

## Evolution and stability of the **G**-matrix during the colonization of a novel environment

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### Abstract

Populations that undergo a process of rapid evolution present excellent opportunities to investigate the mechanisms driving or restraining adaptive divergence. The genetic variance–covariance matrix (**G**) is often considered to constrain adaptation but little is known about its potential to evolve during phenotypic divergence. We compared the **G**-matrices of ancestral and recently established ecotype populations of an aquatic isopod (*Asellus aquaticus*) that have diverged in parallel in two south Swedish lakes. Phenotypic changes after colonization involved a reduction in overall size, lost pigmentation and changes in shape. Comparisons between **G**-matrices reveal close similarity within the same ecotype from different lakes but some degree of differentiation among ecotypes. Phenotypic divergence has apparently not been much influenced by the orientation of **G**. Additive genetic variation in the newly colonized habitats has also decreased substantially. This suggests that a process of adaptation from standing genetic variation has occurred and has probably facilitated phenotypic divergence.

### Introduction

Evolutionary change in a quantitative trait is influenced by the magnitude of standing genetic variation, mutational input during each generation and genetic correlations with other traits and the strength and mode of selection on the traits in question (Agrawal *et al.*, 2001; Sinervo & Svensson, 2002; Barrett & Schluter, 2008). The genetic variance–covariance matrix (**G**) is a fundamental parameter in evolutionary quantitative genetics because **G** is thought to constrain evolution in its early stages and will then bias the short-term evolutionary response towards  $\mathbf{g}_{\max}$ , the genetic line of least resistance (Lande, 1979; Arnold, 1992; Schluter, 1996; but see Berner *et al.*, 2008). The extent to which **G** constrains adaptation is, however, still subject to much discussion among theoretical and empirical evolutionary biologists (Agrawal & Stinchcombe, 2009; Eroukhmanoff, 2009; Hine *et al.*, 2009; Chenoweth *et al.*, 2010). Studying patterns of population

divergence **G** can therefore increase our understanding of the mechanisms underlying phenotypic divergence and how the response to selection might be constrained by genetic architecture during adaptive evolution (Maynard Smith *et al.*, 1985; Schluter, 1996; Stepan *et al.*, 2002; Arnold *et al.*, 2008; Eroukhmanoff, 2009).

One issue that has been much debated in recent past is whether **G** is stable over short time scales of only a few generations, i.e. ecological timescales (Björklund, 1996; Eroukhmanoff, 2009). **G** can change drastically under a variety of selective conditions (Jones *et al.*, 2003, 2004; Blows *et al.*, 2004), but it has been proved theoretically challenging to predict the dynamics of **G** on a generation-time basis (Arnold *et al.*, 2008). Some empirical studies conducted in natural populations have found **G** to be stable on ecological timescales of only a few generations but unstable on evolutionary timescales of several hundreds or thousands of generations (Björklund, 1996; Cano *et al.*, 2004). Temporal changes in **G** stability are still largely unknown and are difficult, or even impossible, to study in natural populations of most organisms. A few laboratory studies on fruitflies (*Drosophila melanogaster*) indicate that **G** can change very rapidly and over only a few generations (Wilkinson *et al.*, 1990; Shaw *et al.*,

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1995; Phillips *et al.*, 2001; Sgro & Blows, 2004). However, the multifarious and stochastically varying selection pressures in natural populations are likely to differ substantially from the more stable and predictable laboratory environments in these previous studies (Arnold *et al.*, 2008). One recent study on wild populations of nematodes (*Acrobeloides nanus*) documented relatively rapid changes in **G** for three life history traits under various environmental conditions (Doroszuk *et al.*, 2008). However, life history traits are likely to be subject to more pronounced nonadditive genetic effects compared with morphological traits (Roff, 2000; Doroszuk *et al.*, 2008) and are also likely to be targets of stronger selection pressures as they are fitness components. These factors are likely to contribute to a lower stability of **G** for life history traits. Therefore, uncertainty remains about whether and how **G** can change over a short timescale in natural populations experiencing divergent natural selection (McGuigan, 2006).

Another important issue with implications for the stability of **G** is the genetic mechanisms by which populations diverge and adapt locally. There are at least two possible mechanisms by which a population can adapt to a novel environment: selection on novel mutations (Linnen *et al.*, 2009) or selection on pre-existing or 'standing' genetic variation (Barrett & Schluter, 2008; Barrett *et al.*, 2008; Schluter & Conte, 2009). The stability of **G** has been explored theoretically for conditions where the effects of new alleles originating from mutations are much larger than already-existing alleles (Turelli, 1985). Yet, little is known empirically about the consequences for **G** stability when local adaptation originates from selection on standing genetic variation (but see Jones *et al.*, 2003, 2004). If genes of major effects segregate in a population, selectively favoured alleles could rapidly increase in frequency, which in turn could result in rapid evolutionary change of phenotypes (Barrett *et al.*, 2008; Teotonio *et al.*, 2009). Evolutionary change caused by selection on genes of major effect might also result in transient changes in **G**-matrix structure (Agrawal *et al.*, 2001). In contrast, most of the classical assumptions underlying **G** stability come from quantitative genetic models assuming small effects of many genes affecting quantitative traits (Agrawal *et al.*, 2001). A dramatic decrease in genetic variation caused by strong selection might lead to the build-up of new genetic correlations (Barrett *et al.*, 2008), and to a quantitative and qualitative reorganization of **G**.

