Rapid Divergence of Genetic Variance-Covariance Matrix within a Natural Population

Agnieszka Doroszuk,[1,*] Marcin W. Wojewodzic,[1,†] Gerrit Gort,[2,‡] and Jan E. Kammenga[1,§]

1. Laboratory of Nematology, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands;
2. Biometris, Wageningen University, Bornsesteeg 47, 6708 PD Wageningen, The Netherlands

Submitted September 12, 2006; Accepted October 24, 2007; Electronically published January 25, 2008

ABSTRACT: The matrix of genetic variances and covariances (**G** matrix) represents the genetic architecture of multiple traits sharing developmental and genetic processes and is central for predicting phenotypic evolution. These predictions require that the **G** matrix be stable. Yet the timescale and conditions promoting **G** matrix stability in natural populations remain unclear. We studied stability of the **G** matrix in a 20-year evolution field experiment, where a population of the cosmopolitan parthenogenetic soil nematode *Acrobeloides nanus* was subjected to drift and divergent selection (benign and stress environments). Selection regime did not influence the level of absolute genetic constraints: under both regimes, two genetic dimensions for three life-history traits were identified. A substantial response to selection in principal components structure and in general matrix pattern was indicated by three statistical methods. **G** structure was also influenced by drift, with higher divergence under benign conditions. These results show that the **G** matrix might evolve rapidly in natural populations. The observed high dynamics of **G** structure probably represents the general feature of asexual species and limits the predictive power of **G** in phenotypic evolution analyses.

Keywords: **G** matrix, genetic covariance, selection, genetic drift, rapid evolution.

* Present address: Institute of Biology, Leiden University, Leiden 2300 RA, The Netherlands; e-mail: a.doroszuk@biology.leidenuniv.nl.

† Present address: Biologisk Institutt, Blindernveien 31, 0316 Oslo, Norway; e-mail: marcin.bazyliszek@gmail.com.

‡ E-mail: gerrit.gort@wur.nl.

§ E-mail: jan.kammenga@wur.nl.

Am. Nat. 2008. Vol. 171, pp. 291–304.
DOI: 10.1086/527478

Evolutionary change in a quantitative trait is commonly accompanied by simultaneous changes in other traits through shared developmental, functional, and genetic processes. The **G** matrix summarizes these relationships and can therefore be used to make predictions of future response to selection of correlated traits or to obtain the estimation of the selection pressure acting in the past (Lande 1979, 1980; Cheetham et al. 1994; Björklund 1996; Phillips and McGuigan 2006). Evolutionary change in quantitative traits can be described by the multivariate extension of the breeder's equation $\Delta\bar{z} = \mathbf{G}\mathbf{P}^{-1}\mathbf{s}$, where $\Delta\bar{z}$ is the vector of population mean responses, **G** is the matrix of additive genetic variances and covariances, **P** is the matrix of phenotypic variances and covariances, and **s** is the vector of selection differentials (Lande 1979; Lande and Arnold 1983). This equation is valid only under the assumption that **G** is stable over evolutionary time. While many studies demonstrated a considerable **G** stability among populations (Spitze et al. 1991; reviewed in Roff and Mousseau 1999) or even among species (Cheverud 1996; Marroig and Cheverud 2001; Begin and Roff 2003), other theoretical and empirical investigations show that stability of **G** cannot be ensured (reviewed in Steppan et al. 2002; McGuigan 2006). Over longer evolutionary times, in particular, **G** matrices are likely to diverge. This is supported by several studies comparing **G** matrices among different taxa (Paulsen 1996; Roff and Mousseau 1999). Additionally, several experimental laboratory studies showed that strong selection or drift may lead to rapid changes in **G** structure (but see Wilkinson et al. 1990; Shaw et al. 1995; Phillips et al. 2001).

Since the problem of **G** stability does not seem to have a definite solution, it should be considered from the perspective of specific genetic conditions and evolutionary forces that might promote **G** stability or act in a destabilizing way (Turelli 1988; Jones et al. 2003). One of the factors affecting **G** stability is recombination frequency and, related to it, mode of reproduction (Phillips and McGuigan 2006). In principle, genomes of asexual species are transmitted to new generations without recombination, and as a result, selection acts on the properties of

the genomes as a whole. In such populations, genetic means, variances, and covariances are influenced by nonadditive genetic effects and by genetic disequilibria to a greater extent than in the corresponding sexual populations (Kelly 1999; Pfrender and Lynch 2000). In cyclical parthenogens, genetic disequilibria are expected to accumulate over the period of asexual reproduction, leading to changes in **G** structure. A single event of sex is likely, however, to make **G** structure return to its original state (Deng and Lynch 1996; Pfrender and Lynch 2000). Although there are no clear predictions concerning **G** matrix stability in obligate parthenogens, the dynamics of linkage disequilibria (understood as correlations among loci) under selection or drift might limit the stability of genetic architecture. To our knowledge, there are no empirical studies addressing the question of **G** stability in species reproducing by strict parthenogenesis, and the patterns of **G** matrix evolution under selection and drift in this group remain unexplored.

Many evolutionary forces (i.e., mutation, migration, selection, and drift) are expected to influence the **G** structure. Dimensionality of **G** is the most general aspect of **G** structure and is related to the number of genetically independent traits represented by a set of phenotypic traits that make up the "phenotypic space." When breeding values are concerned, the genetic traits may fall within the subspace of this phenotypic space, indicating absolute evolutionary constraints (Kirkpatrick and Lofsvold 1992; Mezey and Houle 2005). In such cases, there is no genetic variance for certain phenotypes (trait combinations), and these phenotypes cannot evolve in the population. In relation to other aspects of **G** structure, it has been suggested that random drift induces proportional changes of **G**, while selection is expected to cause nonproportional structural changes (Roff et al. 1999; Roff 2000). However, some researchers have questioned whether these theoretical expectations may serve as a conclusive criterion for distinguishing which process contributed to the observed differences among populations' **G** matrices. Phillips et al. (2001), for example, argued that proportional changes are rather an average response to drift and that individual populations may display a broader range of divergence levels.

Because of the lack of information on the parameter values of the evolutionary forces and their interactive effects, the problem of the influence of different evolutionary processes and genetic conditions on **G** evolution remains analytically intractable (Turelli 1988; Jones et al. 2004; McGuigan 2006). Therefore, empirical studies offer valuable means to investigate the stability of **G** matrices. The empirical studies can be classified into two main groups: comparative studies of natural populations and laboratory evolution studies. The advantage of comparative analyses is that they reveal the direction of the changes in **G** that actually happen in nature. On the other hand, they often suffer from a lack of information on populations' phylogenies and histories. This complicates relating the observed pattern of **G** structure to the timescale of population differentiation (Phillips and McGuigan 2006). In addition, the knowledge of the nature of selection is usually limited. Laboratory studies provide a framework for testing the role of evolutionary processes in shaping **G** structure, however, under unrealistic laboratory conditions. Natural experiments or planned experiments involving natural populations in the field combine the advantages of both approaches. Such experiments could provide a setting for making testable predictions without compromising the realism of field conditions.

We investigated stability of **G** in response to imposed selection and drift in natural populations of a parthenogenetic, soil-dwelling nematode *Acrobeloides nanus* de Man, 1880 (Nematoda, Cephalobidae). *Acrobeloides nanus* can be found in soils of various physical and chemical properties (Bird et al. 1993; Korthals et al. 1996; De Goede and Bongers 1998) and constitutes a large part of soil nematode communities across extremely different habitats, such as deserts of Australia (Bird et al. 1993) and Swedish tundra (Sohlenius and Boström 1999). The analyzed populations originated from an experimental field where combined treatments of two stress factors (pH level and copper) were applied approximately 20 years ago in a replicated and randomized block design and maintained thereafter. In that way, the population of *A. nanus* inhabiting the field before the treatment application was divided into a number of subpopulations exposed to different treatments of the stress factors. We analyzed subpopulations from two extreme treatments (benign treatment, habitat B, and stress treatment, habitat S; four replicate subpopulations per treatment) and demonstrated adaptive divergence of life-history traits in response to benign and stress conditions (Doroszuk et al. 2006).

