Stochasticity and variability in the dynamics and genetics of populations
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Chapter 4

\( G \) spotted in \textit{Rana temporaria}!

The standard selection equations have been taken too literally; and genetic assumptions with little empirical support have gained undue credibility.

Nick Barton and Michael Turelli
Abstract

Plenty of work has focused in understanding the evolution of the genetic covariances ($G$ matrix). Yet this empirical and theoretical knowledge has not fully merged. Thus we lack the big picture about $G$’s evolution. We present a model that considers how $G$ relates to allele frequencies and pleiotropic structure. Averaging over these gives estimators of $G$ that are independent of these variables, but which depend only on mutation rate, selection differentials, population size and allelic effects. The latter may be approximated by average values. The model thus integrates the mechanisms of population with quantitative genetics, but requires only phenotypic (quantitative) information. This is already a significant achievement. However we apply our ideas to previous results in experimental evolution of Rana temporaria, addressing a classical question: which factors affect the diversification of $G$? We give concrete answers on the role of selection, mutation, and drift in the observed experimental patterns.
4.1 INTRODUCTION

Since the pioneering work of Lande (1979) much research has been done to understand the evolution of genetic covariances (Steppan et al., 2002; Blows, 2007; Arnold et al., 2008). These are essential for understanding the evolution of metric traits (Barton and Turelli, 1987; Bürger, 1991). Throughout the evolutionary process, for few generations genetic variation remains unchanged (Turelli, 1988). As with the breeder’s equation, the formula $\Delta \bar{z} = G.P^{-1}.\bar{\beta}$ would allow prediction of the mean of multivariate traits of a population, $\bar{z}$ (Lande, 1979, 1980). $G$ is the genetic covariances (of the traits) matrix, $P$ is the matrix of phenotypic covariances, and $\bar{\beta}$ is a vector of selection differentials. Under certain conditions $G$ can be stable across generations (Brodie, 1993; Roff, 2000; Begin and Roff, 2001, 2003; Nosil et al., 2006; Renaud et al., 2006). However, other observations show that many factors affect $G$’s constancy (Wagner, 1984; Shaw et al., 1995; Roff, 2000; Phillips et al., 2001; Widen et al., 2002; Cano et al., 2004; Kotiaho, 2007; Doroszuk et al., 2008), which are also supported by theoretical understandings (Turelli, 1988; Reeve, 2000; Jones et al., 2003, 2004, 2007). But the theories on the evolution of $G$ are incomplete (Arnold et al., 2008), hence employing measurements of the $G$ at one given time, might not be enough to explain or predict phenotypic variation and diversification in ecological or evolutionary times (Steppan et al., 2002; Blows, 2007; Kotiaho, 2007). Selection aligns $G$ to evolve in a particular direction (Reeve, 2000; Roff, 2000; Steppan et al., 2002; Jones et al., 2004), but random drift make it wobble unpredictably generation after generation (Roff, 2000; Jones et al., 2003; Arnold et al., 2008). Mutation, depending on the degree of pleiotropy and linkage, and migration will act like a torque inducing or reducing the correlations among the traits (Jones et al., 2003,
2004; Guillaume and Whitlock, 2007; Arnold et al., 2008). Despite this knowledge on how different factors affect $G$, the quantitative predictions are raw (Arnold et al., 2008). Hence, empirical quantifications of $G$ are hard to relate to the theoretical knowledge.