Here, we present the results of a study on the quantitative genetic architecture of two ecotypes of an aquatic isopod (*Asellus aquaticus*) in two different lakes in southern Sweden. Recently (i.e. during the past two decades), a new ecotype (stonewort) emerged from a source ecotype (reed) independently and in parallel in both these lakes (Eroukhmanoff *et al.*, 2009a) as well as in several other geographically separated lakes in southern Sweden (Hargeby *et al.*, 2005). The stonewort

populations in the centre of the lakes are thus derived from the reed populations surrounding the lakes. These novel stonewort populations differ substantially and significantly in several important phenotypic traits, such as pigmentation, size and shape (Eroukhmanoff & Svensson, 2009; Eroukhmanoff *et al.*, 2009a,b). Recently, we have demonstrated that isopods of the two newly established ecotypes have evolved substantially different morphologies and pigmentations since 1989, i.e. in only a few decades and a few dozen isopod generations (Hargeby *et al.*, 2004). The selective agent causing these phenotypic changes is likely to be different predator faunas (invertebrates vs. fish) in the reed and stonewort habitats, respectively. Molecular analyses from both nuclear genes and mtDNA have confirmed that these adaptive changes occurred independently in each lake and that the local stonewort populations arose *in situ* (Eroukhmanoff *et al.*, 2009a). Moreover, the differences between the reed and stonewort ecotype populations are not solely a result of phenotypic plasticity but there is a significant additive genetic component between ecotype divergence (Eroukhmanoff *et al.*, 2009b). The purpose of this study was to investigate whether divergent selection between the different ecotypes has resulted in a quantitative genetic signature in the **G**-matrices of the different populations. We demonstrate that in both these lakes, **G** has changed in the new habitats. During this short evolutionary time frame (<40 generations), the overall additive genetic variation has also become dramatically reduced in the stonewort habitat. We discuss the possible consequences of adaptation from standing genetic variation and how it could have affected the stability of **G** in this system, as well as the ecological significance of these phenotypic changes.

## Materials and methods

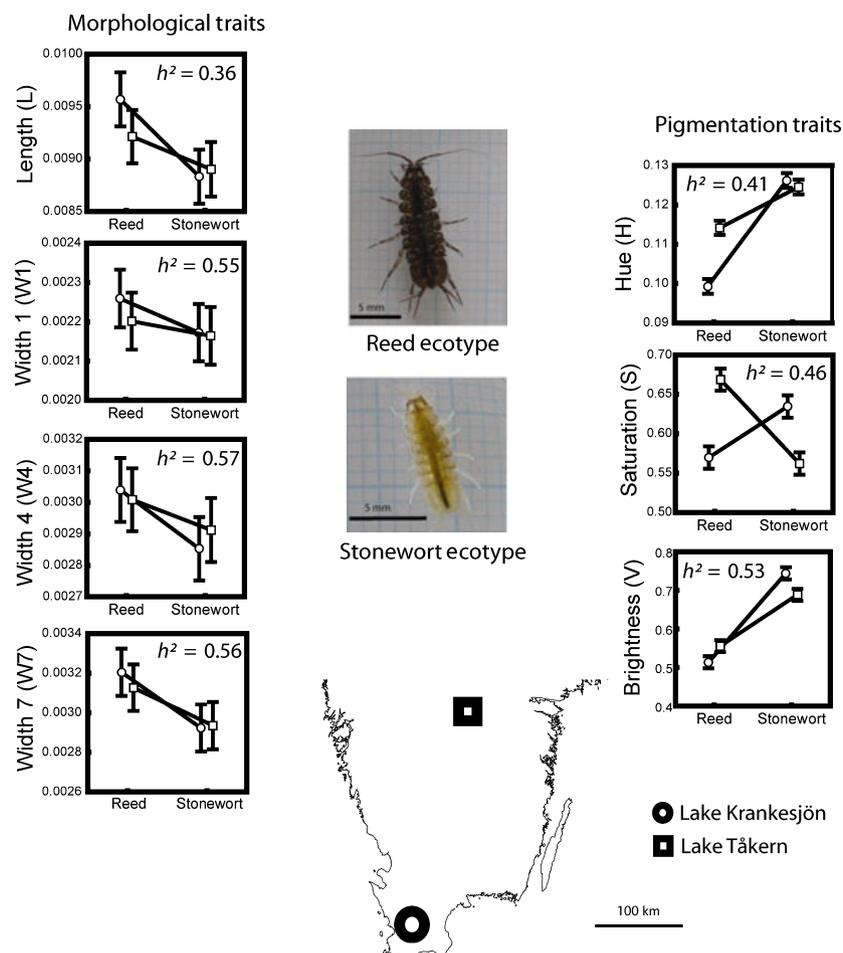
### Study species and study sites

*Asellus aquaticus* is a freshwater isopod that is widespread in lakes and ponds in Europe (Hargeby *et al.*, 2004). Populations of *A. aquaticus* occupy various habitats, but mainly occur in reed stands (*Phragmites australis*) where they feed on decaying leaves (Hargeby *et al.*, 2004). Two south Swedish lakes separated by more than 300 km, Lake Krankesjön (55°42'N, 13°28'E) and Lake Tåkern (58°21'N, 14°50'E), have in the past 20 years (starting in 1987 in Lake Krankesjön and in 2000 in Lake Tåkern) experienced dramatic ecological shifts from a phytoplankton-dominant state towards a macrophyte-dominated state (Hargeby *et al.*, 2007). These ecological shifts resulted in the colonization of sediment lake bottoms by stonewort (*Chara tomentosa*). The substrate in the reed consists of organic detritus that form a black background, whereas the stonewort habitat consists of light green vegetation growing above a light grey mineral substrate. Following the establishment of stonewort, isopods also

colonized this novel vegetative habitat in both lakes and now inhabit it at higher densities than in their source habitat (Karlsson *et al.*, 2010a). In both Lake Tåkern and Lake Krankesjön, habitat-specific changes in pigmentation and size were observed shortly after this colonization (<40 generations in Lake Krankesjön and <15 generations in Lake Tåkern). The isopods became smaller and brighter in the stonewort stands compared with the larger and darker ancestral populations in the reed (Fig. 1). This diversification process might indicate a case of ongoing ecological speciation, as recent data suggest (Eroukhmanoff *et al.*, 2011).

