Evol Ecol (2012) 26:863–878
DOI 10.1007/s10682-011-9542-2

ORIGINAL PAPER

Evolution of the G-matrix in life history traits in the common frog during a recent colonisation of an island system

Frank Johansson · Martin I. Lind · Pär K. Ingvarsson · Folmer Bokma

Received: 3 August 2011 / Accepted: 14 November 2011 / Published online: 27 November 2011

Abstract Studies of genetic correlations between traits that ostensibly channel the path of evolution away from the direction of natural selection require information on key aspects such as ancestral phenotypes, the duration of adaptive evolution, the direction of natural selection, and genetic covariances. In this study we provide such information in a frog population system. We studied adaptation in life history traits to pool drying in frog populations on islands of known age, which have been colonized from a mainland population. The island populations show strong local adaptation in development time and size. We found that the first eigenvector of the variance–covariance matrix ($\mathbf{g}_{max}$) had changed between ancestral mainland populations and newly established island populations. Interestingly, there was no divergence in $\mathbf{g}_{max}$ among island populations that differed in their local adaptation in development time and size. Thus, a major change in the genetic covariance of life-history traits occurred in the colonization of the island system, but subsequent local adaptation in development time took place despite the constraints imposed by the genetic covariance structure.

Keywords **G**-matrix · $\mathbf{g}_{max}$ · Pool drying · Genetic covariance · Life history evolution · *Rana temporaria*

F. Johansson (✉) · M. I. Lind · P. K. Ingvarsson · F. Bokma
Department of Ecology and Environmental Science, Umeå University, 901 87 Umeå, Sweden
e-mail: frank.johansson@ebc.uu.se

F. Johansson
Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, 75236 Uppsala, Sweden

M. I. Lind
Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

P. K. Ingvarsson
Umeå Plant Science Centre, Umeå University, 901 87 Umeå, Sweden

Introduction

An organism can be viewed as an integrated system of different traits, and many traits are part of co-adapted complexes (Pigliucci 2003). Therefore it will often be difficult to predict evolutionary changes in any one trait by studying only that trait, because the trajectory of evolution will be biased by the other traits involved in the co-adapted complex. In other words, the evolution of traits is constrained not only by the genetic variance in the trait itself, but also by the covariance with other traits. These variances and covariances are summarized in the genetic variance–covariance matrix **G** (Lande 1979), which should allow, at least in theory, prediction of the course of evolution using the multivariate breeder's equation (Lande and Arnold 1983).

Character evolution in natural populations has been suggested to be constrained by genetic correlation structure, and to follow the axis of maximum genetic variance, the so-called "genetic lines of least resistance" (Schluter 1996). If a fitness peak is stable and **G** is constant, trait values will evolve along the line of greatest genetic variance, that is $\mathbf{g}_{max}$, which essentially is the first principle component of the **G**-matrix. Interesting questions are how fast changes can occur in trait values constrained by genetic correlation and how quickly the genetic correlations themselves can change: i.e. the stability of the **G**-matrix. This is also highly important for the use of the **G**-matrix as a predictive tool: if the **G**-matrix is not stable; the direction of evolution cannot be predicted (Arnold et al. 2008; Eroukhmanoff 2009).

Several previous studies have attempted to study the evolution of the **G**-matrix at the species and population level (e.g. Arnold and Phillips 1999; Roff et al. 2004; Bégin and Roff 2003; Blows and Higgie 2003; Cano et al. 2004; Marroig and Cheverud 2005; McGuigan et al. 2005; Arnold et al. 2008; Eroukhmanoff and Svensson 2008, 2011; Berner et al. 2010), and much information is needed to make such inferences. For example, the genetic structure of traits (e.g. the **G**-matrix) has to be measured. While the **G**-matrix has been successfully estimated in organisms that are easily raised in the laboratory, such as *Drosophila* (Hansen and Houle 2008; Hine et al. 2009; Chenoweth et al. 2010), many field oriented evolutionary ecology studies have instead estimated the **P**-matrix (Berner et al. 2008, 2010; Eroukhmanoff et al. 2009). The latter studies use phenotypic measurements, on the assumption that phenotypic correlations properly reflect genotypic correlations, but this assumption is not always met (Roff 2002): therefore common garden studies are needed to decompose the phenotypic variance into a genetic and environmental part (Steppan et al. 2002). There are however elegant exceptions that have estimated the **G**-matrix in field-based systems (e.g. Cano et al. 2004; Eroukhmanoff and Svensson 2011).

The stability of the **G**-matrix over time is another important information needed to make inferences about the evolution of the **G**-matrix, and models predict that changes in the **G**-matrix over time can impacted by migration, selection and drift (Jones et al. 2003). While studies on natural populations have found the **G**-matrix to be stable over thousands of generations others have found it to vary on a time scale of only a few generations (Merilä and Björklund 1999; Cano et al. 2004; Doroszuk et al. 2008). Controlled laboratory studies certainly show that the **G**-matrix can change considerable over a few generations (e.g. Sgro and Blows 2004). However, the environmental conditions in the laboratory differ substantially from those in the wild which are much more variably with regard to selection pressures. In summary, more knowledge about how and whether the **G**-matrix can change in wild population over an ecological time scale is needed, and especially for population experiencing divergent natural selection, because in such populations we know that natural selection has and is working on trait divergence (McGuigan 2006).