Here we characterize **G** matrices of three life-history traits of the same subpopulations using common garden laboratory experiment and three methods of matrix comparison: factor-analytic approach (Hine and Blows 2006), Flury hierarchy (Phillips and Arnold 1999), and a modification of the random skewers method (Pielou 1984; Cheverud 1996). We attempt to determine effects of selection on the structure of **G** (i.e., dimensionality, proportionality, common principal components). The insight into the effects of drift is obtained by the comparison of **G** matrices among replicate populations within selection treatments. Using Flury hierarchy, we evaluate the effects of these evolutionary forces on phenotypic (**P**) and environmental (**E**) matrices. The **G** matrix, together with the environmental covariance (**E**) matrix, determines the phe-

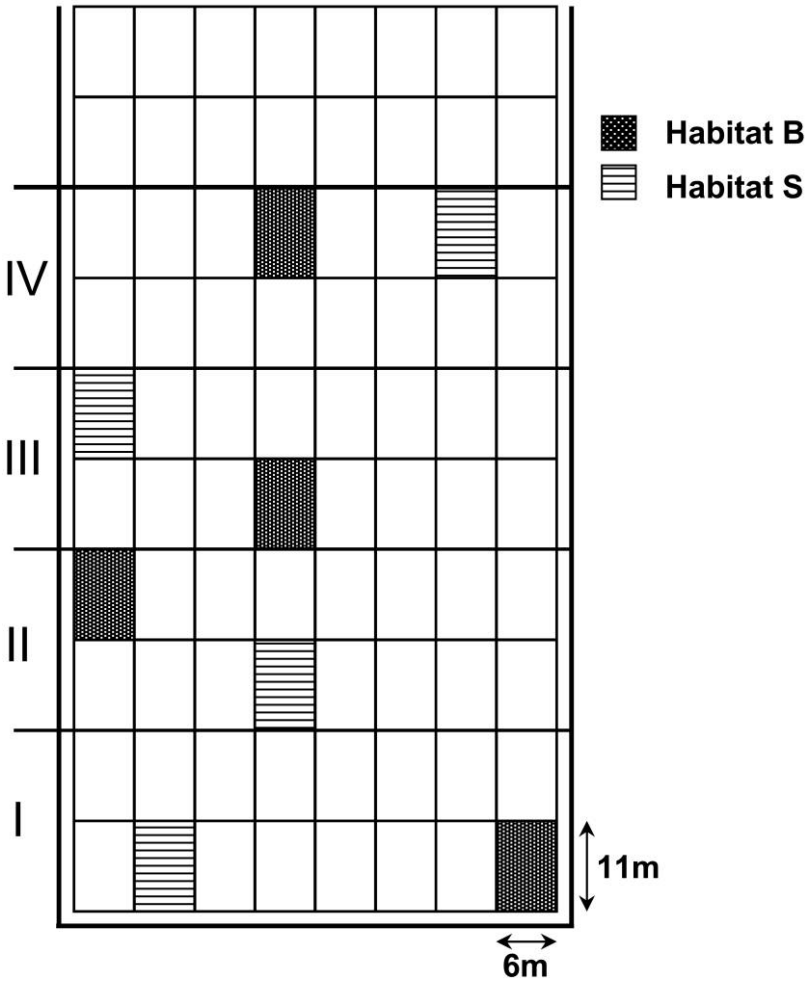

Figure 1: Distribution of treatments at the experimental field. Roman numerals indicate randomized blocks. Habitat B represents benign conditions (pH 6.1; Cu = 0 kg ha^{-1}); habitat S represents stressful conditions (pH 4.0; Cu = 750 kg ha^{-1}).

notypic covariance (**P**) matrix. Although **E** itself is not heritable, the patterns of **E** respond to evolutionary processes such as selection and mutations (Bull 1987). Because the **G** matrix is the main focus of the studies within this field, changes in **E** and their dependence on various evolutionary processes remain unexplored.

Material and Methods

Study Species, Experimental Field, and Sampling

Acrobeloides nanus is a free-living, bacterial-feeding nematode that reproduces by parthenogenesis (Wiegner and Schierenberg 1998; Laugsch and Schierenberg 2004). Under laboratory conditions at 20°C, the juvenile period requires approximately 10 days, and approximately 250 eggs are laid over the period of 35 days. The postreproductive period is nearly 10 days.

The experimental field at Bovenbuurt is located approximately 3 km north-northeast of Wageningen, Netherlands (Korthals et al. 1996). It was created in 1982, when an agricultural field was divided into 128 plots of 6 m × 11 m each, and four copper and pH levels were introduced by a single application of $CuSO_4 \cdot 5H_2O$ and sulphur powder or ground calcitic limestone in a full factorial design. Levels of pH were adjusted every 5 years on average. The plots were arranged into eight blocks (fig. 1), each with a random distribution of all combinations of copper and pH treatments. For our study, we chose two extreme treatments (each represented by four replicate plots): one treatment imposing stress by low pH (4.0) and a copper concentration of 750 kg Cu ha^{-1} (habitat S) and

a benign treatment with pH 6.1 without copper addition (habitat B). More information about *A. nanus* and the experimental field can be found in the study by Doroszuk et al. (2007).

To obtain representative soil samples, we mixed about 30 core samples per plot (diameter 30 mm) from the top 10 cm of soil mineral layer. Nematodes were extracted using standard techniques (Oostenbrink 1960), and individuals identified as *A. nanus* (using a microscope at × 400 total magnification) were placed on petri dishes (one individual per dish) with proteose peptone agar (PPA) medium (2% technical agar, 0.5% proteose peptone) and *Acinetobacter johnsonii* strain 210A (Netherlands Culture Collection of Microorganisms, access no. LMAU A130; Bonting et al. 1992), a soil bacterium, as a food source. In this way, approximately 15 laboratory populations per plot were established. Number of generations per year under field conditions was estimated as 0.7 for the nematodes from habitat S and 6.6 for the nematodes from habitat B (Doroszuk et al. 2007).

Common Garden Experiment

Life-history traits were recorded on the basis of the observation of individual nematodes kept in 24-well plates with PPA. The PPA (200-μL droplets, pH 6.0) was pipetted on the inner side of the lid, while the wells contained 0.5 mL of water to prevent desiccation. On the top of every agar droplet, 2 μL of *A. johnsonii* suspension (2×10^8 cells mL^{-1}) was applied, and the plates were subsequently incubated at 28°C overnight.

The nematodes derived from a single individual isolated from an experimental plot were defined as a clone. We used eight to 15 clones per experimental plot (of both habitats). Six age-synchronized eggs per clone were placed in separate wells. After hatching, individuals were transferred every second day to fresh PPA plates. The eggs used for this experiment were the fourth generation after introduction of the nematodes to the laboratory. For each individual, the duration of reproductive period, total reproduction, and life span were recorded. The 24-well plates with nematodes were incubated at 20°C in dark. The overview of the experimental design is presented in table 1.

Statistical Methods

All traits were standardized to a mean of 0 and standard deviation of 1. The basis of genetic analysis was the use of the eight to 15 clones originating from each experimental plot. Partitioning of the total phenotypic variance into the within- and among-clone variance allowed for estimating total genetic variance. The among-clone variance reflected total genetic variance (additive and nonadditive) together with the maternal effects (Lynch and Walsh 1998). Although we did not assess the magnitude of maternal effects, the influence of maternal effect on the results of the experiment was equalized by rearing the former generation of nematodes in standardized, similar laboratory conditions. Broad-sense heritability (H^2) was estimated as the proportion of total variance due to among-clone variance. Quantitative genetic parameters and phenotypic covariances were estimated with H2boot software (http://www.uoregon.edu/~pphil/software.html).