These differences in isopod morphology and pigmentation are presumably a result of divergent selection

caused by the different visual backgrounds and different predator faunas in the two habitats, and possibly also differences in overall predation risk (Hargeby *et al.*, 2004, 2005; Eroukhmanoff & Svensson, 2009). In support of this adaptive interpretation, similar locally cryptic colour differences has been documented in at least seven other south Swedish lakes, suggesting that the parallel changes observed in Lake Tåkern and Lake Krankesjön are not restricted to these two particular lakes (Hargeby *et al.*, 2005). Recently, we have demonstrated that in the two lakes that were focus of the present study, ecotype divergence occurred independently, as the different ecotype populations within the same lake were genetically closer to each



**Fig. 1** Parallel phenotypic divergence between lakes in their ecotype populations. Mean phenotypic values for wild-caught individuals from both ecotypes and lakes for the seven study traits, four size-related traits to shape (L, W1, W4 and W7) and three pigmentation-related traits (H, S and V). All traits have significantly diverged between ecotypes (Eroukhmanoff *et al.*, 2009a,b), and these changes all occurred in parallel among these two study lakes except for S, leading to a smaller and lighter pigmented morphotype in the stonewort, as can be seen in the two photographs. All these phenotypic traits exhibit significant levels of heritability, suggesting that phenotypic divergence does not simply reflect the effects of phenotypic plasticity. These phenotypic changes occurred independently after the last oligotrophication phase of both lakes which enabled the emergence of stands of submerged vegetation such as stonewort (*Chara tromentosum*), not earlier than in 1987 in southern Sweden (see map).

other (for two types of molecular markers, AFLP and mtDNA) than the populations from different lakes involving either reed or stonewort ecotypes (Eroukhmanoff *et al.*, 2009a). Fishes (e.g. perch, *Perca fluviatilis*) prey on invertebrates, including *A. aquaticus* (Rask & Hiisivouri, 1985), and are much more abundant in the stonewort (Eroukhmanoff & Svensson, 2009). Predation from visually hunting fish is thus likely to be more intense in the stonewort habitat than in the darker reed habitat, partly because of higher densities of perch in the stonewort (Wagner & Hansson, 1998). This ecological difference between the two habitats is likely to select for smaller, brighter isopods in the stonewort habitat, whereas in the reed habitat, invertebrate predators relying on tactile cues (i.e. dragonfly and damselfly larvae) are the main threat towards the isopods (Wagner & Hansson, 1998; Hargeby *et al.*, 2005). Additional support for a role of selection in driving phenotypic divergence between these habitats comes from our recent study where neutral molecular population divergence was compared with genetic divergence for the quantitative phenotypic traits (Eroukhmanoff *et al.*, 2009b). This study revealed that the phenotypic differentiation (measured as  $Q_{ST}$ ) was substantially larger than genetic divergence for putatively neutral markers (AFLP:s, measured as  $F_{ST}$ ; Eroukhmanoff *et al.*, 2009b).

### Common garden experiment and quantitative genetics estimates

We raised families in a common environment with similar food, temperature and water conditions, using a half-sib and full-sib design. Each family was raised in single containers with lake water constantly circulating through all containers, thus creating a common water environment for all families. Parents (sexually active adults) were caught in the early beginning of the breeding season. The adults were kept for 1 day in the laboratory and photographed before being randomly assigned in pairs and then transferred to their individual container. The male was removed from the container once it had inseminated the female. When the offspring hatched, the female was also removed, leaving the offspring to grow alone for several months until they reached sexual maturity. A total of 2131 individuals were measured, representing a total of 359 families, distributed as follows: 68 full-sibships and 28 paternal half-sibships for Krankesjön Reed, 56 full-sibships and 24 paternal half-sibships for Krankesjön Stonewort, 71 full-sibships and 26 paternal half-sibships for Tåkern Reed and 64 full-sibships and 22 paternal half-sibships for Tåkern stonewort. The paternal half-sibships were obtained by sequentially mating sires to two different females with the aim to estimate maternal effects. However, in some cases, the female died after producing offspring with one sire, and hence in these cases, it was not possible to obtain additional half-sibs from that dam.

