathbf{x}_i$. Vice versa,

$$\mathbf{g} = (\mathbf{X}^t\mathbf{X})^{-1}\mathbf{X}^b\mathbf{z} = \mathbf{U}\mathbf{z}. \qquad \text{(A1.1}b\text{)}$$

Note that $\mathbf{U}$ is used and not $\mathbf{X}^{-1}$, since $\mathbf{X}$ need not be a square matrix. For instance, linear reaction norms are described with only two parameters: intercept and slope; the number of distinct environments may be much higher. If the number of distinct environments is smaller than the number of parameters of the reaction norm function, the matrix $(\mathbf{X}^t\mathbf{X})$ is singular so that $\mathbf{U}$ does not exist.

(i) *Genetic covariances*

Since (1.1) involves a linear transformation of scale only, it follows that

$$\mathbf{G}_z = \mathbf{X}\mathbf{G}_g\mathbf{X}^t \qquad \text{(A1.2}a\text{)}$$

$$\mathbf{G}_g = \mathbf{U}\mathbf{G}_z\mathbf{U}^t \qquad \text{(A1.2}b\text{)}$$

with $\mathbf{G}_z$ and $\mathbf{G}_g$ the additive genetic covariance matrices for $\mathbf{z}$ and $\mathbf{g}$, respectively (De Jong, 1994*b*). Thus, additive genetic variation in character states $\mathbf{z}$ can be rewritten in terms of variation in the parameters $\mathbf{g}$ of the reaction norm. The opposite is true provided that $\mathbf{U}$ exists. For polynomial models this is the case if the $\mathbf{x}_i$'s are different and the number of environments is at least equal to the degree of the polynomial (again, if there are fewer environments, higher order terms cannot be estimated because $\mathbf{X}^t\mathbf{X}$ is singular).

(ii) *Selection gradients*

The strength of directional selection is measured by the selection gradient, i.e. the partial derivative of the logarithm of mean fitness on the mean of a trait. If relative fitnesses are constant in time, selection within environments is quantified as the slope of the mean fitness function at the mean character value, $\beta_{zi} = \delta \ln \bar{W}_i / \delta \bar{z}_i$ (Lande, 1979; Via & Lande, 1985). Given the linear transformation between $\mathbf{z}$ and $\mathbf{g}$ (1.1) we can apply the chain rule:

$$\frac{\delta \ln \bar{W}_i}{\delta \bar{g}_j} = \sum_k \frac{\delta \ln \bar{W}_i}{\delta \bar{z}_k} \frac{\delta \bar{z}_k}{\delta \bar{g}_j}$$

to get the selection gradient for reaction norm

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

parameters $g_j, j = 0 \ldots m$, from selection on character states $z_i, i = 1 \ldots n$. If selection only acts on expressed phenotypes, $\delta \bar{W}_i / \delta z_k = 0$ for all $k \neq i$, so that

$$\frac{\delta \ln \bar{W}_i}{\delta \bar{g}_j} = \frac{\delta \ln \bar{W}_i}{\delta \bar{z}_i} \frac{\delta \bar{z}_i}{\delta \bar{g}_j}.$$

Note that if there are costs to plasticity (Van Tienderen, 1991), this assumption of selection acting only at expressed characters may not be valid. Since $\bar{\mathbf{z}} = \mathbf{X}\bar{\mathbf{g}}$, the partial derivative $\delta \bar{z}_i / \delta \bar{g}_j$ is simply the matrix element of $\mathbf{X}$ at row $j+1$, column i.