Comparison of the Matrices

Factor-Analytic Approach. Factor-analytic modeling aims at finding the minimum number of dimensions explaining the pattern of covariances among a number of variables and can be used for fitting genetic principal components (Kirkpatrick and Meyer 2004; Hine and Blows 2006). The estimation of effective dimensionality of **G** for almost any experimental design is performed by likelihood-ratio testing in the restricted maximum likelihood framework. Hine and Blows (2006) outlined the readily available factor-analytic approach implemented in the PROC MIXED in SAS (SAS Institute, Cary, NC). We tested the hypotheses in the following three steps. In the first step, **G** dimensionality was tested separately for each habitat. The reduced-rank covariance model for trait was specified for the clone level nested within plot using the FA0(q) covariance structure (q is the number of dimensions of **G**) of PROC MIXED, assuming independence between the clones. Plot was specified in the random part of the model. An unstructured covariance matrix was assumed for trait at the level of individuals. Three nested hypothesis tests were performed starting from the full model, where the number of factors (dimensions) was equal to the number of traits, and continued by dropping the factors sequen-

Table 1: Overview of the experimental design

	Habitat B				Habitat S			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 1	Plot 2	Plot 3	Plot 4
No. clones	15	8	14	10	13	15	13	14
No. individuals	64	32	53	37	52	72	52	66

Note: Number of individuals per clone was variable (three to six).

tially. After determining the dimensionality of **G** for both habitats, the second step of the analysis was performed, namely, the test for the differences in reduced-rank **G** matrices among replicate plots (within habitats). The differences in FA0(q) structures among plots were allowed by using the "group" statement in PROC MIXED. This model was compared with the model with an equal FA0(q) structure for the plots, using a likelihood ratio test. The third step of the analysis involved the test for the difference of reduced-rank **G** matrices between habitats. The fixed part of the mixed model contained habitat, trait, and their interactions, eliminating mean differences between the combinations. The same random part of the mixed model was specified as in the within-habitat analyses. Initially, the variation in **G** among plots was accommodated in the model. However, the analysis did not converge. Therefore, the final model did not accommodate among plot variation in **G**. The "group" statement was used to assess habitat effect on the reduced-rank **G** matrices.

Flury Hierarchy. The Flury hierarchy method is a principal components–based method that allows comparison of two or more matrices along the hierarchy of hypotheses relating to the level of their similarity (Phillips and Arnold 1999). Compared matrices can have unrelated structures or can share some principal components (eigenvectors). Furthermore, matrices can share all eigenvectors, while their eigenvalues might differ by a single constant indicating proportionality. Finally, matrices can be equal, having the same values at each element. The fit of each model to the observed matrices is determined by the log-likelihood statistics, and the hypotheses are tested with a likelihood ratio against the model of unrelated structure. Testing starts at the bottom (where the first principal component is tested) through all levels toward matrix equality. When a significant deviation from the unrelated structure is encountered, the testing is terminated (jump-up procedure; Phillips and Arnold 1999). In this study, the significance of each test was determined with the use of a randomization procedure (10,000 runs per test), where clones were randomly assigned to different habitats (or plots in case of the within-habitat comparisons) and the matrices were tested for similarity. The obtained distribution of the test statistics was used as the null distribution for test at all hierarchy levels. This analysis was performed using the CPCrand software (http://www.uoregon.edu/~pphil/software.html).

Since this approach does not allow more complicated experimental designs, we performed three separate analyses. In the first analysis, the data for all plots within habitats were pooled in order to investigate the matrix similarity between the habitats. The other two analyses were performed separately for each habitat in order to test the similarity of the **G** matrices among the replicate plots within habitats. Since we encountered the problem of nonpositive matrices in our analyses, it was necessary to apply a "bending" procedure. This procedure adjusts nonpositive eigenvalues in the way that the matrix becomes positive definite.

Random Skewers Method. The random skewers method (Pielou 1984; Cheverud 1996) allows comparison of **G** matrices by comparing the evolutionary responses of these matrices to random selection vectors. Random selection gradient vectors are generated and applied to each of the compared matrices. The vectors of expected evolutionary responses are obtained and subsequently compared with a vector correlation. This procedure is repeated, and the average vector correlation between evolutionary responses to, in this case, 10^5 random selection vectors is used as a measure of the similarity between **G** matrices. When **G** matrices are identical, the average vector correlation will be 1.0. The null hypothesis of no similarity among matrices is tested by comparing the obtained average vector correlation with the distribution of correlations among random vectors. The analysis was performed in two steps. First, the similarity of **G** matrices pooled within habitats was tested. Second, in order to gain more insight into similarity patterns among matrices within and between habitats, all eight matrices (two habitats × four replicate plots) underwent pairwise comparisons. In the presented case (three-element vectors), vector correlations greater than 0.9 are significantly greater than 0. The analysis was performed using skewers software (http://anolis.oeb.harvard.edu/~liam/programs/).

Results

Phenotypic Means, Heritability, and Genetic Correlations

MANOVA with habitat as a fixed factor and plot (replicate population) nested within habitat showed a significant overall effect of habitat on phenotypic means of *Acrobeloides nanus* life-history traits (Wilks's $\lambda = 0.91$, $F = 10.67$, df $= 3, 343$, $P < .0001$) as well as a significant effect of plot (Wilks's $\lambda = 0.91$, $F = 1.84$, df $= 18, 971$, $P = .017$). The most pronounced differences between habitats were observed for total reproduction. For this trait, the nematodes from the plots of habitat B showed lower values. The differences between the habitats in the trait means together with the results of the previous study implementing reaction norm and transplant experiments in the same populations (Doroszuk et al. 2006) provide strong support for an adaptive response to the imposed selection. Such a response is likely to be accompanied by the changes in genetic covariance structure of the analyzed traits.

Broad-sense heritability (H^2) for the data pooled within

habitats was significant for all three life-history traits in habitat S populations. This was not found for populations from habitat B (table 2). For data pooled within habitat S, all three genetic correlations were significant, while for habitat B they were not.

Divergence of **G** *within Habitats*

The factor-analytic approach indicated the best fit of the two-dimensional model FA0(2) for both habitats. Likelihood ratio tests showed significant decreases in the model fits when changing the two-dimensional covariance structures to one-dimensional structures (habitat B: $\chi^2 = 8.8$, df = 2, $P < .012$; habitat S: $\chi^2 = 12.3$, df = 2, $P < .002$), suggesting the best fit of the models with two-dimensional **G** structure. This was supported by the lowest Akaike Information Criterion (AIC) values (table 3). On the basis of these results, two-dimensional **G** matrix structure FA0(2) was incorporated to test for the difference in the reduced-rank **G** among replicate plots. While for habitat S the hypothesis of common reduced-rank **G** structure for all plots was not rejected ($\chi^2 = 15.5$, df = 15, $P = .42$), allowing for different reduced-rank **G** structures for the replicate plots of habitat B resulted in the better fit of the model ($\chi^2 = 25.1$, df = 15, $P = .048$). This shows that the level of **G** matrix divergence within habitats depends on habitat or, in other words, habitat-specific selection regime.

Comparison of **G** matrices for four replicate plots within habitat S using Flury hierarchy resulted in detection of a high level of matrix similarity. Although the jump-up procedure returned a common principal components verdict (i.e., the "CPC" hypothesis was not rejected, while the proportionality was declined; $P = .026$), the same analysis did not lead to the rejection of the hypothesis of matrix equality (table 4), therefore indicating high similarity among **G** structure for replicate plots of habitat S. The corresponding analysis for habitat B could not be carried out because the initial **G** matrix constructed in Flury hierarchy had too many negative eigenvalues, which is likely to be caused by limited genetic variance for life span and reproductive period.