For each family, when it was possible, we randomly picked four males and four females and photographed them. All the individual measurements for the parents and the offspring were made using the software PICSTATS (available from the authors upon request), within the time frame of 2 weeks, and under constant lightning conditions, with six colour scales (yellow, blue and red and white, grey and black) and one metric scale. In total, seven traits were measured on all individuals. Four of these traits were related to size and shape morphology (length and width at segments 1, 4 and 7) and three of the traits were related to body pigmentation (hue, saturation and brightness). In this system, these size-related traits and pigmentation traits are likely to be ecologically important in terms of predator detection (crypsis, Hargeby *et al.*, 2004, 2005) and antipredator strategy (escape speed, which is connected to body shape, Eroukhmanoff & Svensson, 2009). Recent work has shown that all these traits are largely heritable within populations and that there is a substantial amount of additive genetic variation also between ecotypes for most of them (Eroukhmanoff *et al.*, 2009b). An animal model approach including data from all parents and their offspring was used to estimate quantitative genetics parameters (heritabilities, additive genetic variances, etc.) using the REML software WOMBAT (Meyer, 2007). A separate animal model for each population was also run, using phenotypic values for both parents and offspring with sex (fixed effect), and sire and dam as random effects (Eroukhmanoff *et al.*, 2009b). Dams were nested into sires and convergence of the model was obtained in all cases. Additional information about this model and the effect of ecotype and lake on additive genetic variation can be found in a previous study (Eroukhmanoff *et al.*, 2009b).

### G-matrix comparisons

We estimated two important parameters of  $\mathbf{G}$ ,  $\theta$ , which refers to the angle of the first eigenvector  $\mathbf{g}_{\max}$  and  $\epsilon$ , the eccentricity, which is the ratio between the first and the second eigenvalues (Schluter, 1996; Jones *et al.*, 2003). These two parameters describe and capture the main characteristics of  $\mathbf{G}$ , i.e. its orientation and shape, thus its possible influence of the evolutionary direction during adaptation to novel environments (Schluter, 1996; Guillaume & Whitlock, 2007). We also estimated the % of change for each of these parameters between the ancestral reed ecotype and the new stonewort ecotype in each lake. The common principal component approach (CPC) (Phillips & Arnold, 1999) is a general and efficient approach to compare different matrices (Steppan *et al.*, 2002). The CPC approach makes it possible to reveal the hierarchical relationships between matrices, in a hierarchical manner, called 'the Flury hierarchy' (Phillips & Arnold, 1999; Steppan *et al.*, 2002). The steps in the Flury hierarchy include *equality*, *proportionality*, *all*

principal components in common, or *i* principal components in common (*i* ranging from  $n-2$  to 1, with  $n$  being the number of traits) and finally *unrelated* (Phillips & Arnold, 1999; Steppan *et al.*, 2002). We did not manually reorder principal components and used the step-up approach developed by Phillips & Arnold (1999). The  $\chi^2$  value divided by the degrees of freedom provides information about the lack of fit for the amount of information in a given model. The model that minimizes this ratio should be provisionally accepted, given that the associated *P*-value is above 0.05 (Phillips & Arnold, 1999).

In addition, we also used a different statistical approach to compare **G**-matrices: random skewers (Cheverud, 1996; Revell, 2007). The random selection skewers analysis uses a set of randomly generated selection vectors (skewers) to compare the **G**-matrices between populations. The response vector correlation (the correlation between all the norms of the response vectors to many selection skewers from each matrix) measures the similarity of the matrices, with values ranging between 0 (completely different) and 1 (identical). This approach was framed by Cheverud (1996) and the software 'skewers' we used has been developed by Revell (2007). This program calculates the mean random skewers vector correlations for the comparison of two or more genetic variance–covariance matrices. We used 10 000 random skewers for all comparisons we made. We compared **G**-matrices involving the seven study traits for all four populations studied in a pairwise fashion. Our goal with these comparisons was to estimate the differences between the reed and stonewort matrices to investigate whether habitat-specific selection might have affected the configuration of **G**.

### Estimates of additive genetic variation

We estimated the amount of standing genetic variation in each ecotype by two different procedures. First, we calculated coefficients of additive variation of the seven traits studied in each population. This coefficient ( $CV_A$ , %) was computed as:

$$CV_A = 100 \frac{\sqrt{V_A}}{\bar{X}}$$

where  $V_A$  is the genetic variance of a trait and  $\bar{X}$  its phenotypic mean (Houle, 1992). This enabled to assess the average additive genetic variance and how it possibly might have decreased in the stonewort habitat.

To investigate whether genetic variation had not only decreased but had also been reorganized and redistributed along the different eigenvectors of **G**, we also quantified the amount of variation in each population by summing all the eigenvalues of **G**, i.e. the size of **G**. This summed composite measure represents the total amount of additive genetic variation present in each of the populations. We also compared the amount of

genetic variation present on  $\mathbf{g}_{\max}$ , the axis of maximum genetic variation is the first eigenvalue of **G**.