Our first brass ring is to develop a theory that comprehends population and quantitative genetics, that is applicable for multivariate response to selection, mutation, and drift (SMD). A previous approach predicted the evolution of a quantitative characters subject to SMD, considering the influence of the genetic states (allele frequencies), but without making direct reference to them (Barton and de Vladar, 2009; Barton and Coe, 2009). We extend these methods to the multivariate case, with pleiotropic effects. A given trait is affected by a set of genetic variables (e.g. allele frequencies), whose distribution can be described by the Wright-Fisher SMD equilibrium distribution (Crow and Kimura, 1970, pp. 442-445). From this distribution, we can calculate a generating function, which considers all genetic states and averages over them. Thus it is implicitly dependent on the genetic variables, but depends explicitly only on the selective gradients over each trait $\beta$, mutation rate $\mu$, population size $N$ and the additive effects of each locus over the trait, $\gamma$. The expectancies of the mean traits $\bar{z}$, genetic co-variances matrix $G = \{\nu_{ij}\}$, phenotypic covariances, etc. can be calculated from the generating function. Ergo, we offer a method to calculate quantitative aspects of a population’s traits in such a way that genetics is not disregarded, but the knowledge of its details is dispensable for the quantitative description of the population. This merging of the genetic with the quantitative variables has been a riddle for decades, and its failures imbued the understanding of the $G$’s stability. Although we have by no means solved all questions regarding the evolution of $G$, our results are opportune to address some of the relevant aspects
about quantitative evolution.

Much is to be done in the theory of $\mathcal{G}$, but at this point we are encouraged to formulate our questions by empirical motivations. We seek a marriage between the practical needs and the theoretical capabilities. Specifically, we chose to re-evaluate the experimental results of Cano et al. (2004) see also Laurila et al. (2002); Palo et al. (2003); Ovaskainen et al. (2008) from which the non-constancy of $\mathcal{G}$ has been verified for four correlated traits in *Rana temporaria*: development time, mass, body length, and tail length. Employing suitable experimental design and statistical analyses the authors verified that the $\mathcal{G}$ matrices of two populations were statistically different (Cano et al., 2004; Ovaskainen et al., 2008). However Jones et al. (2003), identified that drift is a major source of fluctuations of $\mathcal{G}$. Whilst selection effectively affects $\mathcal{G}$, it would have lamer and predictable repercussions than drift (Roff, 2000). Our second goal is to appraise the roles of selection and drift from the data of Cano et al. (2004). Employing the proposed theoretical construct we will characterize from the trait data the conditions maintained at two distinct selection-mutation-drift (SMD) equilibria. Then we will predict the corresponding $\mathcal{G}$-matrices, which we compare to the empirical estimations. Randomly sampling the distribution of allele frequencies and computing $\mathcal{G}$ for these, illustrates the variability that the genetic covariances can show, and whether it is (or not) a plausible explanation for the observed diversification in $\mathcal{G}$, instead or along with selection.

4.2 THEORETICAL BACKGROUND

Throughout this paper, we will assume that the populations are in Hardy-Weinberg equilibrium. Consider $M$ autosomal traits, affected by $N$ independent loci (i.e. in linkage equilibrium), each
trait of them is determined by the contribution of the diploid set at each locus $x^\ell_f$ and $x^\ell_m$, $z_m = \sum_{\ell=1}^n \gamma_{m\ell} \left(x^\ell_f + x^\ell_m - 1\right)$, where $x^\ell_f$ and $x^\ell_m$ are either 0 or 1 (unfavorable or favorable copies of the alleles). Averaging $x_\ell$ over the population, and calling $p$ ($q$) the frequency of $x = 1$ ($x = 0$) in a population, the mean trait results in

$$z_m = \sum_{\ell=1}^n \gamma_{m\ell} (p_\ell - q_\ell) \ , m = 1, 2, \ldots, M$$

(4.1) where $\gamma_{m\ell}$ is the effect of locus $l$ over the trait $m$. We consider only additive on all traits (there is neither epistasis nor dominance), but pleiotropic effects are present (unless $\gamma_{m\ell} = 0$). We consider selection over all traits to be directional and of exponential nature (Kingsolver et al., 2001; Hoekstra et al., 2001):

$$\bar{W} = \exp \left[ \bar{b} \cdot \bar{z} \right], \ \bar{b} \cdot \bar{z} = \beta_1 \bar{z}_1 + \beta_2 \bar{z}_2 + \ldots \beta_M \bar{z}_M.$$ The gradient of log-mean fitness is

$$\frac{\partial}{\partial p_{\ell}} \log \left[ \bar{W} \right] = \beta_m \gamma_{m\ell},$$

where $\beta_m$ is the intensity of selection over the trait $m$. The rate of change of the frequency $p$ at every locus, including SMD is given by the Wright-Fisher model (Wright, 1938; Kimura, 1955):

$$\frac{\partial \psi}{\partial t} = M_{\delta p} \frac{\partial \psi}{\partial t} + \frac{1}{2} V_{\delta p} \frac{\partial^2 \psi}{\partial t^2}$$