The random skewers vector correlations obtained for pairwise comparisons of **G** matrices for eight plots (table 5) were used to test for the differences between habitats in the similarity level among replicate plots. **G** matrices were more similar in habitat S, with average vector correlation of 0.490 (SE = 0.128), than in habitat B (Mann-Whitney $U = 3$; $Z = -2.4$; $P = .016$), where the average vector correlation was −0.038 (SE = 0.151). These results are consistent with the results obtained with the factor-analytic approach and suggest an overall lower divergence of **G** matrices among replicate plots of habitat S, where strong selective pressure was applied. It needs to be noted that according to the significance tests for the random skewers vector correlations, the hypothesis of no similarity was sustained for all pairwise comparisons.

Table 3: Fit statistics for the nested series of factor-analytic models testing the dimensionality of **G** matrices of *Acrobeloides nanus* from both habitats

	Habitat B		Habitat S		No. covariance parameters
	−2LL	AIC	−2LL	AIC	
FA0(3)	1,140.6	1,166.6	1,415.3	1,441.3	13
FA0(2)	1,141.0	1,163.0	1,416.1	1,440.1	12
FA0(1)	1,149.8	1,169.8	1,428.4	1,448.4	10

Note: Tests for habitat S and habitat B performed separately. LL = log likelihood, AIC = Akaike Information Criterion.

Table 2: Genetic correlations, covariances, broad-sense heritability, and genetic variance for life-history traits of the populations of *Acrobeloides nanus* from different habitats

	Total reproduction	Life span	Reproductive period	H^2	V_G
Total reproduction:					
Habitat B	…	−.24 (1.16)	.44 (.73)	.28 (.10)***	.25 (.12)***
Habitat S	…	.92 (.91)*	.82 (.25)*	.20 (.07)**	.22 (.08)**
Life span:					
Habitat B	−.04 (.05)	…	.40 (3.60)	.01 (.07)	.02 (.09)
Habitat S	.16 (.08)*	…	.98 (.82)**	.14 (.08)*	.18 (.1)*
Reproductive period:					
Habitat B	.08 (.08)	−.03 (.08)	…	.10 (.07)	.08 (.06)
Habitat S	.14 (.06)*	.13 (.06)*	…	.18 (.06)**	.13 (.05)*

Note: Data within habitats pooled. Genetic covariances are shown below diagonal and genetic correlations above diagonal. Standard errors are given in parentheses. Asterisks indicate significance level when different from 0.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

Table 4: Comparisons of **G**, **E**, and **P** matrices with Flury hierarchy

	G similarity (*P*)	**E** similarity (*P*)	**P** similarity (*P*)
Between habitats	Unrelated	Unrelated	Unrelated
Among populations within habitats:			
Habitat B	Unable to estimate	Unable to estimate	Unable to estimate
Habitat S	Equal (.085)	Proportional (.069)	Equal (.32)

Note: Results are based on 10,000 randomizations over clones (CPCrand software). *P* values indicate that the similarity hypothesis could not be rejected.

Effect of Divergent Selection on G Matrices

Since two-dimensional covariance structure FA0(2) was supported by the factor-analytic approach for both habitats, the subsequent analysis used FA0(2) to test for the differences between the habitats in the reduced-rank matrices. The likelihood ratio test indicated that the hypothesis of common structure of **G** matrices for both habitats could not be rejected at the significance level of 0.05 ($\chi^2 = 10.2$, df = 5, $P = .068$). However, different conclusions can be derived from the inspection of AIC: the reduced model resulted in AIC = 2,610.7, while the full model resulted in AIC = 2,607.5.

Flury hierarchy indicated a highly divergent **G** structure between habitats (matrices pooled across replicate plots). The verdict of unrelated structure was reached, since the hypotheses at all similarity levels were rejected (table 4). These substantial changes in **G** matrices (fig. 2) can most likely be attributed to the applied divergent selective pressure.

Similar results were obtained with the random skewers method. Low similarity in **G** structure between habitats (pooled across replicate plots) was indicated (average vector correlation = 0.387), and the hypothesis of no similarity of **G** was maintained ($P = .30$). In addition, the level of **G** similarity between habitats was also compared with the one observed within habitats using the average random skewers vector correlations (hereafter called vector correlations) from pairwise **G** comparisons for eight plots (two habitats × four replicate plots). The values presented in table 5 were divided into two classes, depending on whether they concerned within- or between-habitat comparison. The average of vector correlations for the between-habitat comparisons was 0.05 (SE = 0.132), while that for the within-habitat comparisons was 0.23 (SE = 0.123). Mann-Whitney *U*-test (distribution of vector correlations departed from normality) indicated that the level of matrix similarity within and between habitats did not differ significantly (Mann-Whitney $U = 80$; $Z = -0.74$; $P = .46$). This suggests that the level of **G** matrix divergence due to selection regime was not much greater than the divergence among replicate plots.

Environmental and Phenotypic (Co)variance Matrices

Pooled environmental variance-covariance matrices (**E**) showed unrelated structure between habitats (table 4). The hypothesis of single shared principal component was rejected ($P = .006$). The same analysis resulted also in the rejection of the hypotheses on all other levels of similarity hierarchy (in all cases $P < .05$), which confirms the verdict of high divergence in **E** between habitats. In contrast, **E** matrices of replicate populations within habitat S showed a considerable level of similarity. Namely, the results of Flury hierarchy suggest proportionality of **E** among replicate plots by sustaining the hypothesis on this level ($P = .069$) and rejecting the equality hypothesis ($P = .045$). The corresponding analysis for habitat B could not be carried out because the initial **E** matrix had too many negative eigenvalues. Pooled phenotypic matrices (**P**) showed high divergence between habitats. The hypotheses for all the levels of similarity, including the one of a single shared principal component ($P = .032$), were rejected, indicating unrelated structure of the matrices. The **P** comparison of replicate populations of habitat S indicated their equality ($P = .324$)

Discussion

We demonstrated a rapid divergence (20 years) of **G** structure within a natural population of the parthenogenetic nematode *Acrobeloides nanus* in response to experimentally imposed divergent selection and drift. To our knowledge, this study is the first attempt to combine the realism of field conditions with the control of an evolution experiment where selective agent and evolutionary time frame are defined by experimental manipulation. Documented examples of **G** matrix evolution in natural populations commonly consider the time spans of thousands or millions of years (Arnold and Phillips 1999; Cano et al. 2004). The time of divergence of genetic architecture reported in this study is more comparable with the ones observed in laboratory studies (Bryant and Meffert 1988; Wilkinson et al. 1990; Shaw et al. 1995; Phillips et al. 2001). We are not aware of any other reports of such high dynamics of **G**

Table 5: Average vector correlations between **G** matrices responses to 100,000 random selection vectors for all pairwise comparisons between plots

	Habitat B				Habitat S			
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 1	Plot 2	Plot 3	Plot 4
Habitat B:								
Plot 1	1.000							
Plot 2	.267	1.000						
Plot 3	.281	−.709	1.000					
Plot 4	−.122	.153	−.096	1.000				
Habitat S:								
Plot 1	−.36	−.604	.389	−.461	1.000			
Plot 2	.702	.042	.431	−.347	.042	1.000		
Plot 3	.548	−.366	.804	−.279	.318	.743	1.000	
Plot 4	.442	−.558	.894	−.339	.342	.600	.895	1.000

Note: For significant comparison at the significance level of 0.05, the average vector correlation should be >0.9.

structure (involving nontransient changes) in natural populations.