## Results

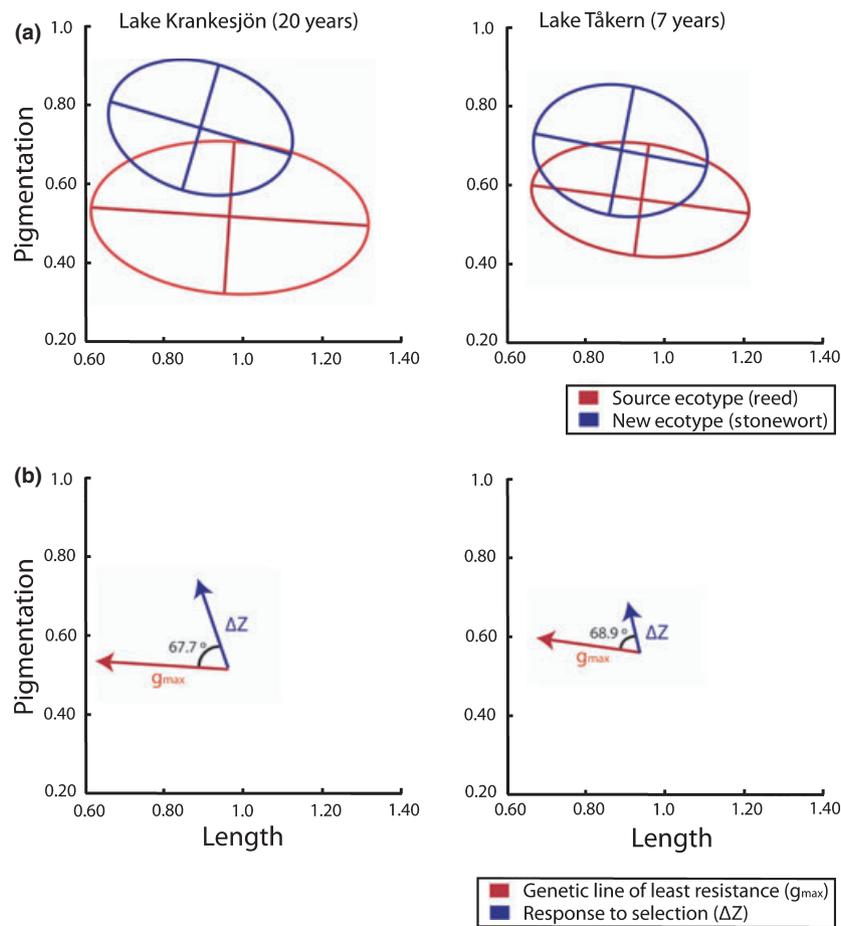
### G-matrix comparisons

Heritability estimates and maternal effects have already been discussed in our previous study, although we reaffirm that all traits we have studied are significantly heritable in all the four study populations (Fig. 1; Eroukhmanoff *et al.*, 2009b). We found only weak and nonsignificant maternal effects for all traits (Eroukhmanoff *et al.*, 2009b). It is thus reasonable to assume that differences between ecotypes in maternal effects are unlikely to have influenced divergence in their covariance structures (Roff, 2000). The four estimated **G**-matrices obtained are presented in Appendix S1. These matrices have diverged in terms of shape, orientation and size (Table 1; Fig. 2). It is especially important to notice that the eccentricity of **G**, which is an estimate of overall shape, has almost changed by a factor of two in the stonewort ecotypes of both lakes. Isopods from the stonewort habitat are smaller and brighter in pigmentation than reed isopods (Hargeby *et al.*, 2004; Eroukhmanoff *et al.*, 2009a). These parallel changes in both lakes have apparently not been in the direction of  $\mathbf{g}_{\max}$ , the axis of maximum genetic variation (Fig. 2). The deviation of the vector of phenotypic differentiation (or response to selection in the new ecotype) from  $\mathbf{g}_{\max}$  amounts to almost 70° in both lakes, when projecting on the bivariate plan of length and pigmentation brightness. The pairwise comparisons and their associated statistics are shown in Fig. 3.

Although the reed **G**-matrices were significantly different from each other, they both had all their seven principal components in common (Fig. 3). However, the matrices have changed in general structure in the stonewort habitat, and the **G**-matrices in stonewort

**Table 1** Comparisons of the shape and orientation of **G** in the reed source ecotypes and the new colonized stonewort ecotypes.  $\theta$  refers to the angle between the first eigenvector  $\mathbf{g}_{\max}$  and the *X*-axis (orientation) and  $\epsilon$  to the eccentricity of **G** (shape), which is the ratio between the first and the second eigenvalues. Change refers to the difference in **G**-parameter (in %) after the colonization of the stonewort habitat by the reed isopod populations within each lake.

Trait	Population		
	Ancestral (Reed)	Novel (Stonewort)	Change (%)
<b>G</b> -matrix divergence	Lake Krankesjön (20 years or 40 generations)		
Orientation ( $\theta$ , °)	69.2338	59.6183	–13.88
Eccentricity ( $\epsilon$ )	3.3595	1.7915	–46.67
	Lake Tåkern (7 years or 14 generations)		
Orientation ( $\theta$ , °)	61.7697	66.8837	8.28
Eccentricity ( $\epsilon$ )	3.7153	2.0638	–44.45



**Fig. 2** Divergence in genetic variance–covariance structure exemplified by the projected covariance ellipsoids of  $\mathbf{G}$  on the length and brightness axis (a). In each lake, the size, the eccentricity and the orientation of  $\mathbf{G}$  changed following the new habitat selective pressures (the ellipses corresponding to  $\mathbf{G}$  in the reed ecotype are larger and flatter in both lakes). The eigenvectors and the eigenvalues of each matrix were used to plot  $\mathbf{G}$ , which were centred through the respective population means for length and pigmentation brightness ( $V$ ) and represent 95% confidence ellipses. Note that for illustration, these ellipses are plotted on a bivariate plane but are part of a multivariate data set and consequently should not be entirely elliptical, although they are here shown as such for illustrative purposes. (b) The response to selection following colonization for pigmentation brightness and length compared with the axis of maximum variation ( $\mathbf{g}_{\max}$ ) or the genetic lines of least resistance. In both lakes, evolution has proceeded against  $\mathbf{g}_{\max}$ . The angle between  $\mathbf{g}_{\max}$  and  $\Delta\mathbf{Z}$ , when projected on the bivariate plan of length and pigmentation brightness, is similar in both lakes and close to  $70^\circ$ , indicating that the direction followed by the evolution is almost orthogonal to  $\mathbf{g}_{\max}$ . These values are also close to the same angles when considering all trait dimensions ( $64^\circ$  for Lake Krankesjön and  $74^\circ$  for Lake Tåkern), underscoring the importance of pigmentation brightness [a trait shown to be under strong divergent selection between ecotypes (Eroukhmanoff *et al.*, 2009b)] and length [a trait known to have role in mate choice (Bertin & Cezilly, 2003), mate guarding (Karlsson *et al.*, 2010b) and reproductive isolation between ecotypes (Hargeby & Erlandsson, 2006)].