Few studies have been done on systems where both the ancestral phenotype and the direction of natural selection is known using a **G**-matrix approach, but see e.g. Chenoweth et al. (2010). Here we investigate a system where we know the ancestral phenotype and the direction of natural selection: we study populations of the common frog *Rana temporaria* adapting to pool drying regimes on a time series of islands, the oldest being about 800 years and the youngest only a couple of 100 years old (Lind 2009). These populations all derive from mainland populations, which very likely represent the ancestral phenotype (Lind et al. 2011). Previous studies have shown that natural selection has acted on developmental time and size at metamorphosis in these populations as Q_{st} for the life history traits is larger than F_{st} (i.e. divergent selection is present): populations have adapted to the local pool drying conditions (Lind et al. 2011). Populations in time-constrained island pools are selected for fast development, since frogs need to complete their development before the end of the season or before the pool dries out (Rowe and Ludwig 1991). Selection also acts on size at maturity, since large size at metamorphosis has positive impact on fitness in the adult stage (Berven 1990; Altwegg and Reyer 2003). However, age and size at maturation show a negative trade-off: populations from temporary pools have a shorter developmental time and a lower mass at metamorphosis than frogs from permanent pools, not only in the wild but also in common garden experiments (Lind et al. 2008), which suggests conflict between the directions of natural selection and the direction of genetic covariance when adapting to time-stressed environments. Here we study **G**-matrices of the island and mainland populations to test for such conflict. We investigate whether $\mathbf{g}_{max}$ change in orientation when comparing ancestral and derived populations, and whether island populations which differ genetically in life history characters differ in **G**-matrices.

Materials and methods

Study system

As a study system, we used 14 populations of the common frog, *R. temporaria,* from islands in the Gulf of Bothnia (Fig. 1). These island populations are genetically differentiated since they have limited gene flow and Q_{st} values for life history traits are larger than F_{st} values (Lind et al. 2011). These islands were formed by isostatic rebound (post-glacial land rise): as the land continued to rise, ever more islands emerged, and became subsequently colonized. Thus, just like the islands on which they live, the frog populations provide a time gradient, ranging in age from 70 to 800 years (Johansson et al. 2005). On the mainland the common frog commonly breeds in temporary and permanent waters (Gasc et al. 1997). We also included frogs from 4 locations on the mainland in this study, and we consider these present-day mainland frogs to represent the ancestral population from which the island populations descended. When the glacial ice withdrew 7,000 years ago, the land area of these mainland populations became available for colonisation and hence these populations are probably approximately 7,000 years old.

Some of the island populations inhabit temporary pools, which dry out every summer. This imposes a time constraint on the frogs: to survive, they must reach a critical developmental stage and size before the pool dries. Some pools dry faster than others, and the time constraint is relaxed the longer it takes before a pool is dry. Following Lind and Johansson (2007), we distinguish three categories of island populations based on drying time of the pools in which they live. In order of increasing time to drying we have

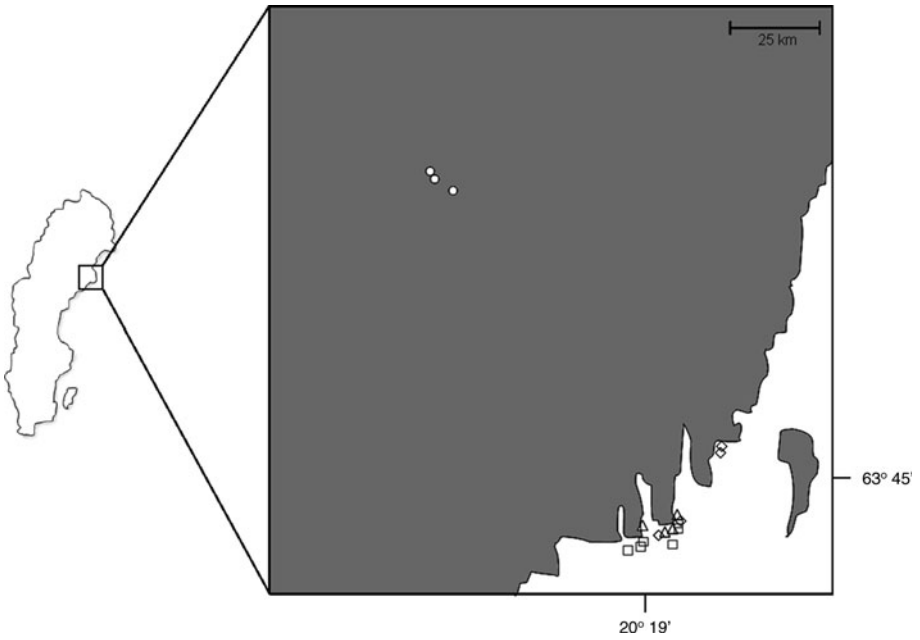

Fig. 1 Map showing the location of the populations studied. Four different kind of population with regard to pool permanence and geographic position were used, and they are indicated with different symbols on the map. Inland populations with unknown drying regime are denoted with *circles*, *diamonds* are island population with temporary pools, *triangles* island populations with permanent pools and *squares* are island populations with intermediate drying regimes. The map to the *left* shows Sweden. A detailed map of the island system is available in Lind et al. (2011)

temporary pools (4 populations), intermediate pools (6 populations), and permanent pools (4 populations) that never dry completely. The 4 mainland pools are permanent, since they are deeper than the permanent pools on the islands (unpubl data), and form a separate, fourth category. The intermediate populations were included only in comparisons between mainland versus all island populations.

To meet the time constraints imposed by pool drying, frogs could speed up development and start metamorphosis at a smaller size, but size at metamorphosis is an important life-history trait (Roff 1992) with small size at metamorphosis negatively affecting fitness in the adult stage (Berven 1990; Altwegg and Reyer 2003). The alternative strategy, to speed up development so as to reach the same size in a shorter period of time, does not seem to exist (perhaps for obvious reasons): fast development usually comes at a cost of smaller size (Ball and Baker 1996; Laurila and Kujasalo 1999). In other words, there is a trade-off between size and development time. This trade-off is present in all organisms, but more accentuated (and therefore easier to study) in organisms developing under time constraints. Indeed, the frogs studied here are locally adapted to the drying regime of their pools (Lind and Johansson 2007). In common garden studies, developmental time of tadpoles correlates with the drying rate of their pool. Hence, tadpoles from the study populations differ genetically with respect to both developmental time and size at maturation, and these traits are genetically correlated (Lind and Johansson 2007).