The mean fitness $\bar{W}$ over all environments depends on the mode of selection and the frequencies of the environments. If $\bar{W}$ is the geometric mean fitness over environments, $\bar{W} = \prod_i \bar{W}_i^{c_i}$ (soft selection, see Via & Lande, 1985; Van Tienderen, 1991), it follows that

$$(\delta \bar{W}/\delta \bar{z}_1 \ldots \delta \bar{W}/\delta \bar{z}_n)^t = \nabla_{\bar{z}} \ln \bar{W} = \nabla_{\bar{z}} \ln \prod_i \bar{W}_i^{c_i}$$

$$= \nabla_{\bar{z}} \sum_i c_i \ln \bar{W}_i = \sum_i c_i \nabla_{\bar{z}} \ln \bar{W}_i$$

$$= \mathbf{Q}\boldsymbol{\beta}_z$$

with $\mathbf{Q}$ a diagonal matrix of environmental frequencies, $\mathbf{Q} = \mathrm{diag}(c_1 \ldots c_n)$, and $\boldsymbol{\beta}_z = (\delta \bar{W}_1/\delta \bar{z}_1 \ldots \delta \bar{W}_n/\delta \bar{z}_n)^t$ the vector of selective forces within environments. If fitness is the arithmetic mean over environments (hard selection), $\bar{W} = \sum_i c_i \bar{W}_i$, it follows that

$$\nabla_{\bar{z}} \ln \bar{W} = \bar{W}^{-1} \nabla_{\bar{z}} \bar{W} = \bar{W}^{-1} \nabla_{\bar{z}} \sum_i c_i \bar{W}_i$$

$$= \sum_i c_i \bar{W}_i \bar{W}^{-1} \nabla_{\bar{z}} \ln \bar{W}_i = \mathbf{Q}\boldsymbol{\beta}_z$$

with a matrix $\mathbf{Q} = \mathrm{diag}(c_1 \bar{W}_1 \bar{W}^{-1} \ldots c_n \bar{W}_n \bar{W}^{-1})$.

Soft and hard selection only differ in the elements of $\mathbf{Q}$. In both cases the elements of $\mathbf{Q}$ can be interpreted as the relative contribution of each environment to the next generation. Applying the chain rule, we get the relationship between the selection gradient for reaction norm parameters, $\boldsymbol{\beta}_g = \nabla_{\bar{g}} \ln \bar{W} = (\delta \bar{W}/\delta g_0 \ldots \delta \bar{W}/\delta g_m)^t$:

$$\boldsymbol{\beta}_g = \mathbf{X}^t \nabla_{\bar{z}} \ln \bar{W} = \mathbf{X}^t \mathbf{Q} \boldsymbol{\beta}_z. \qquad \text{(A1.3)}$$

The selection gradient for the parameters of the reaction norm function can be found from the selective forces on characters within environments by this simple equation, that involves the reaction norm function chosen and the contributions of the different environments.

(iii) *Selection response*

From (1.1) it follows that

$$\begin{aligned} \Delta \bar{\mathbf{z}} &= \bar{\mathbf{z}}_{(t+1)} - \bar{\mathbf{z}}_{(t)} \\ &= \mathbf{X}\bar{\mathbf{g}}_{(t+1)} - \mathbf{X}\bar{\mathbf{g}}_{(t)} \\ &= \mathbf{X}\{\bar{\mathbf{g}}_{(t+1)} - \bar{\mathbf{g}}_{(t)}\} \\ &= \mathbf{X}\,\Delta\bar{\mathbf{g}}. \end{aligned} \qquad \text{(A1.4)}$$

This equality describes how the per generation change in the mean breeding value for the character is related to change in the mean breeding value of the function parameter.

So far, eqn 1.1–1.4 are independent of genetic details, and followed from transformation from $\mathbf{g}$ to $\mathbf{z}$. For a multivariate Gaussian model, Via & Lande (1985) derived the equation for the selection response due to one generation of selection:

$$\Delta \bar{\mathbf{z}} = \mathbf{G}_z \mathbf{Q} \boldsymbol{\beta}_z. \qquad \text{(A1.5)}$$

Using (1.2*a*), (1.3) and (1.4) it follows that selection on the reaction norm parameters becomes

$$\Delta \bar{\mathbf{g}} = \mathbf{G}_g \boldsymbol{\beta}_g. \qquad \text{(A1.6)}$$

Thus, the standard multivariate Gaussian model of selection (Lande, 1979; Gavrilets & Scheiner, 1993*b*) resurfaces, because multivariate normality in $\mathbf{z}$ implies multivariate normality in $\mathbf{g}$.