(4.2)

$$M_{\delta p} = \underbrace{pq \beta}_{\text{selection}} + \underbrace{\mu (2p - 1)}_{\text{mutation}}$$

(4.3)

$$V_{\delta p} = \underbrace{\sqrt{pq}}_{\text{drift}} N$$

(4.4)

where $q = 1 - p$, $\mu$ is the mutation rate, and $\zeta$ represents the drift, as a normal distribution with variance $\frac{pq}{2N}$, and $N$ the size of the population. This leads to the classical equilibrium distribution of joint allele frequencies (Wright, 1938; Crow and
4.2. THEORETICAL BACKGROUND


\[ \psi = \frac{Z^{-1}}{V_{\delta p}} \exp \left[ \int \frac{M_{\delta p} dp}{V_{\delta p}} \right] \quad (4.5) \]

Here \( Z \) is the normalizing constant:

\[ Z = \int \exp \left[ 2N\bar{\beta}.\bar{z} + 2N\mu U \right] / \Pi_{\ell=1} \frac{n}{\pi} p_{\ell q_{\ell}} d\bar{p}. \quad (4.6) \]

where with \( U = 2\sum_{\ell=1}^{n} \log (p_{\ell q_{\ell}}) \), the contribution by mutation of all loci to the quantitative evolutionary potential. Notice that beyond just normalizing, it is a generating function; taking derivatives of \( \log(Z) \) with respect to \( \beta_{m} \) and \( \mu \), leads to the expected values of the traits, and of the mutation effects \( U \):

\[
\frac{\partial \log Z}{2N\partial \beta_{m}} = \langle \bar{z}_{m} \rangle \\
= \frac{1}{Z} \int \bar{z}_{m} \exp \left[ 2N\bar{\beta}.\bar{z} + 2N\mu U \right] / \Pi_{\ell=1} \frac{n}{\pi} p_{\ell q_{\ell}} d\bar{p}
\]

\[
\frac{\partial \log Z}{2N\partial \mu} = \langle U \rangle \\
= \frac{1}{Z} \int U \exp \left[ 2N\bar{\beta}.\bar{z} + 2N\mu U \right] / \Pi_{\ell=1} \frac{n}{\pi} p_{\ell q_{\ell}} d\bar{p}
\]

The angle brackets \( \langle \ldots \rangle \) indicate statistical expectation over drift. The reader can check that the second derivatives correspond to variances and covariances of the population means. The interesting issue is that if there is an algebraic expression for \( Z \), the expectations can be calculated explicitly. Indeed, \( Z \) is:

\[ Z = \prod_{\ell=1}^{n} Z_{\ell} \left( \mu, \bar{\beta}.\bar{\gamma}_{\ell} \right), \quad (4.9) \]

\[ Z_{\ell} = \sqrt{\pi} 2^{1-8N\mu} \Gamma(4N\mu)_{0} \tilde{F}_{1} \left( 4N\mu + 1/2; N\bar{\beta}.\bar{\gamma}_{\ell} \right) \]

where \( \bar{\gamma}_{\ell} = (\gamma_{1\ell}, \gamma_{2\ell}, \ldots, \gamma_{M\ell}) \) is the vector of effects of locus \( \ell \) over each trait. In the last expression, \( \Gamma \) is the Gamma function, and \( \tilde{F}_{1} \) is the regularized hypergeometric of order \( (0,1) \).
4. Evolution of the $G$-Matrix