Genetic covariances between size and time at metamorphosis (**G**-matrices) were for every population calculated from full sib families: 2 sibs per family and 10 families per

population. Using full-sibs is less powerful than using a formal breeding design, since maternal effects cannot be controlled for. However, when using a North-Carolina II half-sib breeding design in one of the island populations, Lind and Johansson (2007) found that maternal effects only account for 5% of the phenotypic variation in development time and metamorphic weight.

Hence, we assume that the maternal effects, which we cannot consider here due to our breeding design, are of little importance. We also acknowledge that dominance effects are not taken into account in our breeding design. (This and other limitations of our breeding design are considered in the discussion.) Individuals were genotyped at 5 microsatellite loci (data from Lind et al. 2011) in order to estimate population divergence. We defined time at maturation as the time needed to reach Gosner stage 42 (front legs visible). Size at maturation was measured as wet weight when stage 42 was reached.

Data collection

Eggs were collected during the first 2 weeks of May 2005, from up to 10 clutches per population. One clutch represents offspring from one female, and because females of *R. temporaria* in northern Sweden only lay one egg clump (Elmberg 1991a, b) and multiple paternities within egg clutches is rare (Laurila and Seppä 1998), we treat the sampled offspring as full sibs (Lind 2009). This is conservative with respect to estimating genetic variances and covariances, even if a few offspring pairs would turn out to be half-sibs (Lynch and Walsh 1998). In the laboratory, eggs were kept in a walk-in thermo constant room at 4°C, until all eggs had been collected. At this temperature no development occurs. Then we raised the temperature to 22°C with a light: dark cycle of 16 h:6 h, which corresponds to natural light regime at the latitude of origin of the populations. When the larvae reached Gosner stage 23 (active swimming) two siblings from each clutch were introduced individually into small plastic containers (9.5 × 9.5 cm, height 10 cm), filled with 750 ml aerated and non-chlorinated tap water, which was replaced every fourth day before feeding. Tadpoles were fed a mixture (1:2) of finely ground fish flakes and rabbit show, and the food levels were increased according to the following schedule: 15 mg from Day 1–8, 30 mg from Day 9–12; 45 mg from Day 13–16; 60 mg from Day 17–20; and 75 mg from Day 21 until metamorphosis. At Gosner stage 42 the experiment was terminated. Differentiation among populations and population categories (temporary, permanent etc.) in the two life history traits were tested with ANOVAs and nested ANOVAs.

Matrix computation and matrix comparisons

We analyzed the two traits, size at maturation and developmental time, using an animal model:

$$z_{ij} = u + a_i + e_{ij} \quad (1)$$

where z_{ij} is the phenotype of the jth individual from the ith maternal family, u denotes the grand mean, a_i the maternal breeding value of the i-th maternal family, and e_{ij} is the residual, or error term. We used inverse Wishart priors for the variances and covariances, specified to be non-informative as outlined in Hadfield (2010). The fixed effects all used diffuse normal priors centred around zero and with very large variance (10^8). All MCMC analyses were run for 110,000 steps, with the first 10,000 discarded as burn-in, and chains were thinned by selecting every 100 steps, yielding a total of 1,000 data points for each

analysis. The convergence of chains was checked and visualized using the coda package in R (Plummer et al. 2006).

We first tested for genetic differentiation among populations by fitting a univariate model that allows for separate genetic variances in each population. This model was contrasted with a model that constrains the genetic variation to be equal in all populations. Model comparisons were made based on the Deviance Information Criteria (DIC), (Hadfield 2010).

To estimate genetic variance and covariances between traits we implemented a bivariate version of the animal model (Wilson et al. 2009) described by Eq. 1 in *MCMCglmm* (Hadfield 2010). In this model, the phenotypic matrix **P** contains the phenotypic variances and co-variances between traits. This matrix can be decomposed into an additive genetic matrix (**G**) and a residual (or environmental) matrix (**R**), so that $\mathbf{P} = \mathbf{G} + \mathbf{R}$.

$$\begin{bmatrix} V_{P(size)} & COV_{P(size)P(age)} \\ COV_{P(size)P(age)} & V_{P(age)} \end{bmatrix} = \begin{bmatrix} V_{G(size)} & COV_{G(size)G(age)} \\ COV_{G(size)G(age)} & V_{G(age)} \end{bmatrix} + \begin{bmatrix} V_{R(size)} & COV_{R(size)R(age)} \\ COV_{R(size)R(age)} & V_{R(age)} \end{bmatrix} \quad (2)$$

Model (2) was fit to the data from each population separately. We used posterior modes of parameters as our point estimates of genetic variances and covariances and these were then used to construct a **G**-matrix for each population. We also estimated covariance and their uncertainty.

While early studies of **G**-matrix divergence used the first principal component ($\mathbf{g}_{max}$) of the **G**-matrix (Schluter 1996), later developments in matrix comparisons enabled the comparisons of all dimensions of the **G**-matrix (Phillips and Arnold 1999; Roff 2002). Therefore, many recent studies have extended the analyses to compare more aspects of the **G**-matrix (e.g. Blows and Higgie 2003; Cano et al. 2004). However, if $\mathbf{g}_{max}$ explains most of the variation in the **G**-matrix, there is little reason to use matrix comparison methods. In addition, if only two characters are used, as in our case, interpretation is relatively simple (Hansen and Houle 2008). We found that the first principal component ($\mathbf{g}_{max}$) explained almost all variation in the **G**-matrix (>90%), and therefore we analysed population divergence in $\mathbf{g}_{max}$, following Schluter (1996).