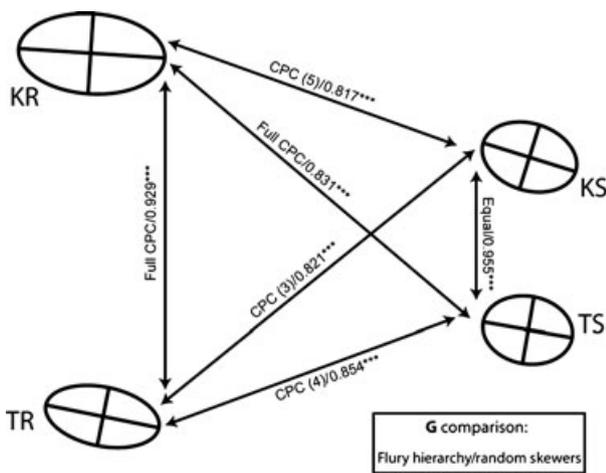
ecotype in each lake presented a very similar eigenstructure (Fig. 3, right).

It is important to keep in mind that these six pairwise comparisons are not statistically independent. We also performed an additional CPC comparison by simultaneously including all four  $\mathbf{G}$ -matrices. We found that they all shared some degree of similarity (five principal components in common) although they were certainly not identical. Another issue with CPC analyses is that they can lead to results biased towards higher similarities under low sample sizes; however, given the high number of individuals and families we used in our design, this

problem seems unlikely here (Mezey & Houle, 2003). The random skewers approach confirmed this picture, and the two stonewort  $\mathbf{G}$ -matrices were almost identical (vector correlation: 0.955), whereas the  $\mathbf{G}$ -matrices comparisons between ecotypes revealed a lesser degree of vector correlation (values ranged between 0.817 and 0.854) (Fig. 3).

#### Decrease and redistribution of the genetic variation

The average coefficient of additive genetic variation ( $CV_A$ ) has decreased in the newly established stonewort

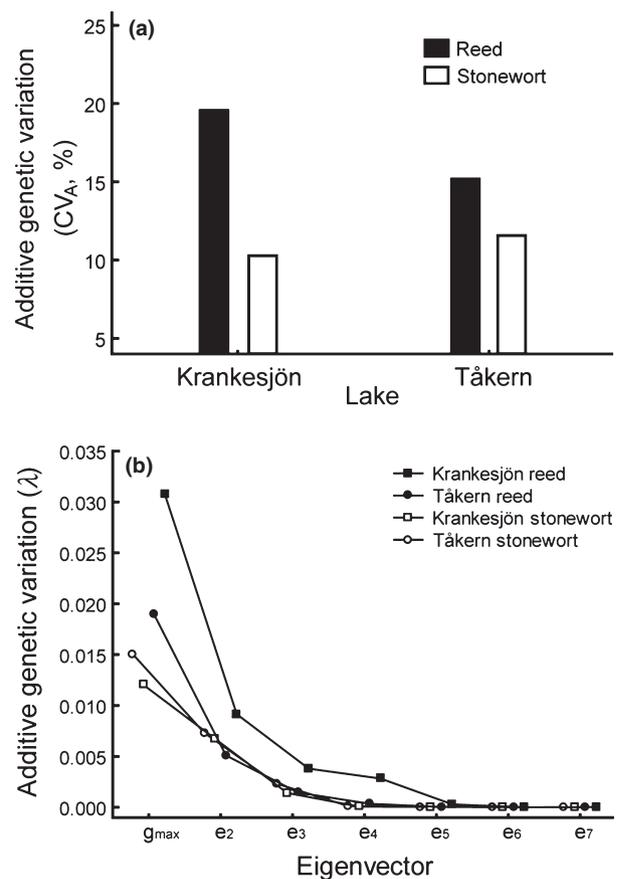


**Fig. 3** Pairwise comparisons of the genetic variance–covariance matrices of the four study populations, using both the common principal component analysis based on the Flury Hierarchy and the random skewers method. Population Abbreviations: KR, Krankesjön Reed; TR, Tåkern Reed; KS, Krankesjön Stonewort; TS, Tåkern Stonewort. \*\*\*The skewer correlation was significant ( $P < 0.001$ ).

ecotype (Fig. 4). However, genetic variation decreased more in Lake Krankesjön (46.5%) than in Lake Tåkern (22.1%). The total amount of additive genetic variance (the sum of all the eigenvalues) was lower in the stonewort populations (Fig. 4). On average, the additive genetic variance has decreased by 44% after colonization of the stonewort habitats. However, this decrease did not affect all the axis of variation in the same way. Most of this decrease in genetic variation occurred along the first eigenvector ( $\mathbf{g}_{\max}$ , the axis of maximum genetic variation) (Fig. 4b). The amount of genetic variation along  $\mathbf{g}_{\max}$  was lower in the stonewort ecotype in both lakes (60.8% of the genetic variation for  $\mathbf{g}_{\max}$  was lost in Lake Krankesjön and 20.5% in Lake Tåkern).