Acrobeloides nanus reproduces by obligate parthenogenesis (Wiegner and Schierenberg 1998; Laugsch and Schierenberg 2004). Although it has been acknowledged that the mode of reproduction (recombination frequency) influences the behavior of **G** matrices (Kelly 1999; Phillips and McGuigan 2006), little is known about the dynamics of genetic covariance in asexual populations. Analytic and stochastic studies taking into account genetic factors relevant in asexual populations (such as genetic disequilibria) are scarce (but see Bulmer 1980; Lynch and Gabriel 1983; Lynch and Deng 1994; Deng and Lynch 1996; Kelly 1999). It should be noted that genetic covariances are determined mainly by pleiotropy and genetic disequilibria (both gametic-phase and Hardy-Weinberg disequilibria). Clonal reproduction generates nonrandom associations between loci, because it mimics complete physical linkage over the entire genome (Tibayrenc et al. 1991; Awadalla 2003). Therefore, it is expected that genetic disequilibria have a bigger influence on the patterns of genetic covariance in clonal than in sexual species. Some theoretical predictions and experimental data on this topic are available for cyclical parthenogens (Lynch and Deng 1994; Deng and Lynch 1996; Pfrender and Lynch 2000). These studies focus predominantly on the changes of genetic variances and covariances due to accumulation of genetic disequilibria during clonal propagation and on effects of subsequent sexual reproduction. While they usually indicate a large influence of genetic disequilibria on stability of genetic covariances in cyclically clonal species, the expectations regarding obligate parthenogens, where genetic disequilibria have different dynamics, remain unclear. In obligate asexuals, selection favors specific multilocus genotypes, which is expected to change correlations among loci (pattern of linkage disequilibrium) in the population. This is likely to influence the levels of expressed genetic variance and covariance (Lynch and Deng 1994) and introduce changes into **G** matrices.

The interpretation of our results would be easier with the information on how dynamics of genetic disequilibria influence the dynamics of **G**. Currently, however, we are not aware of any study (theoretical or empirical) where the problem of **G** stability in asexual populations under selection is directly approached. Overall, the results of our study support the general view of destabilizing effects of genetic disequilibria on the **G** matrix. The results imply also that the predictive value of **G** for analyzing phenotypic evolution in asexual species or species with rare recombination events might be limited.

Analytical and simulation studies (Lande 1980; Turelli 1988; Barton and Turelli 1989; Reeve 2000; Jones et al. 2003, 2004) resulted in formulation of conditions promoting stability of **G** structure. **G** stability is expected to be enhanced by, among others, large population sizes and the presence of strong correlations of mutational effects (Jones et al. 2003). In relation to population size, we found that a population inhabiting a single plot (6 m × 11 m), regardless of habitat type, consisted of approximately 2×10^5–5×10^5 individuals (Doroszuk et al. 2007). These numbers, which are likely to be representative for effective population size (Balloux et al. 2003), are relatively high. Therefore, population size is not likely to be an important factor driving the divergence of **G** among the populations. The influence of correlational selection and mutation remain unclear, since these aspects were not analyzed in our system. For another nematode, *Caenorhabditis elegans*, effects of mutations on life-history traits showed positive correlation (Keightley et al. 2000; Estes et al. 2005; Estes and Phillips 2006). It is predicted that some types of traits might have more stable **G** matrices than the others; namely, for life-history parameters, lower stability of **G** is expected.

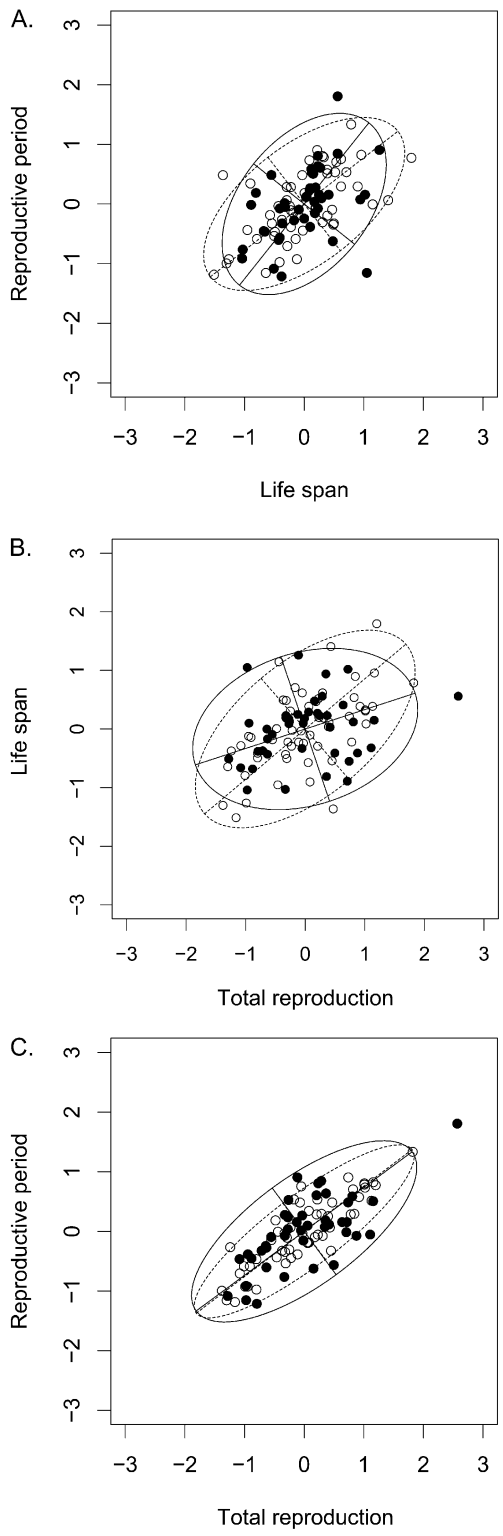

Figure 2: Genetic covariances of life-history traits for the populations of *Acrobeloides nanus* from habitat B (*solid lines*) and from habitat S (*dashed lines*). Each graph represents different trait combinations. Ellipses of 95% confidence intervals were calculated on the basis of the standardized means (overall mean = 0, SD = 1) for clones pooled within habitat type. Principal components of the matrices were used to orient the ellipses in the plane. The ellipses were centered through the origin of principal axes. The clones' means are represented by solid circles for habitat B and open circles for habitat S. Note that each graph represents a bivariate plane that is a part of a three-dimensional data set.

On the basis of the results of their simulation study, Jones et al. (2003) suggested that morphological traits, especially the ones that are bilaterally symmetrical, are likely to be highly stable because of strong correlation of mutational effects and strong correlational selection. The opposite is expected for **G** matrices of fitness components that are under persistent directional selection and are characterized more often than other traits by negative genetic correlations (Roff 1996). This mechanism, however, is not likely to play an important role in destabilizing **G** matrices in *A. nanus*, since the genetic correlations between life-history traits in the analyzed populations were positive. Although life-history characters are expected to show often negative genetic correlations, the finding of positive genetic correlations in *A. nanus* is not an exception. Positive genetic correlations of life-history traits have been reported in many studies (reviewed in Roff 1996). In addition, as noted earlier, major effect mutations in *C. elegans* appear to have positive correlated effects on life-history traits (Keightley et al. 2000; Estes et al. 2005; Estes and Phillips 2006). Another characteristic of life-history traits is that they are under a greater influence of nonadditive effects as compared with other traits. This property is likely to contribute to lower stability of **G** and limited predictability of its responses under specific evolutionary forces such as short episodes of genetic drift (Roff 2000). Overall, although stability of **G** matrices in *A. nanus* could have been influenced by the described factors, their role was possibly minor in comparison with the influence of the reproductive mode.

Using the random skewers method, Flury hierarchy, and the factor-analytic approach, we showed divergence of **G** structure in *A. nanus* due to the applied divergent selection. The detected differences concern both the magnitude of matrix elements and the orientation of genetic covariances and are therefore in concordance with the theoretical predictions of nonproportional changes in **G** in response to strong selection (Roff 2000). Phillips et al. (2001) argued that theoretical predictions regarding response of **G** matrix to specific evolutionary forces can be treated as expectations, namely, the mean over all possible outcomes. In this context, looking at the average responses of pooled **G** matrices to divergent selection allows determination of whether the average divergence patterns correspond to the

predictions for selection effects or rather represent the effects predicted for drift.