To asses **G**-matrix divergence between populations we calculated the angle between $\mathbf{g}_{max}$ for matrices from different populations, where $\mathbf{g}_{max}$ is the direction of maximum genetic variance, that is, the first principal component of the **G**-matrix, also known as the "genetic line of least resistance" (Schluter 1996). The angle between two populations, θ, was calculated as:

$$\theta = \arccos(\mathbf{e}_1\mathbf{e}_2)/(||\mathbf{e}_1||||\mathbf{e}_2||) \quad (3)$$

where $\mathbf{e}_1$ and $\mathbf{e}_2$ are the first eigenvectors of the two **G**-matrices, and where $\|\mathbf{e}_i\|$ is the norm of the eigenvectors $\mathbf{e}_i$. To compare angles among population categories we calculated the mean and the 95% confidence intervals of θ and compared θ pairwise among populations. Because the same populations are involved in multiple comparisons, the values used for calculating the average angle between populations are not strictly independent. To alleviate this problem we estimated the average angle among populations and the standard errors using a permutation-based re-sampling procedure with 1,000 replications for each comparison. We also estimated two other parameters of the **G**-matrix. Eccentricity, which is the ratio between the first and the second eigenvector and size, which is the sum of the eigenvectors. These are given to facilitate comparisons with other studies and are not

discussed in detail because our purpose is to compare divergence in $\mathbf{g}_{max}$ among populations.

Results

Population differentiation

First, to make sure the sampling locations can indeed be treated as separate populations, we used the microsatellite genotypes to calculate global F_{st} for the each category of populations, using FSTAT 2.9.3.2 (Goudet 1995). According to expectation, population differentiation was significant in all groups except the mainland populations (Fig. 2). The highest F_{st} values were found between mainland and island populations, which is expected because they are situated far away from each other.

We observed significant differences among populations in mean developmental time ($F_{17,246} = 3.27$, $P < 0.001$) and mean size at metamorphosis ($F_{17,246} = 9.90$, $P < 0.001$)

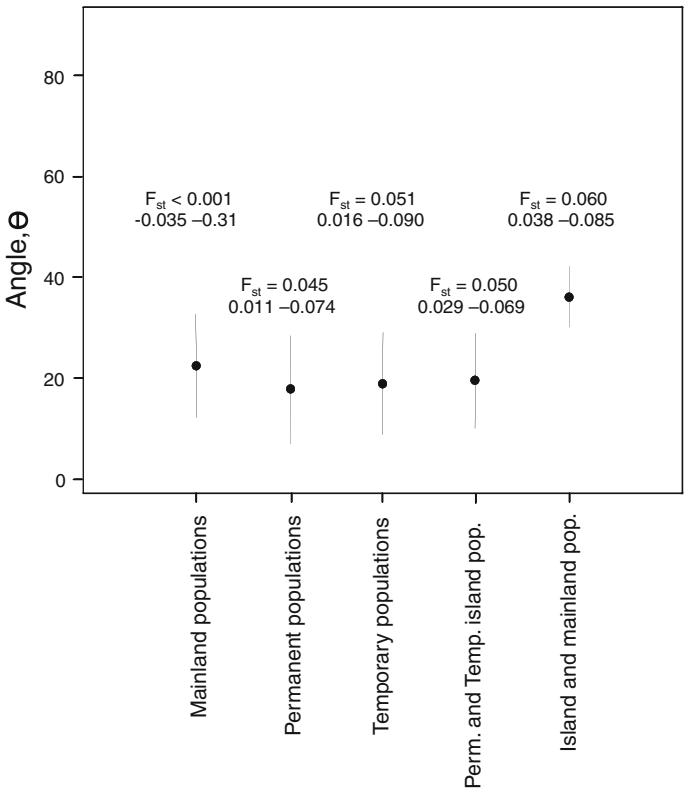

Fig. 2 Average angle between the direction of maximum genetic variance (θ) among populations for five different categories of populations. *Error bars* denote 95% confidence intervals for θ obtained using a resampling procedure. Values above the bars denote global F_{st} values for the populations compared, with 95% confidence intervals in *parentheses*

(Fig. 3). Island populations from permanent pools had a faster development than those from temporary pools ($F_{1,6} = 2.84$, $P = 0.047$), but they did not differ in size ($F_{1,6} = 0.04$, $P = 0.85$) (Fig. 3). Temporary island pool populations also had a significantly faster development time and smaller size than mainland populations ($F_{1,6} = 4.59$, $P = 0.03$ and $F_{1,6} = 73.22$, $P < 0.001$ for developmental time and size at metamorphosis, respectively) (Fig. 3). Finally, island populations had faster development time and a smaller size at metamorphosis compared to mainland populations (0.57 g (±0.01 SD) versus 0.48 g (±0.03 SD)), respectively; $F_{1,15} = 28.02$; $P = 0.0009$ (Fig. 3).