## Discussion

Cases of rapid parallel evolution in between two different ecotypes provide excellent opportunities to evaluate the role of quantitative genetic variation and the possible constraining effect of genetic architecture as a bias on evolutionary divergence (Eroukhmanoff, 2009). Our recent work has confirmed that these two different ecotypes are under divergent selection for at least three traits (all pigmentation traits) of the seven quantitative traits that are included also in the current study (Eroukhmanoff *et al.*, 2009b). Our recent work also suggested that divergent selection is likely to be weaker or non-existent on the size-related traits (Eroukhmanoff *et al.*, 2009b), although it is important to underscore the low statistical power in such studies when using such indirect inferences of selection based on comparisons between phenotypic and molecular divergence (Whitlock, 2008).



**Fig. 4** Decrease in additive genetic variance following the colonization of the new stonewort habitat. (a) The average additive genetic variation for all seven traits studied for the four study populations estimated with the coefficient of additive variation ( $CV_A$ , in %). The average additive genetic variation has decreased by 44% in the newly established stonewort ecotypes. (b) The respective amount of additive genetic variation associated with the eigenvectors of the  $\mathbf{G}$ -matrices for the four study populations. The total magnitude of genetic variation (the sum of all the eigenvalues) has significantly decreased in the stonewort habitat (on average by 30%). The amount of additive genetic variation that was associated with the first eigenvector,  $\mathbf{g}_{\max}$ , the axis of maximum genetic variation, has decreased considerably in the new stonewort ecotypes (on average by 41%).

Our previous work has also revealed a very limited role of historical contingency during this diversifying process (Eroukhmanoff & Svensson, 2009; Eroukhmanoff *et al.*, 2009a). For instance, there is a remarkably strong parallelism between the lakes in ecotype divergence in terms of sexual and nonsexual behaviour, physical performance, predator avoidance and morphology (Eroukhmanoff & Svensson, 2009; Eroukhmanoff *et al.*, 2009a). Other recent studies have also reported cases of limited role for historical contingency during older and more long-term diversification processes (Langerhans &

DeWitt, 2004; Vermeij, 2006). Genetic constraints on adaptive divergence are generally thought to be stronger in the early stages of divergence, and it should therefore be easier to detect a historical signature on quantitative genetic architecture in younger systems like these isopod populations (cf. Schluter, 1996).

Furthermore, the effects of genetic constraints on evolution might sometimes be transient. McGuigan *et al.* (2005) showed that in some cases, evolution could be biased by genetic constraints in populations that have been diverging for relatively long periods. Here, we have showed that divergence between ecotypes has been rapid, and there is no clear historical imprint of  $\mathbf{G}$  in the reed populations on phenotypic divergence in the recently invaded stonewort populations. Moreover,  $\mathbf{G}$  might have been influenced by the change in habitat-specific pressures that are quite similar across both lakes, because similar novel stonewort ecotype populations seem to share a similar covariance structure (Fig. 3). A previous study involving cave and surface populations of amphipods also found remarkable similarities in the genetic correlations of important sets of traits between different populations occupying the same habitat, although in that study, the genetic correlations of other traits also exhibited a strong historical signature (Jernigan *et al.*, 1994). Thus, this and a few other systems support the idea that the stability of  $\mathbf{G}$  might be strongly influenced by local ecology and evolve fast, which is in line with recent suggestions from more phenotypically oriented studies (Berner *et al.*, 2008, 2009).

$\mathbf{G}$  might of course not only influence the future response to selection but also is also likely to have been shaped by past selective pressures. Thus,  $\mathbf{G}$  might, at least under certain conditions, be moulded by selection and could therefore 'store' some long-term information about past environments and historical selection pressures (Eroukhmanoff, 2009). In this particular case, the stonewort ecotype populations in both Lake Tåkern and Lake Krankesjön are derived from reed populations, which experience different selective pressures in terms of predators than the novel stonewort habitat (Hargeby *et al.*, 2004; Eroukhmanoff & Svensson, 2009; Eroukhmanoff *et al.*, 2009b). Recent studies in both natural populations (Barrett *et al.*, 2008) and laboratory evolution experiments (Teotonio *et al.*, 2009) have suggested a role for standing genetic variation in rapid adaptive divergence, in contrast to the more classical and widely recognized scenario where adaptation is mainly fuelled by selection on novel mutations (Feldman *et al.*, 2009; Linnen *et al.*, 2009). Our findings of lower genetic variance along  $\mathbf{g}_{\max}$  in the recently invaded stonewort habitat (Fig. 4b) is certainly consistent with adaptation being mainly driven by standing genetic variation that was abundant already in the reed environment, especially in the light of the short time frame of these changes, i.e. a few decades (Hargeby *et al.*, 2004). Clearly, more empirical work is needed to determine whether

and to what extent  $\mathbf{G}$  could accurately be used to predict future responses to selection. If  $\mathbf{G}$  can change over the short time frame of a few dozen generations (<40 generations in our case), caution should certainly be used when using retrospective selection analyses to infer the strength of divergent selection when comparing populations that have been isolated for a long time (e.g. Dudley, 1996).

We have demonstrated that  $\mathbf{G}$  can change rapidly in terms of its size, shape and orientation in the context of colonization and local adaptation of a new environment, whereas strong similarities can be found across population belonging to the same ecotype and living in the same habitat. This finding adds to the increasing empirical evidence that  $\mathbf{G}$  might not be as stable as previously thought (Doroszuk *et al.*, 2008), not even over short (ecological) timescales. These results also suggest that short-term evolutionary divergence might not necessarily be biased towards  $\mathbf{g}_{\max}$ , the genetic line of least resistance (Schluter, 1996). Indeed, we found that not only has  $\mathbf{G}$  changed its orientation and shape after colonization in both lakes (Fig. 4a), but the response to selection ( $\Delta\mathbf{Z}$ ) is also almost orthogonal to  $\mathbf{g}_{\max}$  (Fig. 4b). This result suggests that the influence of  $\mathbf{G}$  might be much more limited than previously thought (Merila & Björklund, 1999), and adaptation to novel environments might not be as severely constrained by genetic architecture, or at least not in this system.