The level of divergence of **G** among replicate populations depended strongly on habitat type. Populations of habitat B showed higher levels of divergence than the populations of habitat S. This result was consistent across all methods, with the limitation that Flury hierarchy comparison for habitat B could not be carried out. According to theoretical predictions, a lack of major differences in selective pressures across populations within the same habitat should conserve **G** structure, and the effect of drift is expected to be restricted to proportional changes in **G** (Roff 2000). Therefore, the level of shared structure of **G** matrices in populations in habitat B is lower than expected. It has been indicated, however, that proportional change in response to drift is rather an average effect and that individual populations are likely to show a whole range of structural changes of **G** (Phillips et al. 2001; Widen et al. 2002). Moreover, our result of higher **G** divergence among replicate plots within habitat B is consistent with observations made by Cohan (1984), who demonstrated that replicate populations are likely to diverge via drift when uniform selection acting on them is weak. Another possible explanation of the difference between habitats in **G** divergence level might be related to the difference in generation time of the nematodes. In our previous study, we estimated the number of generations per year as 6.6 for habitat B and 0.7 for habitat S (Doroszuk et al. 2007), which indicates that populations of habitat B were subjected to drift for longer "evolutionary time." Alternatively, the divergence within habitats might reflect not only the effect of drift but also possible effects of local selective pressures and their interactive effects, which could have more pronounced effects on the populations of habitat B.

Interpreting our results, we assumed that **G** matrices had been uniform across the experimental populations before the treatment application 20 years ago. While we cannot exclude the possibility that there could be some initial local differences in **G**, we believe that their influence on our conclusions is minor. First, randomized block design used in the experimental field and the spatial distribution of the experimental plots (fig. 1) minimized the possibility of the occurrence of such artifacts. Second, before the treatment application, the experimental field was used as an agricultural field with uniform management practices and crops that reduce environmental heterogeneity (Benton et al. 2003) and potential differences in **G**.

Although the average levels of heritability in *A. nanus* were similar across habitats, the individual traits differed in their heritability patterns. For total reproduction, higher heritability was observed in populations from habitat B, while the same populations showed lower heritability for life span and reproductive period. It needs to be noted that the largest adaptive phenotypic differences between habitats were observed for total reproduction (Doroszuk et al. 2006). Therefore, in case of this trait, our observations are consistent with the general expectation that strong directional selection (e.g., stress) depletes genetic variance (Fisher 1930; Merilä and Sheldon 1999). Higher levels of heritability for the other two traits in habitat S could be partly explained by the influence of mutation and environmental variance (Lynch and Gabriel 1983). While we have no data on mutation rate in *A. nanus* under different environmental conditions, studies on other species showed that stress can elevate mutation rate (Metzgar and Wills 2000; Bjedov et al. 2003) and cause higher heritability levels under such conditions. Moreover, our study indicated higher levels of environmental variance for habitat B for these traits (data not shown), which reduced heritability values.

Two genetic dimensions were found to represent sufficiently three life-history traits of *A. nanus* from both habitats, indicating an intermediate level of absolute constraints. It should be noted that this analysis concerns bidirectional absolute constraints, which occur when there is no genetic variance for phenotypically variable traits (Mezey and Houle 2005). Our results of **G** showing lower rank than the dimensions measured are not very surprising. Indirect evidence suggests that for life-history traits in *A. nanus*, some bidirectional constraints might exist. Although life-history traits are determined by a large number of genes, it is unlikely that all these genes have distinct effects. In fact, life-history traits are often negatively correlated (Barton and Turelli 1989; Roff 1996), and it has been postulated that loci contributing to trade-offs between life-history traits show antagonistic pleiotropy (Stearns 1992). Our previous study (Doroszuk et al. 2006) showed that divergent selection (habitats S and B) acting on the same populations caused an evolutionary change of almost all analyzed life-history traits. It suggests a correlated response rather than independent, fine-grained responses of each trait. Our findings of reduced-rank **G** matrices are similar to the findings of Kirkpatrick and Lofsvold (1992) and Hine and Blows (2006) and support the idea that absolute constraints could be a common feature of traits' genetic architecture. On the other hand, the evidence of very low levels of absolute bidirectional constraints obtained for *Drosophila melanogaster* wing shape (Mezey and Houle 2005) suggests cautiousness in generalizing these conclusions for all traits.

Phenotypic variances and covariances are generated by genetic and environmental effects. One of these two components may entirely determine phenotypic covariances or any of their intermediate combination. It is important to realize that although environmental covariance is not heritable, the susceptibility to environmental effects is often

genetically determined and therefore might evolve (Bull 1987). We found that **E** matrices of *A. nanus* significantly diverged between habitats, as indicated by the verdict of unrelated matrices returned by the Flury hierarchy. It has been demonstrated that patterns of selection might have a profound effect on environmental variances and covariances (Slatkin and Lande 1976; Bull 1987). While stabilizing selection is expected to reduce environmental variance around the genotypic mean near the optimum, fluctuating selection favors higher environmental variance in phenotypes. Different selection regimes in two habitat types might promote other levels of phenotypic and environmental variation and result in divergence of **E** structure. Mutational effects might also contribute to the reduction of **E** stability under weakly conserved developmental programs (Arnold and Phillips 1999). Embryonic studies have demonstrated that developmental decisions are made relatively late in *A. nanus*, and the sequence of developmental events is not as strictly ordered as in *C. elegans* (reviewed in Schierenberg 2001). This suggests limited conservation in developmental programs, which might result in potential greater influence of mutational effects on **E** structure. The latter scenario is also supported by the divergence of **E** among replicate plots of habitat S.

It has often been suggested that phenotypic matrices are a reliable surrogate of **G** (Steppan et al. 2002). Although we did not compare **P** with **G** directly, our results show that the response of phenotypic matrices to the imposed selection and drift differed from the one found for **G** matrices. Consequently, we are of the opinion that the interpretation of the patterns of **P** as representative for genetic matrices should be treated with caution.

Although there are many statistical methods for **G** matrix comparison (Cheverud 1996; Phillips and Arnold 1999; Roff 2002; Widen et al. 2002; Begin and Roff 2003), no single statistical test offers an ideal solution. Therefore, a simultaneous use of multiple methods is currently the option providing most accurate insights into the patterns of **G** evolution (Steppan et al. 2002; Begin and Roff 2004). The problem of appropriate statistical testing is particularly apparent in studies with complicated experimental designs. In such studies (including ours), experimental designs cannot be readily implemented in statistical analysis, which leads to less direct approaches and applications of series of pairwise comparisons. We decided to use three methods of matrix comparison: Flury hierarchy, factor-analytic approach, and random skewers method. While Flury hierarchy is currently one of the most popular methods in **G** matrix studies, interpretation of the results returned by these methods requires consideration of several points. Small sample sizes and small numbers of analyzed traits are expected to result in detection of **G** structure similarity (Phillips and Arnold 1999). In our study, these limitations do not need to be considered as a source of a serious bias in **G** comparison between habitats, since in this case a significant divergence in **G** was found. On the other hand, we cannot exclude the possibility that the result of **G** equality within habitat S was influenced by relatively small sample size and application of "bending" procedure. As emphasized by some authors (Marroig and Cheverud 2001; Houle et al. 2002), by focusing on orthogonal structures represented by CPC, the Flury method might underestimate the degree of structure shared by different matrices, especially at the intermediate similarity levels. Implementation of "bending" procedure is known to change the error structure of the comparison matrix, and it is likely to bias results obtained with the Flury method. It is, however, uncertain what kind of bias might be expected when this procedure is applied. The other principal components–based method applied in this study, the factor-analytic modeling, was introduced in quantitative genetics to study primarily effective dimensionality of **G** matrices (Meyer and Kirkpatrick 2005; Hine and Blows 2006). The implementation of this method into the framework of the PROC MIXED procedure of SAS software enables direct testing of the eigenvectors' differences among populations (Kraft et al. 2006; Blows 2007). These two different tests (dimensionality and difference among reduced-rank matrices) provide valuable information on various aspects of **G** evolution, which is a great advantage of this method. For finding the statistically supported number of **G** dimensions, almost any experimental design can be implemented. However, testing for the differences among **G** structures in complicated experimental designs requires the construction of multistep hypotheses, which might lead to difficulties in interpretation. Our study showed slight discrepancies between the results obtained with both principal components–based methods. While factor-analytic modeling indicated the level of divergence of reduced-rank **G** matrices that was at the border of statistical significance ($P = .068$; AIC smaller for the model with separate **G** structures for both habitats), Flury hierarchy showed unrelated **G** structures between habitats. Higher level of divergence, as compared with factor-analytic modeling, was also indicated by Flury hierarchy for replicate plots of habitat S. The results returned by the random skewers method are in general agreement with the results obtained with the other two methods. This method differs from the other two in two main aspects. First, it can be broadly classified as a matrix correlation method, where the matrices are not directly compared but their evolutionary responses to random selection vectors are. Second, it tests the null hypothesis of no similarity among matrices, which is an informative approach from the perspective of studying evolution of **G** (Marroig and