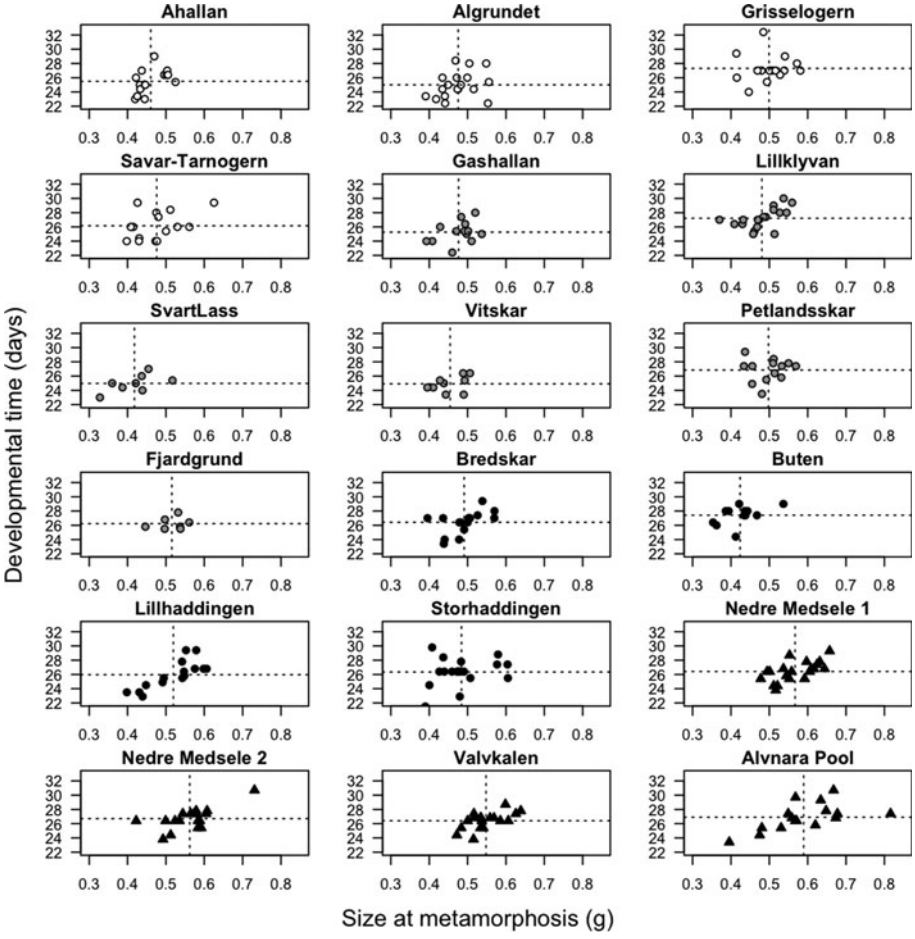

Fig. 3 The relationship between size at metamorphosis and development time to metamorphosis in the 18 frog populations studied. *Dots* represent island population and triangles mainland populations. *Unfilled*, *shaded* and *black dots*, represents temporary, intermediate and permanent water regime populations respectively. Mean developmental time and size at metamorphosis at each island are shown as *horizontal* and *vertical dashed lines*

$\mathbf{g}_{max}$ and θ difference among populations

We observed significant differences in genetic variance (V_G) among populations, as indicated by a better fit of a model allowing for separate estimates of V_G in each population compared to a model constraining V_G to be equal across populations (single V_G: DIC = 698.2, separate V_G: DIC = 667.9 for developmental time and single V_G: DIC = 702.0, separate V_G: DIC = 657.4 for size at metamorphosis).

The first eigenvector accounted for the majority (range 91–94%) of the total variance in all populations, indicating strong genetic correlation between developmental time and size at maturation in all populations. Overall, development time and size at metamorphosis showed a genetic correlation of $r_g = 0.42$. In other words, **G** is represented by an ellipsoid rather than a circle (Schluter 1996), which is an important prerequisite when testing whether the course of evolution has been affected by the genetic covariance structure of traits. **G**-matrices for individual populations are given in Table 1, and show high 95% confidence intervals for V_G, rG (COV_G), and h^2, probably a result of few replicates and some large point estimates within each population. Since our main interest was comparisons among population categories, we provide similar information for these comparisons as well ("Appendix").

There was a significant divergence in covariance structure between populations since confidence intervals of differences between the directions of maximum genetic variance $\mathbf{g}_{max}$ of populations do not overlap zero (Fig. 2). The greatest angle θ of about 44 degrees we found between the directions of maximum genetic variance $\mathbf{g}_{max}$ of the mainland and the combined island populations. This difference was substantially greater than in any of the other comparisons (Fig. 2), suggesting that populations have diverged in **G**. However, comparisons in all population categories showed angles that were, although smaller, significantly larger than zero, indicating that $\mathbf{g}_{max}$ have changed direction. The average θ among the three categories of island populations and the mainland population was about 23° and there was no significant difference in the angle among these four population comparisons, since their confidence intervals overlapped substantially. Eccentricity was somewhat higher in island populations (Table 2), but an F test showed no significant difference between mainland and island populations ($F_{1,16} = 2.31$, $P = 0.15$). The size of **G** was greater in the island populations (Table 2), and this difference was significant ($F_{1,16} = 7.01$, $P = 0.018$).

Discussion

We found divergence in the structure of the **G**-matrix in natural populations of the common frog, since the major axis of the genetic variance- covariance matrix ($\mathbf{g}_{max}$) has changed orientation between ancestral and derived populations of the common frog (*R. temporaria*). Together with a study on the colonisation of a novel environment by aquatic isopods (Eroukhmanoff and Svensson 2011), this is one of the few systems where natural populations with known optimal life history traits have been studied with regard to changes in the **G**-matrix. These changes in the **G**-matrix occurred within 7,000 years, and assuming a generation time of 3 years: within less than 2,000 generations. However, this assumes a direct dispersal from our mainland populations to the island which probably did not occur. If we assume that mainland populations have been connected by gene flow since the colonisation of the mainland after the last glaciation, the evolution of the **G**-matrix

Table 1 Genetic variance (V_G), covariance (rG [COV_G]) and heritability (h^2) for life history traits in each population