The equations 1.5 and 1.6 are equivalent if both $\boldsymbol{\beta}_z$ and $\boldsymbol{\beta}_g$ are measured at the level of breeding values. Selection gradients at the phenotypic level are not necessarily equal to gradients at the genetic level (Rausher, 1992); they may differ due to strong selection and non-linear fitnesses (De Jong, 1994*a*). Also, the phenotypic gradient may be a poor predictor of selection at the genetic level, if the genetic or environmental component of the phenotype is correlated with fitness rather than the phenotype itself; this can be checked by comparing selection at the phenotypic and genetic level (Rausher, 1992; Van Tienderen & De Jong, 1994). This derivation slightly differs from Gavrilets & Scheiner's model (1993*b*), since they did not use the frequencies of environments explicitly (and have adopted the hard selection scheme).

Appendix 2. Model selection and variance components

(i) *Genetic covariance matrices*

Equation 1.1 concerned the transformation from character values to function parameters at the level of breeding values. Phenotypic values also include variation due to non-additive genetic effects, and error variation unrelated to the (macro)environmental factors studied. To interpret our data we need to fit a particular function to the actual observations, e.g. linear reaction norms, and test how well such a model fits the data. The phenotype of the jth individual of a particular sibgroup in environment i is denoted as:

$$z_{ij} = z_i^* + e_{ij} \qquad \text{(A2.1)}$$

with z_i^* the sibgroup effect, and e_{ij} the deviation from the sibgroup effect (due to mixed genetic/environmental causes). The asterisk is used to denote that z^*

https://doi.org/10.1017/S0016672300032729 Published online by Cambridge University Press

pertains to a sibgroup, and is not a breeding value as in Appendix 1. The next step is to estimate variances and covariances among sibgroups by equating expected and observed mean squares and crossproducts or by maximum-likelihood methods (Shaw, 1987; Fry, 1992). From these estimates and the relatedness of sibgroup members (e.g. full-sibs, half-sibs, clones) the genetic covariance matrix of breeding values $\mathbf{G}_z$ can be constructed (Falconer, 1989).

The relationship between character and function parameters in breeding values is written as

$$\mathbf{z} = \mathbf{c} + \mathbf{X}\mathbf{g} + \boldsymbol{\varepsilon}. \quad \text{(A2.2)}$$

Now $\mathbf{z}$ is a vector of breeding values, $\mathbf{X}$ is the transition matrix as in Appendix 1, and $\mathbf{g}$ is a vector of function parameters. The vector $\mathbf{c}$ is optional, and contains fixed effects that are the same for all sibgroups; consequently $\mathbf{c}$ does not contribute to the (co)variance among sibgroups (cf. Gavrilets & Scheiner, 1993*b*). For instance, $\mathbf{c}$ may contain the mean performance in each environment, or in a model of parallel reaction norms, $\mathbf{c}$ contains the mean trend over environments. The error component of the model for the sibgroup is denoted as $\boldsymbol{\varepsilon}$. Equation 2.2 is similar to finding a vector of unknown parameters $\mathbf{g}$ from a set of breeding values $\mathbf{z}$ or $\mathbf{z}-\mathbf{c}$ given a design matrix $\mathbf{X}$. The $\mathbf{g}$ values that minimize the squared error component $\boldsymbol{\varepsilon}$ follow from

$$\begin{aligned}\mathbf{g} &= (\mathbf{X}^t\mathbf{X})^{-1}\mathbf{X}^t(\mathbf{z}-\mathbf{c}) \\ &= \mathbf{U}(\mathbf{z}-\mathbf{c}). \quad \text{(A2.3)}\end{aligned}$$