Cheverud 2001). It has been reported that matrix correlation methods might provide different results in comparison to principal component methods (Ackermann and Cheverud 2000; Begin and Roff 2001). In this study, however, all used methods generally support each other. The random skewers method, unlike the other two, allows only for pairwise matrix comparisons, which limits the advantages of applied experimental designs. In our study, we performed Mann-Whitney *U*-tests in order to investigate the patterns of **G** similarity within and between habitats. Although this way of analyzing the data does not provide an ideal statistical approach, since the vector correlation data are not fully independent, studying the patterns of pairwise matrix similarities can provide important insights into the patterns of **G** divergence.

The reliability of laboratory estimates of genetic parameters has been questioned in several studies (Charmantier and Garant 2005; Pigliucci 2006). Environmental influence on genetic covariance structure has been demonstrated on several occasions (Guntrip et al. 1997; Cano et al. 2004) and led to the general conclusions that genetic architecture is likely to breakdown in novel environments, thus also under laboratory conditions (Charmantier and Garant 2005). Under novel but less variable laboratory conditions, environmental variance is expected to be reduced and the expression of genetic variance might increase (Sgrò and Hoffmann 1998), leading to an overestimation of heritability and the elements of **G**. Although our results might suffer from similar bias, the observed differences between the habitats are likely to remain valid. In fact, if the genetic parameters were estimated under field conditions, the detected difference could be even larger than the one based on the laboratory estimates. Stressful environments are usually more variable than favorable ones (Charmantier and Garant 2005). Therefore, laboratory estimates of heritability and elements of **G** are expected to be more inflated for the populations from habitat S, which would imply that the real differences between habitats are larger than observed.

Understanding patterns of phenotypic variation produced by selection and ability to make predictions of future phenotypic responses depends on our knowledge of the stability of genetic variance-covariance matrices. The information on how the structure of **G** evolves and what conditions promote these changes would allow us to decide whether an evolution analysis with the breeder's equation could be performed (Phillips and McGuigan 2006). The high dynamics of the changes in genetic architecture reported in this study is in evident contrast with most of the existing studies on natural populations. This difference is likely to be associated with an asexual mode of reproduction in *A. nanus* and the following dynamics of genetic disequilibria. If low stability of the **G** matrix in asexual species or the species with rare events of sexual reproduction is their general feature, the predictive power of **G** for their phenotypic evolution will be limited.

Acknowledgments

We thank A. W. G. van der Wurff and B. Sheldon for their valuable comments on the previous version of the manuscript. The work was supported by the TRIAS Program (TRIpartite Approach to Soil System Processes) of Delft Cluster, the SKB (Center for Soil Quality Management and Knowledge Transfer), and the NWO (Netherlands Organization for Scientific Research) as project 835.80.008.

Literature Cited

Ackermann, R. R., and J. M. Cheverud. 2000. Phenotypic covariance structure in tamarins (genus *Saguinus*): a comparison of variation patterns using matrix correlation and common principal component analysis. American Journal of Physical Anthropology 111: 489–501.

Arnold, S. J., and P. C. Phillips. 1999. Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. Evolution 53:1516–1527.

Awadalla, P. 2003. The evolutionary genomics of pathogen recombination. Nature Reviews Genetics 4:50–60.

Balloux, F., L. Lehmann, and T. de Meeus. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164:1635–1644.

Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative genetics: how little do we know? Annual Review of Genetics 23:337–370.

Begin, M., and D. A. Roff. 2001. An analysis of **G** matrix variation in two closely related cricket species, *Gryllus firmus* and *G. pennsylvanicus*. Journal of Evolutionary Biology 14:1–13.

———. 2003. The constancy of the **G** matrix through species divergence and the effects of quantitative genetic constraints on phenotypic evolution: a case study in crickets. Evolution 57:1107–1120.

———. 2004. From micro- to macroevolution through quantitative genetic variation: positive evidence from field crickets. Evolution 58:2287–2304.

Benton, T. G., J. A. Vickery, and J. D. Wilson. 2003. Farmland biodiversity: is habitat heterogeneity the key? Trends in Ecology & Evolution 18:182–188.

Bird, A. F., P. De Ley, and J. Bird. 1993. Morphology, oviposition and embryogenesis in an Australian population of *Acrobeloides nanus*. Journal of Nematology 25:607–615.

Bjedov, I., O. Tenaillon, B. Gerard, V. Souza, E. Denamur, M. Radman, F. Taddei, and I. Matic. 2003. Stress-induced mutagenesis in bacteria. Science 300:1404–1409.

Björklund, M. 1996. The importance of evolutionary constraints in ecological time scales. Evolutionary Ecology 10:423–431.

Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. Journal of Evolutionary Biology 20:1–8.

Bonting, C. F. C., B. M. F. Willemsen, W. Akkermans-van Vliet, P. J. M. Bouvet, G. J. J. Kortstee, and A. J. B. Zehnder. 1992. Additional characteristics of the polyphosphate-accumulating *Aci-*

netobacter strain 210a and its identification as *Acinetobacter johnsonii*. FEMS Microbiology Letters 102:57–64.

Bryant, E. H., and L. M. Meffert. 1988. Effect of an experimental bottleneck on morphological integration in the housefly. Evolution 42:698–707.

Bull, J. J. 1987. Evolution of phenotypic variance. Evolution 41:303–315.

Bulmer, M. G. 1980. The mathematical theory of quantitative genetics. Clarendon, Oxford.

Cano, J. M., A. Laurila, J. Palo, and J. Merilä. 2004. Population differentiation in **G** matrix structure due to natural selection in *Rana temporaria*. Evolution 58:2013–2020.

Charmantier, A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proceedings of the Royal Society B: Biological Sciences 272:1415–1425.

Cheetham, A. H., J. B. C. Jackson, and L. A. C. Hayek. 1994. Quantitative genetics of Bryozoan phenotypic evolution. 2. Analysis of selection and random change in fossil species using reconstructed genetic parameters. Evolution 48:360–375.

Cheverud, J. M. 1996. Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins. Journal of Evolutionary Biology 9:5–42.

Cohan, F. M. 1984. Can uniform selection retard random genetic divergence between isolated conspecific populations? Evolution 38: 495–504.

De Goede, R. G. M., and T. Bongers. 1998. Nematode communities of northern temperate grassland ecosystems. Focus, Giessen.

Deng, H. W., and M. Lynch. 1996. Change of genetic architecture in response to sex. Genetics 143:203–212.

Doroszuk, A., M. W. Wojewodzic, and J. E. Kammenga. 2006. Rapid adaptive divergence of life-history traits in response to abiotic stress within a natural population of a parthenogenetic nematode. Proceedings of the Royal Society B: Biological Sciences 273:2611–2618.