Population	V_G (size)	95% CI		V_G (age)	95% CI		rG (size, age)	95% CI		h^2(size)	95% CI		h^2(age)	95% CI	
Ahallan.IT	0.65	0.20	2.32	0.94	0.33	3.09	0.50	−0.37	0.87	0.41	0.14	0.73	0.69	0.31	0.90
Algrund.IT	0.64	0.22	2.05	0.75	0.26	2.11	0.52	−0.25	0.85	0.47	0.19	0.75	0.58	0.24	0.82
Grissl.IT	0.69	0.21	2.65	0.64	0.20	2.10	0.02	−0.70	0.70	0.46	0.15	0.83	0.45	0.15	0.75
Savar-T.IT	0.54	0.17	1.79	0.72	0.25	2.41	0.47	−0.37	0.85	0.34	0.11	0.66	0.56	0.21	0.84
Fjardg.II	1.21	0.26	9.65	1.08	0.25	10.11	0.19	−0.82	0.92	0.54	0.13	0.92	0.44	0.11	0.89
Gashall.II	0.81	0.25	3.37	0.82	0.26	3.37	0.26	−0.58	0.82	0.55	0.19	0.86	0.58	0.21	0.86
Lillkly.II	0.62	0.23	1.86	0.70	0.26	2.05	0.39	−0.36	0.81	0.47	0.17	0.78	0.57	0.24	0.83
Petlan.II	0.63	0.17	2.39	0.78	0.28	2.84	0.08	−0.69	0.76	0.39	0.11	0.75	0.57	0.21	0.86
Svart.L.II	0.90	0.22	4.24	1.06	0.27	6.38	0.34	−0.69	0.91	0.47	0.12	0.85	0.53	0.14	0.89
Vitskar.II	0.91	0.21	5.55	0.96	0.23	5.31	0.35	−0.64	0.89	0.45	0.12	0.86	0.48	0.13	0.87
Bredska.IP	0.58	0.19	1.86	0.96	0.37	2.58	0.54	−0.24	0.87	0.39	0.13	0.71	0.75	0.40	0.92
Buten.IP	0.62	0.19	2.62	0.61	0.20	2.39	0.39	−0.53	0.85	0.38	0.11	0.74	0.39	0.13	0.73
Lillha.IP	0.44	0.15	1.85	0.50	0.17	1.93	0.33	−0.48	0.83	0.28	0.09	0.64	0.33	0.11	0.69
Storha.IP	0.47	0.17	1.56	0.57	0.18	1.88	0.06	−0.58	0.70	0.33	0.12	0.63	0.41	0.14	0.75
Alvnarp.MP	0.56	0.18	1.88	0.50	0.16	1.60	0.26	−0.53	0.80	0.38	0.13	0.74	0.32	0.11	0.66
NedreM2.MP	0.53	0.18	1.61	0.46	0.16	1.69	0.45	−0.27	0.85	0.37	0.13	0.70	0.32	0.11	0.65
NedreM3.MP	0.44	0.16	1.44	0.51	0.19	1.76	0.30	−0.49	0.80	0.30	0.11	0.62	0.40	0.15	0.71
Valvkal.MP	0.54	0.18	1.70	0.43	0.15	1.58	0.09	−0.63	0.72	0.36	0.14	0.69	0.27	0.10	0.60

Abbreviations after population name are as follows: IT, II and IP are temporary, intermediate and permanent water regime island populations respectively, and MP is permanent water mainland populations

Table 2 Comparison of eigenvalues (λ), eccentricity and size of **G** in the frog populations

Population	$\lambda 1$	$\lambda 2$	Eccentricity ($\lambda 1/\lambda 2$)	Size ($\lambda 1 + \lambda 2$)
Ahallan.IT	1.32	0.27	4.80	1.60
Algrund.IT	1.22	0.17	6.89	1.40
Grissl.IT	0.70	0.62	1.12	1.33
Savar-T.IT	1.11	0.15	7.16	1.27
Fjardg.II	1.34	0.94	1.42	2.29
Gashall.II	1.08	0.55	1.97	1.63
Lillkly.II	1.06	0.26	3.99	1.33
Petlan.II	0.82	0.59	1.38	1.42
Svart.L.II	1.33	0.63	2.11	1.96
Vitskar.II	1.29	0.58	2.21	1.87
Bredska.IP	1.34	0.20	6.69	1.54
Buten.IP	1.01	0.22	4.60	1.23
Lillha.IP	0.80	0.14	5.67	0.94
Storha.IP	0.61	0.44	1.39	1.05
Alvnarp.MP	0.79	0.27	2.92	1.06
NedreM2.MP	0.95	0.04	21.43	1.00
NedreM3.MP	0.78	0.18	4.36	0.96
Valvkal.MP	0.59	0.37	1.59	0.97

Abbreviations after population name are as follows: IT, II and IP are temporary, intermediate and permanent water regime island populations respectively, and MP is permanent water mainland populations

associated with the more recent colonisation of the island system has probably taken place during the last 800 years (270 generations), when the oldest island emerged by isostatic uplift.

Our results are in agreement with previous studies, where $\mathbf{g}_{max}$ or the **G**-matrix has changed orientation (e.g. Bégin and Roff 2003; Blows and Higgie 2003; Cano et al. 2004; Roff et al. 2004; Blows and Hoffmann 2005; Doroszuk et al. 2008; Berner et al. 2010; Chenoweth et al. 2010). The comparison of covariance matrices between the mainland populations and the Baltic Sea island populations showed the largest angle of divergence in $\mathbf{g}_{max}$. This difference was significantly larger than in the other four categories of population comparisons, suggesting a strong divergence between the former population categories following the invasion of the island system. In contrast, subsequent local adaptation in the island system does not seem to have caused substantial divergence in $\mathbf{g}_{max}$ since island category comparisons have broadly similar angle (θ) (Fig. 2). The absence of a divergence among island populations might be surprising, as we previously have found strong divergent selection in development time to match the local pool-drying conditions present on the islands (Lind and Johansson 2007; Lind et al. 2011). However, the island populations are young (23–267 generations, Johansson et al. 2005) and given enough time a divergence in the **G**-matrix might occur. Our study is in line with the results of Eroukhmanoff and Svensson (2008), and Eroukhmanoff et al. (2009) who found modest divergence in the **P**-matrix among populations of the same species but more substantial divergence between species.