(this can also be written as Bessel functions, see Barton and de Vladar (2009)). Explicit formulas for the values of the mean traits follow from the derivatives:

$$\langle \bar{z}_m \rangle = \sum_{\ell=1}^{n} \frac{I_{4N\mu+1/2} \left( 2N\tilde{\beta}, \tilde{\gamma}_\ell \right)}{I_{4N\mu-1/2} \left( 2N\tilde{\beta}, \tilde{\gamma}_\ell \right)} \gamma_{ml},$$  \hspace{2cm} (4.10)

The elements of $G$ are,

$$\nu_{mr} = 2 \sum_{\ell=i}^{n} \gamma_{ml}\gamma_{rl}p_{\ell}q_{\ell},$$  \hspace{2cm} (4.11)

whose expectations are can also be given explicitly:

$$\langle \nu_{mr} \rangle = 2N\mu \sum_{\ell=1}^{n} \frac{\gamma_{ml}\gamma_{rl}}{N\tilde{\beta}, \tilde{\gamma}_\ell} \frac{I_{4N\mu+1/2} \left( 2N\tilde{\beta}, \tilde{\gamma}_\ell \right)}{I_{4N\mu-1/2} \left( 2N\tilde{\beta}, \tilde{\gamma}_\ell \right)}. \hspace{2cm} (4.12)$$

We point out for the reader, that beyond the mathematics, the relevance of the expressions (4.10 and 4.12) is that they consider the genetic states by construction, but the expressions themselves are not explicitly dependent on the allele frequencies. Thus the expectations embed the genetic variables with the quantitative traits, merging both levels of description.

If the algebraic equations above are not of much insight, the reader may still notice that they can be used for making estimations from the data. We presented only those formulas of immediate interest for this study, but any other statistic can be calculated from direct integration (at worse numerically), or by derivatives of $\log(Z)$ (hints: covariance, higher moments of the trait, like skewness or kurtosis, etc. see Barton and de Vladar (2009)).

The dynamics of the expectations of the trait, can be calculated substituting the formulas of $\bar{z}_m$ and $U$ in Eqns. 4.7 and
4.3. MATERIALS AND METHODS

4.8 and using the rule of chain with Eq. 4.2 to calculate the rates of change. Details are presented in Barton and de Vladar (2009). In short:

\[
\frac{d}{dt} \left\langle z_U \right\rangle = \left\langle G \ z H \right\rangle \cdot \left( \beta \mu \right)
\] (4.13)

\( U \) is included because together with log-mean fitness (in this case, the traits) couple the effects of mutation to the change of \( G \) at all time points (Eq. 4.2), and \( H \) is the genetic variance of the mutation effects \( U \). This treatment of the effects of mutations is somehow different to the mutation matrix \( M \) (e.g. Jones et al. (2007)). We are for the moments uncertain about the relationship between \( M \) and \( U \) and \( H \). However we know that Eq. 4.13 faithfully leads to long-term predictions Barton and de Vladar (2009). We approximate the genetic variances \( G \) and \( H \), by the corresponding expectancies (e.g. Eqns. 4.10 - 4.12). Notice that the parameters \( \beta \) and \( \mu \) (Barton and de Vladar, 2009; Barton and Coe, 2009), are not bound to be constant, but are allowed to change in order to keep the distribution of allele frequencies coupled to the evolutionary dynamics. Details on this method can be found in (Barton and de Vladar, 2009).

4.3 MATERIALS AND METHODS

Summary of the Data

In the original study, Laurila et al. (2002), collected female and male frogs from two Swedish populations of \textit{Rana temporaria}. The Kiruna ‘Northern’ population lives in a stream that rarely (if ever) experiences desiccation. The Lund, or ‘Southern’ population, is situated in a pond that dries up frequently (Laurila et al., 2002). For each location, eggs of four females were ar-
tificially fertilized with sperm of five males for total of 45 full-sib families. Once the tadpoles reached certain developmental stage, 18 of them from each cross were individually dispensed to vials with 0.75 L of water and allowed them to develop until metamorphosis. In the meantime, each tadpole was exposed to one of the three desiccation treatments: control (constant water level), slow (reduction of water level by 15% at each water change), and fast (reduction of water level by 30% at each water change). At metamorphosis the tadpoles were weighed and the body and tail length of the individuals were measured. Their development time (days elapsed from the start of the experiment until metamorphosis) was also measured. To avoid scaling effects and to homogenize variances, the natural logarithm of the trait values was used in the analyses (Further details can be found in Laurila et al., 2002; Cano et al., 2004).