Since $\mathbf{c}$ is a constant, the variance covariance matrix of $\mathbf{g}$ for the population of sibgroups becomes:

$$\mathbf{G}_g = \mathbf{U}\mathbf{G}_z\mathbf{U}^t. \quad \text{(A2.4)}$$

In general $\mathbf{U}$ cannot be inverted, so that the original $\mathbf{G}_z$ cannot be recovered from the model matrix $\mathbf{G}_g$. Initial (co)variances among characters cannot be recovered from the (co)variance of function parameters, because they contain an error component not included in $\mathbf{U}$. However, from (2.2) we can evaluate the matrix of (co)variances of expected values $\hat{\mathbf{z}} = \mathbf{c} + \mathbf{X}\mathbf{g}$, estimated as

$$\mathbf{G}_{\hat{z}} = \mathbf{X}\mathbf{G}_g\mathbf{X}^t \quad \text{(A2.5)}$$

(see also Gomulkiewicz & Kirkpatrick, 1992; Gavrilets & Scheiner, 1993*b*). This matrix could be compared to the initial observed, 'raw' matrix $\mathbf{G}_z$ to check whether the reduced reaction norm model gives a similar pattern of covariances across environments.

(ii) *Selection gradients*

Selection gradients within environments can be estimated from regression of relative fitness on the expressed character state. From these data, selection on the parameters of the reaction norm function can be calculated using equation (1.3). Alternatively, direct estimates of phenotypic selection on the parameters of reaction norms is much more complex, as each individual usually experiences only one environment concurrently.

References

Antonovics, J. & Van Tienderen, P. H. (1991). Ontoecogenophyloconstraints? The chaos of constraint terminology. *Trends in Ecology and Evolution* **6**, 166–168.

Barton, N. H. & Turelli, M. (1987). Adaptive landscapes, genetic distance and the evolution of quantitative characters. *Genetical Research* **49**, 157–173.

Charlesworth, B. (1990). Optimization models, quantitative genetics, and mutation. *Evolution* **44**, 520–538.

Clark, A. G. (1987). Genetic correlation: the quantitative genetics of evolutionary constraints. In *Genetic Constraints on Adaptive Evolution* (ed. V. Loeschcke), pp. 25–45. Berlin: Springer Verlag.

De Jong, G. (1990*a*). Quantitative genetics of reaction norms. *Journal of Evolutionary Biology* **3**, 447–468.

De Jong, G. (1990*b*). Genotype-by-environment interaction and the genetic covariances between environments: multilocus genetics. *Genetica* **81**, 171–177.

De Jong, G. (1994*a*). The fitness of fitness concepts and the description of natural selection. *The Quarterly Review of Biology* **69**, 3–29.

De Jong, G. (1994*b*). Phenotypic plasticity as a product of selection in a variable environment. *American Naturalist* (in the Press).

De Jong, G. & Van Noordwijk, A. J. (1992). Acquisition and allocation of resources: genetic (co)variances, selection and life-histories. *American Naturalist* **139**, 749–770.

Endler, J. A. (1986). Natural selection in the wild. Monographs in Population Biology, vol. 21. Princeton: Princeton University Press.

Falconer, D. S. (1952). The problem of environment and selection. *American Naturalist* **86**, 293–298.

Falconer, D. S. (1989). Introduction to quantitative genetics. London: Longman.

Fry, J. D. (1992). The mixed-model analysis of variance applied to quantitative genetics: biological meaning of the parameters. *Evolution* **46**, 540–550.

Gavrilets, S. (1986). An approach to modeling the evolution of populations with consideration of genotype-environment interaction. *Soviet Genetics* **22**, 28–36.

Gavrilets, S. & Scheiner, S. M. (1993*a*). The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *Journal of Evolutionary Biology* **6**, 31–48.