Despite the marked differences in development time among the island populations resulting from divergent selection (Lind et al. 2011), this local adaptation to the different island habitats seem to have proceeded with minor changes of the genetic covariance

structure. This interpretation is also supported by the finding that local adaptation among the island populations seems to be caused by selection on standing genetic variation on development and growth rate and not by novel strategies (Lind and Johansson 2011). Hence it seems as if time is an important factor for divergence in $\mathbf{g}_{max}$. However, we did find that $\mathbf{g}_{max}$ have changed in orientation also in the island system, since θ differed from zero in the island population categories. One reason for this could be genetic drift caused by the small effective population sizes on the islands. Numbers of individuals per island population may be about 100, and sometimes only around 20. A larger effective population size will make genetic drift a less important factor and will usually lead to a more stable **G**-matrix (Jones et al. 2003; Arnold et al. 2008). We do not know the optimal life history traits for the mainland populations, but it is likely that the frogs breeding in these pools do not experience pool drying in the same degree as the island populations that breed in pools in rocky depressions, because the mainland pools are considerably deeper than pools on the islands. We therefore expect mainland frogs to be less time constrained and to be selected for a longer developmental time which allows a larger size at maturity. Indeed, the common garden experiments showed that at metamorphosis, mainland frogs are significantly larger than island frogs and development time is longer than in temporary island populations (Fig. 3).

Past studies have found conflicting evidence whether the **G**-matrix constrain responses to natural selection or not. For example, Eroukhmanoff and Svensson (2008) showed that population of *Calopteryx* damselflies showed little evidence of divergence in covariance structure among populations (using a **P**-matrix approach) and Chenoweth et al. (2010) found that evolution was in the direction of major axes of genetic variance in characters among populations of *Drosophila* (using a **G**-matrix approach). In contrast, Berner et al. (2008) and Berner et al. (2010) (using a **P**-matrix approach) found that trait divergence among population of sticklebacks was unrelated to the (co)variance structure. Perhaps such differences between studies are due to time since population have diverged, because the **G**-matrix is most likely to constrain evolution during the early phase of divergence (Schluter 1996; Arnold et al. 2008). Hence an absence in of a divergence in the **G**-matrix among our island populations is not surprising. But, the finding that substantial local adaptation in development time has taken place despite constrains imposed by the genetic covariance structure is in itself quite remarkable.

We studied only two traits, and only two offspring per family, rendering a low power to detect statistical differences. Despite this we found a significant divergence in the **G**-matrix. The use of full-sibs means that the genetic variances we estimated may be inflated to an unknown degree by dominance and maternal effects, with the former potentially overestimating additive genetic variance (Merilä and Sheldon 1999). Despite these limitations our study is one of the few where the **G**-matrix rather than the **P**-matrix has been used to study the (co)variance structure and selection on it in recently diverged population with a known optimum. Hence although our study has limitations, the use of a **G**-matrix approach in natural populations with known origin and selection pressures gives interesting insights in the role of genetic correlations during recent population divergence. Clearly, as we obtained significant results, statistical power was sufficient despite the small number of families. We note that eccentricity did not differ between mainland and island populations but that the size of the **G**-matrix was larger in island populations compared to mainland populations. The larger size suggests that the total amount of genetic variation and covariation has increased as the islands were colonized. We also note that strong divergence in the **G**-matrix of life history traits have also been found between locally adapted populations of *R. temporaria* from the time-stressed, alpine northern Scandinavia and the

less time-constrained southern Scandinavia (Cano et al. 2004), but these populations were situated much further apart, with longer time available for divergence.

Perhaps the most intuitive evidence that the course of evolution has been affected by genetic structure is the observation that decreased developmental time as a response to adaptation to time stressed habitats was accompanied by a decrease in size at metamorphosis, even though size at maturity is positively related with fitness in this species. This is the typical sign of a trade-off and offers evidence that genetic structure channels the course of evolution, although it is not certain that age at maturity is the only factor involved. It is most likely, however, that a large number of factors prevent accelerated maturation at constant size at maturation, as life history traits are believed to be influenced by many loci, many of which show antagonistic pleiotropy (Stearns 1992). Nevertheless, our study highlights the need for case studies where the genetic covariance matrix is estimated in multiple populations that differ in their adaptive optimum. Such studies have appeared recently but are limited to the **P**-matrix (Eroukhmanoff and Svensson 2008; Berner et al. 2008, 2010), but see Chenoweth et al. (2010) and Eroukhmanoff and Svensson (2011).

Acknowledgments We thank J. Almfelt for help with egg collection and laboratory experiments, and two anonymous reviewers for very constructive comments that improved this article. The Swedish Research Council and the Swedish Research Council FORMAS funded the research.

Appendix

See Table 3.

Table 3 Genetic variance (V_G), covariance (rG [COV_G]) and heritability (h^2) for life history traits in each population category

Population category	V_G (size)	95% CI		V_G (age)	95% CI		rG (size, age)	95% CI		h^2 (size)	95% CI		h^2 (age)	95% CI	
Mainland	0.23	0.11	0.53	0.27	0.11	0.65	0.38	−0.68	0.89	0.20	0.10	0.36	0.24	0.11	0.44
Temporary	0.39	0.18	0.75	0.29	0.14	0.71	0.65	0.28	0.83	0.36	0.16	0.55	0.26	0.12	0.56
Permanent	0.63	0.34	1.21	0.39	0.18	0.84	0.65	0.24	0.83	0.59	0.24	0.75	0.34	0.17	0.52
Intermed	0.68	0.39	1.31	0.53	0.27	1.05	0.57	0.20	0.78	0.64	0.43	0.78	0.49	0.27	0.63

References

Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882

Arnold SJ, Phillips PC (1999) Hierarchical comparison of genetic variance-covariance matrices. II. Coastal-inland divergence in the garter snake, Thamnophis elegans. Evolution 53:1516–1527

Arnold SJ, Burger R, Holenhole PA, Beverley CA, Jones AG (2008) Understanding the evolution and stability of the G-matrix. Evolution 62:2451–2461

Ball SL, Baker RL (1996) Predator-induced life history changes: antipredatore behavior costs or facultative life history shifts? Ecology 77:1116–1124

Bégin M, Roff DA (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

Berner D, Adams DC, Grandchamp A-C, Hendry AP (2008) Natural selection drives patterns of lake – stream divergence in stickleback foraging morphology. J Evol Biol 21:1653–1665

Berner D, Stutz WE, Bolnick DI (2010) Foarging trait (co)variances in stickleback evolve deterministically and do not predict trajectories of adaptive diversification. Evolution 64:2265–2277