Doroszuk, A., E. Te Brake, D. Crespo-Gonzalez, and J. E. Kammenga. 2007. Response of secondary production and its components to multiple stressors in nematode field populations. Journal of Applied Ecology 44:446–455.

Estes, S., and P. C. Phillips. 2006. Variation in pleiotropy and the mutational underpinnings of the **G**-matrix. Evolution 60:2655–2660.

Estes, S., B. C. Ajie, M. Lynch, and P. C. Phillips. 2005. Spontaneous mutational correlations for life-history, morphological and behavioral characters in *Caenorhabditis elegans*. Genetics 170:645–653.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Guntrip, J., R. M. Sibly, and G. J. Holloway. 1997. The effect of novel environment and sex on the additive genetic variation and covariation in and between emergence body weight and development period in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera, Bruchidae). Heredity 78:158–165.

Hine, E., and M. W. Blows. 2006. Determining the effective dimensionality of the genetic variance-covariance matrix. Genetics 173: 1135–1144.

Houle, D., J. Mezey, and P. Galpern. 2002. Interpretation of the results of common principal components analyses. Evolution 56:433–440.

Jones, A. G., S. J. Arnold, and R. Borger. 2003. Stability of the **G**-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. Evolution 57:1747–1760.

———. 2004. Evolution and stability of the **G**-matrix on a landscape with a moving optimum. Evolution 58:1639–1654.

Keightley, P. D., E. K. Davies, A. D. Peters, and R. G. Shaw. 2000. Properties of ethylmethane sulfonate-induced mutations affecting life-history traits in *Caenorhabditis elegans* and inferences about bivariate distributions of mutation effects. Genetics 156:143–154.

Kelly, J. K. 1999. Response to selection in partially self-fertilizing populations. II. Selection on multiple traits. Evolution 53:350–357.

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

Kirkpatrick, M., and K. Meyer. 2004. Direct estimation of genetic principal components: simplified analysis of complex phenotypes. Genetics 168:2295–2306.

Korthals, G. W., A. D. Alexiev, T. M. Lexmond, J. E. Kammenga, and A. M. T. Bongers. 1996. Long-term effects of copper and pH on the nematode community in an agroecosystem. Environmental Toxicology and Chemistry 15:979–985.

Kraft, P. G., R. S. Wilson, C. E. Franklin, and M. W. Blows. 2006. Substantial changes in the genetic basis of tadpole morphology of *Rana lessonae* in the presence of predators. Journal of Evolutionary Biology 19:1813–1818.

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

———. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203–215.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Laugsch, M., and E. Schierenberg. 2004. Differences in maternal supply and early development of closely related nematode species. International Journal of Developmental Biology 48:655–662.

Lynch, M., and H. W. Deng. 1994. Genetic slippage in response to sex. American Naturalist 144:242–261.

Lynch, M., and W. Gabriel. 1983. Phenotypic evolution and parthenogenesis. American Naturalist 122:745–764.

Lynch, M., and J. B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Marroig, G., and J. M. Cheverud. 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of New World monkeys. Evolution 55:2576–2600.

McGuigan, K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. Molecular Ecology 15:883–896.

Merilä, J., and B. C. Sheldon. 1999. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. Heredity 83:103–109.

Metzgar, D., and C. Wills. 2000. Evidence for the adaptive evolution of mutation rates. Cell 101:581–584.

Meyer, K., and M. Kirkpatrick. 2005. Restricted maximum likelihood estimation of genetic principal components and smoothed covariance matrices. Genetics Selection Evolution 37:1–30.

Mezey, J. G., and D. Houle. 2005. The dimensionality of genetic variation for wing shape in *Drosophila melanogaster*. Evolution 59: 1027–1038.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85–102 *in* J. N. Sasser and W. R. Jenkins, eds. Nematology. University of North Carolina Press, Chapel Hill.

Paulsen, S. M. 1996. Quantitative genetics of the wing color pattern in the buckeye butterfly (*Precis coenia* and *Precis evarete*): evidence against the constancy of **G**. Evolution 50:1585–1597.

Pfrender, M. E., and M. Lynch. 2000. Quantitative genetic variation in *Daphnia*: temporal changes in genetic architecture. Evolution 54:1502–1509.

Phillips, P. C., and S. J. Arnold. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. Evolution 53:1506–1515.

Phillips, P. C., and K. L. McGuigan. 2006. Evolution of genetic variance-covariance structure. Pages 310–325 *in* C. W. Fox and J. B. Wolf, eds. Evolutionary genetics, concepts and case studies. Oxford University Press, Oxford.

Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137–1145.

Pielou, E. C. 1984. Probing multivariate data with random skewers: a preliminary to direct gradient analysis. Oikos 42:161–165.

Pigliucci, M. 2006. Genetic variance-covariance matrices: a critique of the evolutionary quantitative genetics research program. Biology and Philosophy 21:1–23.

Reeve, J. P. 2000. Predicting long-term response to selection. Genetical Research 75:83–94.

Roff, D. A. 1996. The evolution of genetic correlations: an analysis of patterns. Evolution 50:1392–1403.

———. 2000. The evolution of the **G** matrix: selection or drift? Heredity 84:135–142.

———. 2002. Comparing **G** matrices: a MANOVA approach. Evolution 56:1286–1291.

Roff, D. A., and T. A. Mousseau. 1999. Does natural selection alter genetic architecture? an evaluation of quantitative genetic variation among populations of *Allonemobius socius* and *A. fasciatus*. Journal of Evolutionary Biology 12:361–369.

Roff, D. A., T. A. Mousseau, and D. J. Howard. 1999. Variation in genetic architecture of calling song among populations of *Allonemobius socius*, *A. fasciatus*, and a hybrid population: drift or selection? Evolution 53:216–224.

Schierenberg, E. 2001. Three sons of fortune: early embryogenesis, evolution and ecology of nematodes. Bioessays 23:841–847.

Sgrò, C. M., and A. A. Hoffmann. 1998. Effects of stress combinations on the expression of additive genetic variation for fecundity in *Drosophila melanogaster*. Genetical Research 72:13–18.

Shaw, F. H., R. G. Shaw, G. S. Wilkinson, and M. Turelli. 1995. Changes in genetic variances and covariances: **G** whiz! Evolution 49:1260–1267.

Slatkin, M., and R. Lande. 1976. Niche width in a fluctuating environment-density independent model. American Naturalist 110: 31–55.

Sohlenius, B., and S. Boström. 1999. Effects of global warming on nematode diversity in a Swedish tundra soil: a soil transplantation experiment. Nematology 1:695–709.

Spitze, K., J. Burnson, and M. Lynch. 1991. The covariance structure of life-history characters in *Daphnia pulex*. Evolution 45:1081–1090.

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

Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the **G** matrix. Trends in Ecology & Evolution 17:320–327.

Tibayrenc, M., F. Kjellberg, J. Arnaud, B. Oury, S. F. Breniere, M. L. Darde, and F. J. Ayala. 1991. Are eukaryotic microorganisms clonal or sexual? a population-genetics vantage. Proceedings of the National Academy of Sciences of the USA 88:5129–5133.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variance. Evolution 42:1342–1347.

Widen, B., S. Andersson, G. Y. Rao, and M. Widen. 2002. Population divergence of genetic (co)variance matrices in a subdivided plant species, *Brassica cretica*. Journal of Evolutionary Biology 15:961–970.

Wiegner, O., and E. Schierenberg. 1998. Specification of gut cell fate differs significantly between the nematodes *Acrobeloides nanus* and *Caenorhabditis elegans*. Developmental Biology 204:3–14.

Wilkinson, G. S., K. Fowler, and L. Partridge. 1990. Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster*. Evolution 44:1990–2003.

Associate Editor: Mark W. Blows
Editor: Michael C. Whitlock