To estimate \( G \) the authors fitted a linear animal model (Lynch and Walsh, 1998, pp. 755-758) that considered the additive genetic effects of the pedigree structure, additive genetic effects, maternal identities in the pedigree, and nonadditive genetic effects (i.e., dominance and epistasis). T-tests were employed to verify whether the estimations of the heritabilities and genetic correlations were significantly different from zero. The \( G \)-matrix values (and other estimations) are reported in Cano et al. (2004) study (but see also Ovaskainen et al. (2008)).

**Quantitative Estimations**

Typically inbreeding experiments are used to estimate the number of loci and their effect over the traits (Wright, 1968; Lande, 1981; Ollivier and Janss, 1993). But we proceeded by a different method, since these experiments are at the moments not available. We calculate the minimal number of loci and their
average effect over a trait to be respectively:

\[ n = \frac{(\bar{z})^2}{\tilde{\nu}}, \quad \tilde{\gamma} = \frac{2\tilde{\nu}}{\bar{z}}. \]

Where \( \bar{z} \) and \( \tilde{\nu} \) are the maximal meant trait and genetic variance. We pooled all the data of the populations, and performed a bootstrap analysis to estimate \( \bar{z} \). Also assuming the pooling of data, the maximal genetic variance was calculated as \( \text{Var}_{\text{tot}} = \text{Mean}(\nu) + \text{Var}(\bar{z}) \). Both quantities were compared to the actual occurring maxima in the individual samples. See Supplementary information for further details on these estimations.

There can be many possible patterns of pleiotropic interactions affecting the traits for a given number of loci and their effects. We performed a random Monte-Carlo generator to sample the space of pleiotropic architectures (see Supplementary Material). For each of these architectures, we numerically computed the solution to \( \langle \bar{z}_m \rangle_{(\bar{z}|N,\mu)} = \tilde{z}_m \), to obtain the variables \( \bar{\beta} \) (one selection differential for each measured mean trait). For this we assumed a population size \( N \) and mutation rate \( \mu \). The left side of the equation is derived from the generating function, Eq. 4.10, and the right hand side are empirical estimations from Laurila et al. (2002).

To asses the effects of drift, we resampled the distribution of allele frequencies Eq. 4.5 to generate a hypothetical populations for each of the estimated scenarios. For each of these samples of the allele frequencies, the \( G \) matrix was calculated and plotted. Each element of \( G \) is computed from the definition of \( \nu \), Eq. 4.11.

A similar procedure was employed to estimate the effects of sampling within a population (simulating a field sampling procedure), but instead of randomly choosing values with the distribution 4.5, we resampled one particular \( \hat{p} \) (which we assumed
as the expectancy \( \langle \hat{p} \rangle = \frac{1}{2} (\langle \hat{z} \rangle + 1) \). Each iteration simulated the effects of sampling a particular population, in which we end up with an array of ‘measurements’ for particular hypothetical individuals. For each of these populations we re-estimate the allele frequencies (i.e. \( \hat{p} \)), and computed and plotted \( \mathcal{G} \).

## 4.4 RESULTS

Table 4.1 reports the results for the maximum values of the mean traits and genetic variances, effective number of loci, and average effect of the alleles. The reader is deferred to the Supplementary Material for details on the results of the bootstrap analyses, and Monte Carlo search in patterns of pleiotropic interactions. For each of the 444 resulting pleiotropic architectures, we calculated the values of \( \vec{\beta} \) that match the empirical mean traits with Eq. 4.10. Pleiotropic architectures that were not compatible with the observed values, at a mutation rate of \( 10^{-3} \) and population size \( N=300 \) (following Palo et al. (2003)), were discarded. The distribution of \( \vec{\beta} \) was different for different treatments and specially for different locations (see Supplementary Material). Incidentally, not all pleiotropic structures allowed solutions for the given empirical values. Thus solving Eq. 4.13 not only resulted in the identification of the SMD conditions, but also discriminated the possible pleiotropic structures which that are consistent with the data. We found 57 pleiotropic overlaps that are consistent with data (see Supplementary Material), and which in turn happened to be common for the estimations at both locations and all treatments.