Berven KA (1990) Factors affecting population fluctuations in larval and adult stages of the wood frog (*R. sylvatica*). Ecology 71:1599–1608

Blows MW, Higgie M (2003) Genetic constraints on the evolution of mate recognition under Natural Selection. Am Nat 161:240–253

Blows MW, Hoffmann AA (2005) A reassessment of genetic limits to evolutionary change. Ecology 86:1371–1384

Cano JM, Laurila A, Palo J, Merilä J (2004) Population differentiation in **G** matrix structure due to natural selection in *R. temporaria*. Evolution 58:2013–2020

Chenoweth SF, Rundle HD, Blows MW (2010) The contribution of selection and genetic constraints to phenotypic divergence. Am Nat 175:186–196

Doroszuk A, Wojewodzic MW, Gort G, Kammenga JE (2008) Rapid divergence of genetic variance-covariance matrix within a natural population. Am Nat 171:291–304

Elmberg J (1991a) Factors affecting male yearly mating success in the common frog, *R. temporaria*. Beh Ecol Sociobiol 28:125–131

Elmberg J (1991b) Ovarian cyclicity and fecundity in boreal common frogs *R. temporaria* L. along a climatic gradient. Funct Ecol 5:340–350

Eroukhmanoff F (2009) Just how much is the G-matrix actually constraining adaptation? Evol Biol 36:323–326

Eroukhmanoff F, Svensson EI (2008) Phenotypic integration and conserved covariance structure in calopterygid damselflies. J Evol Biol 21:514–526

Eroukhmanoff F, Svensson EI (2011) Evolution and stability of the G-matrix during colonization of a novel envirnonment. J Evol Biol 24:1363–1373

Eroukhmanoff F, Outomuro D, Ochoran FJ, Svensson EI (2009) Patterns of phenotypic divergence in wing covariance structure of calopterygid damselflies. Evol Biol 36:214–224

Gasc J et al (1997) Atlas of amphibians and reptiles in Europe, Societas Europea Herpetologica & Museum National d'Histoire Naturelle, Paris

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Heredity 86:485–486

Hadfield JD (2010) MCMC Methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw 33:1–22

Hansen TF, Houle D (2008) Measuring and comparing evolvability and constraints in multivariate characters. J Evol Biol 21:1201–1219

Hine E, Chenoweth S, Rundle HD, Blows MW (2009) Characterizing the evolution of genetic covariance tensors. Phil Trans R Soc B 364:1567–1578

Johansson F, Hjelm J, Giles BE (2005) Life history and morphology of *R. temporaria* in response to pool permanence. Evol Ecol Res 7:1025–1038

Jones AG, Arnold SJ, Burger R (2003) Stability of the G-matrix in a population experiencing pleitropic mutation, stabilizing selection and genetic drift. Evolution 57:1747–1760

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

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Laurila A, Kujasalo J (1999) Habitat duration, predation risk and phenotypic plasticity in common frog (*R. temporaria*) tadpoles. J Anim Ecol 68:1123–1132

Laurila A, Seppä P (1998) Multiple paternity in the common frog (*R. temporaria*): genetic evidence from tadpole kin groups. Biol J Linn Soc 63:221–232
Lind MI (2009) Phenotypic plasticity and local adaptation in island populations of *R. temporaria*. PhD Thesis, Umeå University
Lind MI, Johansson F (2007) The degree of adaptive phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *R. temporaria*. J Evol Biol 20:1288–1297
Lind MI, Johansson F (2011) Testing the role of phenotypic plasticity for local adaptation: growth and development in time-constrained *R. temporaria* populations. J Evol Biol Online Early. doi: 10.1111/j.1420-9101.2011.02393.x
Lind MI, Persbo F, Johansson F (2008) Pool desiccation and developmental thresholds in the common frog, *R. temporaria*. Proc R Soc B 275:1071–1080
Lind MI, Ingvarsson PK, Johansson H, Hall D, Johansson F (2011) Gene flow and selection on phenotypic plasticity in an island system. Evolution 65:684–697
Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates Inc, Sunderland
Marroig G, Cheverud JM (2005) Size as a line of least evolutionary resistance: diet and adaptive morphological radiation in new world monkeys. Evolution 59:1128–1142
McGuigan K (2006) Studying evolution using multivariate quantitative genetics. Mol Ecol 15:883–896
McGuigan K, Chenoweth SF, Blows MW (2005) Phenotypic divergence along lines of genetic variance. Am Nat 165:32–43
Merilä J, Björklund M (1999) Population divergence and morphometric integration in the greenfinch (*Carduelis chloris*)—evolution against the lines of least resistance. J Evol Biol 12:103–112
Merilä J, Sheldon BC (1999) Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. Heredity 83:103–109
Phillips PC, Arnold SJ (1999) Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. Evolution 53:1506–1515
Pigliucci M (2003) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Ecol Lett 6:265–272
Plummer M, Best N, Cowles K, Vines K (2006) CODA: convergence diagnosis and output analysis for MCMC. R News 6:7–11
Roff DA (1992) The evolution of life histories: theory and analysis. Chapman and Hall, New York
Roff D (2002) Comparing G matrices: a MANOVA approach. Evolution 56:1286–1291
Roff DA, Mousseau T, Møller AP, deLope F, Saino N (2004) Geographic variation in the G matrices of wild populations of the barn swallow. Heredity 93:8–14
Rowe L, Ludwig D (1991) Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology 72:413–427
Schluter D (1996) Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774
Sgro CM, Blows MW (2004) The genetic covariance among clinal environments after adaptation to an environmental gradient in Drosophila serrate. Genetics 167:1281–1291
Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford
Steppan SJ, Phillips PC, Houle D (2002) Comparative quantitative genetics: evolution of the G matrix. Trends Ecol Evol 17:320–327
Wilson AJ et al (2009) An ecologist guide to the animal model. J Anim Ecol 79:13–26