Gavrilets, S. & Scheiner, S. M. (1993*b*). The genetics of phenotypic plasticity. VI. Theoretical predictions for directional selection. *Journal of Evolutionary Biology* **6**, 49–68.

Gomulkiewicz, R. & Kirkpatrick, M. (1992). Quantitative genetics and the evolution of reaction norms. *Evolution* **46**, 390–411.

Hayes, J. F. & Hill, W. G. (1981). Modification of estimation of parameters in the construction of genetic selection indices ('bending'). *Biometrics* **37**, 483–493.

Hill, W. G. & Thompson, R. (1978). Probabilities of non-positive definite between-group or genetic covariance matrices. *Biometrics* **34**, 429–439.

Houle, D. (1991). Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* **45**, 630–648.

Kirkpatrick, M. & Heckman, N. (1989). A quantitative

genetic model for growth, shape and other infinite-dimensional characters. *Journal of Mathematical Biology* **27**, 429–450.

Kirkpatrick, M., Lofsvold, D. & Bulmer, M. (1990). Analysis of the inheritance, selection and evolution of growth trajectories. *Genetics* **124**, 979–993.

Kirkpatrick, M. & Lofsvold, D. (1992). Measuring selection and constraint in the evolution of growth. *Evolution* **46**, 954–971.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* **33**, 402–416.

Lande, R. & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226.

Lenski, R. E. (1988*a*). Environmental studies of pleiotropy and epistasis in Escherichia coli. I. Variation in competitive fitness among mutants resistant to virus T4. *Evolution* **42**, 425–432.

Lenski, R. E. (1988*b*). Environmental studies of pleiotropy and epistasis in Escherichia coli. II. Compensation for maladaptive effects associated with resistance to virus T4. *Evolution* **42**, 433–440.

Maynard Smith, J., Burian, R., Kaufman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. & Wolpert, L. (1985). Developmental constraints and evolution. *The Quarterly Review of Biology* **60**, 265–287.

Rausher, M. D. (1992). The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**, 616–626.

Scheiner, S. M. (1993*a*). Plasticity as a selectable trait: reply to Via. *American Naturalist* **142**, 371–373.

Scheiner, S. M. (1993*b*). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and systematics* **24**, 35–68.

Schlichting, C. D. & Pigliucci, M. (1993). Control of phenotypic plasticity via regulatory genes. *American Naturalist* **142**, 366–370.

Shaw, R. G. (1987). Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution* **41**, 812–826.

Stearns, S. C. (1992). The evolution of life histories. Oxford University Press.

Turelli, M. & Barton, N. H. (1990). Dynamics of polygenic characters under selection. *Theoretical Population Biology* **38**, 1–57.

Van Noordwijk, A. J. & De Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist* **128**, 137–142.

Van Tienderen, P. H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**, 1317–1331.

Van Tienderen, P. H. & De Jong, G. (1994). A general model for the relation between phenotypic selection and genetic response. *Journal of Evolutionary Biology* **6**, 1–12.

Via, S. (1987). Genetic constraints on the evolution of phenotypic plasticity. In *Genetic Constraints on Adaptive Evolution* (ed. V. Loeschcke), pp. 47–71. Berlin: Springer Verlag.

Via, S. (1993*a*). Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? *American Naturalist* **142**, 352–365.

Via, S. (1993*b*). Regulatory genes and reaction norms. *American Naturalist* **142**, 374–378.

Via, S. & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505–522.

Via, S. & Lande, R. (1987). Evolution of genetic variability in a spatially heterogeneous environment: effects of genotype-environment interaction. *Genetical Research* **49**, 147–156.

Woltereck, R. (1909). Weitere experimentelle Untersuchungen über Artveränderung, speziell über das Wesen quantitativer Artunterschiede bei Daphniden. *Verhandlungen der Deutschen Zoologischen Gesellschaft* **19**, 110–172.