Then we forecasted the \( \mathcal{G} \) -matrices for each estimations of \( \vec{\beta} \) and their pleiotropic structures, and averaged over the latter. The eigenstructure of these averages are in good agreement with those of the empirical \( \mathcal{G} \)’s (Table 4.1), specially for
### Table 4.1: Quantitative Estimations from the data, and theoretical predictions of $G$.

<table>
<thead>
<tr>
<th>Trait&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$\tilde{z}_{max}$</th>
<th>$\nu_{max}$</th>
<th>$n$</th>
<th>$\bar{\gamma}$</th>
<th>$\bar{\nu}$</th>
<th>$\langle \nu \rangle$</th>
<th>$\dot{\nu}$</th>
<th>$\langle \nu \rangle$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.51</td>
<td>0.9</td>
<td>4</td>
<td>0.627</td>
<td>0.64</td>
<td>0.94</td>
<td>0.9</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>0.52</td>
<td>$1.6 \times 10^{-3}$</td>
<td>85</td>
<td>$6.1 \times 10^{-3}$</td>
<td>$1.3 \times 10^{-3}$</td>
<td>$1.8 \times 10^{-3}$</td>
<td>5 $10^{-3}$</td>
<td>1.8 $10^{-3}$</td>
</tr>
<tr>
<td>3</td>
<td>1.99</td>
<td>0.11</td>
<td>19</td>
<td>0.105</td>
<td>0.064</td>
<td>0.13</td>
<td>0.026</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>2.47</td>
<td>1.35</td>
<td>3</td>
<td>0.822</td>
<td>0.92</td>
<td>1.21</td>
<td>0.32</td>
<td>1.21</td>
</tr>
</tbody>
</table>

<sup>a</sup>(1) Development time, (2) mass, (3) body length, (4) tail length.
the leading eigenvalues, although some deviations are obvious, specially in the third eigenvalue (see Supplementary Material).

The expectancies of the genetic covariances, according to our theory, show little difference between the two populations, at most 0.8% in one of the genetic variances (Fig. 4.1, middle panel), although the expectancies for the traits are actually different (by construction, since they were fixed in the estimation).

Then, for each estimation, we randomly sampled the distribution of allele frequencies generating 10 populations per pleiotropic structure (4440 choices in total). We assumed a sample size of 300, as in the experimental design (and coincidentally, the population size). The resulting \( G \) matrices are shown in Fig. 4.2.

In this way it was revealed that the differences in \( G \) across the populations might be attributable to sampling effects and drift. The following sections dissect this conclusion according to our logic.

**Figure 4.1:** (Opposite page) Evolution of genetic variances with distinct mutation rates. (A) \( \mu = 10^{-2} \) (B) \( \mu = 10^{-3} \) (C) \( \mu = 10^{-4} \). In all cases, time is scaled as \( t = 2N\mu t_g \) where \( t_g \) is the time in generations. Solid lines: developmental time; large dashes: mass; short dashes: body length; dots: tail length. Selection is weak, starting from the conditions estimated for the Northern population (Kiruna), \( N\vec{\beta} \simeq (-0.11, 0.99, 0.07, 0.004) \), and evolved towards an equilibrium defined by the conditions estimated for the Southern population (Lund) \( N\vec{\beta} \simeq (0.025, 14.1, -0.73, 0.037) \). Population size is 300. The dynamics are based on Eq. 4.13 and Barton and de Vladar’s (2008) method.
4.4. RESULTS

(A)

(B)

(C)
4.5 DISCUSSION

Mutational Variance

Based on neutral microsatellite diversity $F_{ST}$, Palo et al. (2003) estimated that the mutation rate in $R.\, temporaria$ is $6 \cdot 10^{-3}$ with a population size (estimated from capture-recapture field studies) of 141 individuals, but they also point out that this number might be biased by local migration (Ellegren, 2000). Thus another possibility they discuss is that keeping the same value of $F_{ST}$, assuming that $\mu = 10^{-3}$ and absence of migration, the size of the populations is of 300 individuals; this is the scenario we have used, since we still did not develop the theory to include migration effects. In any case at the moments it seems that there is no decisive argument to precise neither $N$ nor $\mu$. Estimates for the other mutational scenario awaits for an extension of the theory to migration factors. Nevertheless, since the effects of mutation is an open question, we can give a brief theoretical account on its effects over $G$.

The expected time to achieve changes in $G$ increases when diminishing the mutation rates. Assume for the moments that mutation rate is $10^{-4}$. One generation in $R.\, temporaria$ takes about 4 yrs, which is equivalent to $t = 0.0015$ (time is scaled as $2N\mu t$). At this rate, about 1700 generations (6.7 millennia) would be needed to reach MSD balance in $G$ (Fig. 4.1, top pannel). Contrast this result at mutation rates of $10^{-3}$, when we should about 17 generations (less than 70 years) of continuous selection are needed to reach a SMD equilibrium in $G$ (Fig. 4.1, middle pannel). Actually, these mutation rate scenarios (Ellegren, 2000) fit very well to the range of time that Laurila et al. (2002) suggest for the divergence of the two populations, which had to happen during the last 10 millenia.
However, notice that for low mutation rates, the overall change in the genetic covariances is very low. The predictions of $\langle G \rangle$ in both populations differ in at most 0.8%, which is a difference too small to be detected. Nevertheless, phenotypic changes are conspicuous between the traits of both populations of *R. temporaria*. But even when these phenotypic changes have indeed taken place, they are not accompanied by a big change in the covariances. This is what raises our doubts in that selection is the responsible factor for the observed differences in $G$.

**Action of Selection**

The original study about the evolution of the $G$-matrix in *R. temporaria* reported that the index of quantitative variation ($Q_{ST}$) deviated significantly from $F_{ST}$, the index of differentiation at neutral genetic markers (estimated by microsatellite analyses Palo et al., 2003). This shows that selection is acting, and inducing phenotypic diversification. The empirical estimations of the $G$-matrices for different treatments and locations were analyzed statistically (Cano et al., 2004) to reveal that the additive genetic co-variances, for most traits, are non-equal between the two locations. This lead to optimism that selection is the cause for such variation.

The distribution of selective gradients $\vec{\beta}$ (estimated from the data, for each pleiotropic structure) between both populations is different, supporting that the diversification between the two population was driven by selection. Yet the expectancies of $\langle G \rangle$ do not show such contrasting differences as the reported empirical $G$-matrices. The eigenstructures of $\langle G \rangle$ in both populations are highly similar (Supplementary Material: Table 4.1 and Fig. 4.2). Even though our theory supports that selection has acted to shift the phenotypic values, it reveals has not acted strongly enough to shift the genetic covariances.
If selection for a character proceeds in the opposite direction as in the natural equilibrium conditions that maintain SMD, there can be a transitory increase in the genetic covariances, due to pleiotropic effects. This seems to have happened in the recent diversification of the two populations (Fig. 4.1, middle panel). We presume that this happened during the first two or three centuries after the populations separated, provided that the ecological conditions were such that selection and population size remained, in average, constant.

**Effects of Genetic Drift and Random Sampling**

The estimations based on our theory indicates that the observed changes in \( G \) are most likely attributable to drift, rather than to mutation or selection. The amount of individuals employed in the experiments allow for significant deviations by genetic drift. Each locus contributed to the variance of drift \( \sigma^2 \) by an amount of \( pq(2p-1)^2/2N \), which has a maximum value of \((32N)^{-1}1.210^{-4}\). If we account for all loci, and for a population of size 300, we can have a range of percentile standard error (\( \sigma_z/\bar{z} \)) from 8\% to 220\%. Thus the power to discern selection from drift can be rather low.

This should not be confused with the power of the statistical analyses in Cano et al. (2004). Their analyses have enough power to discern differences in the \( G \)-matrix structures, supported by a good experimental design (Lynch and Walsh, 1998). Our argument is that the cause of \( G \)'s differentiation is genetic drift.

To assess this possibility, we sampled the distribution of allele frequencies. For each sample of allele frequencies, say \( \hat{p} \), the covariance matrix, \( \hat{G} \) was calculated. Figure 4.2 (top panel) illustrates that the variation pattern on \( G \). Notice how \( G \) is distributed in a bimodal fashion. Furthermore, the empirical \( G \) for both populations seem to fit well in these distributions.
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Figure 4.2: Drift and sampling on $G$ (two principal components: development time and tail length) in two Swedish populations of *Rana temporaria*. Top row: effects of genetic drift on $G$ evaluated by randomly sampling the allele frequencies (gray ellipses). Black ellipses: expectancies $\langle G \rangle$. Bottom row: effects of sampling individuals from a particular population (gray ellipses). Dotted black ellipses: ‘empirical’ $G$s (Cano et al., 2004). Samples include the 10 realizations of each pleiotropic combination of genetic effects, along with their respective $\beta$. Mutation rate is $\mu = 10^{-3}$. Population and sample sizes are 300 for the expectations, and 72 for the drift samples. The representations of $G$ follow the conventions by Arnold et al. (2008): the semi-axes of the ellipse are eigenvectors of the components, with length $1.96\sqrt{\lambda}$, where $\lambda$ is the eigenvalue of the component.
But on top of this stochasticity, there is also randomness due to experimental sampling. One population is in itself a sample, \( \hat{\rho} \), of the distribution of allele frequencies. Thus the genetic states of an individual follows a Bernoulli distribution (assuming two alleles at each locus) with certain probability \( \hat{\rho} \). Hence the \( G \) matrix associated to a population, is restricted to a particular realization of the distribution of allele frequencies. Figure 4.2 (lower panel) shows the sampled genetic covariances along with the empirical \( G \)'s. It is clear to our eyes, that the differences in \( G \) from both populations are attributable first to drift, and second to sampling, rather than to selection.

**How far are we?**

The details that affect the evolution of \( G \) are vast. The quantitative trait loci, and the effect that each of these can have over the traits, are in general a real puzzle. We are able to access so little information about the genetic and epigenetic effects, that we are unable to predict how \( G \) will respond in their presence. We have assumed very restrictive conditions, like Hardy-Weinberg equilibrium, which thanks to the appropriate experimental design of the data herein used, can safely be assumed. Also, selection was assumed to be directional and the estimations show it is fairly weak. This allows the possibility that, to some extent, the effects of linkage can be disregarded (Barton and Turelli, 1991; Kirkpatrick et al., 2002). But other factors with potential consequences in our predictions were left out. Namely recombination, epistasis, and dominance, to mention popular ones. Still, the specific model we have herein introduced allowed us to blur much of the information that is experimentally tedious to obtain. The technical details of the method are discussed by Barton and de Vladar (2009), some of which are subtle. But essentially we have shown that we can dispense of many de-
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grees of freedom, which were thought to be required to forecast evolution.

The theories on the evolution of \( G \), and its empirical studies have remained separated. We have merged some facets of population and quantitative genetics. We still have some degree of uncertainty, with respect of biological factors. If, for example, we were able to precise \( N \) and \( \mu \), our estimations would lead to predictions of the \( G \) matrix, which are testable. Thus appropriate experimental design in line to the assumptions of our calculations can properly help to discern what precisely is affecting \( G \)'s evolution.

This work is an exercise illustrating that the line of modeling that we are following, that is to study the evolution of the expectancies of the quantities of interest, can be rewarding. This is still to be done for realistic eco-evolutionary scenarios; but so far so good.