It is also important to underscore that the genetic correlations between length and pigmentation brightness, although all negative, have decreased in the stonewort ecotypes in both lakes (Appendix S1). Isopods from the stonewort have become smaller and lighter (Figs 1 and 2), presumably as a result of selection for increased crypsis to avoid detection from visually hunting predators like fish (Eroukhmanoff & Svensson, 2009). This negative correlation between brightness and body length has previously also been documented in a number of lakes and ponds across habitats at the phenotypic level (Hargeby *et al.*, 2005). This parallel and repeated strengthening of a negative genetic correlation when invading a novel habitat is certainly consistent with some role for correlational selection (Jernigan *et al.*, 1994). For instance, strong predation from visually hunting fish in the novel stonewort habitat is likely cause correlational selection between colour and size, as predators are known selective agents that often drive correlational selection (Sinervo & Svensson, 2002). Strong correlational selection might alter the eigenstructure of  $\mathbf{G}$ , as it has been theoretically shown under certain conditions (Revell, 2007).

Size-related traits also show high levels of genetic integration in all populations, most of their genetic correlations are close to one (Appendix S1). It is possible that these strong genetic correlations simply reflect the effects of pleiotropy and general size factors affecting overall morphology. These correlations could also have

been further strengthened and maintained by correlational selection to preserve high swimming functionality (e.g. escaping predators, Eroukhmanoff & Svensson, 2009). Genetic and phenotypic covariances could thus be conserved across species and populations because of long-term correlational selection (Zeng, 1988), perhaps because closely related species and populations inhabit similar environments, i.e. niche conservatism (Wiens, 2004). Moreover, mate choice is dependent on both size and shape in this system (Bertin *et al.*, 2002; Bertin & Cezilly, 2003; Hargeby & Erlandsson, 2006) and might influence mate-guarding behaviour (Karlsson *et al.*, 2010b). Thus, it is possible that size-related traits and the way they covary with each other might be under stabilizing or correlational sexual selection, and the correlations have subsequently been maintained and modified during ecotype divergence.

One additional factor that might have led to **G** similarity in eigenstructure in the stonewort ecotypes is migration and gene flow between populations (Guillaume & Whitlock, 2007). However, we have shown in a previous study that the emergence of the stonewort ecotypes and their subsequent phenotypic divergence has occurred independently in both lakes (Eroukhmanoff *et al.*, 2009a). Although our mtDNA haplotype network revealed some level of migration between the lakes and the reed populations, this was less clear for stonewort populations (Eroukhmanoff *et al.*, 2009a). Thus, although we cannot completely rule out some role for gene flow between any of these populations, gene flow unlikely explains the similarities in **G**-matrix structure in the stonewort ecotypes of these two geographically well-separated lakes (Lake Tåkern and Krankesjön). It is more plausible that the similarities between the two stonewort populations we found here is largely a result of shared and parallel correlational selection, which favoured similar trait combinations in the two different stonewort populations (Eroukhmanoff & Svensson, 2009).

Following colonization, a population can become locally adapted through recruitment of genetic variation in two ways: either through novel mutations (Feldman *et al.*, 2009; Linnen *et al.*, 2009) or through standing genetic variation (Barrett *et al.*, 2008). Local adaptation from standing genetic variation is expected to be more rapid because pre-existing alleles will be present at a higher frequency and have a larger fitness advantage than alleles generated from mutations. Moreover, such pre-existing alleles have already been filtered through past selective pressures in previous environments (Schluter *et al.*, 2004; Barrett & Schluter, 2008; Teotonio *et al.*, 2009). This might even lead to rapid ecological speciation if ecological divergence is accompanied by reproductive isolation (Schluter & Conte, 2009). One expected signature of this kind of process is a decrease in genetic variation following adaptation (Barrett & Schluter, 2008). Although we found no such decrease

for neutral molecular genetic variation (Eroukhmanoff *et al.*, 2009a), in the present study we found that the additive genetic variation for the seven phenotypic traits has decreased by nearly 50% in the stonewort habitat compared with the reed habitat (Fig. 4). The hypothesis that adaptation was enabled by standing genetic variation is also corroborated by previous findings that suggested phenotype sorting (Eroukhmanoff *et al.*, 2009a) and high rates of evolution and strong divergent selection on several pigmentation traits (Eroukhmanoff *et al.*, 2009b). These findings might suggest that relatively few pre-existing alleles in the reed populations might have increased in frequency in the stonewort because they were locally beneficial in this novel habitat. As a result, the dimensionality of **G** was altered and genetic variation has become redistributed along different trait combinations. The consequences for quantitative genetic architecture from selection on standing genetic variation has been theoretically investigated by Agrawal *et al.* (2001), but we are not aware of any other empirical studies that have addressed the question of the stability of **G** in this particular ecological and genetic context. The results in the present study suggest that selection on standing genetic variation might affect the size, the orientation and the eccentricity of **G**. In return, these changes in **G** might further diminish its constraining effects on adaptation. The short time frame over which the eigenstructure of **G** has diverged in both lakes could therefore be explained by the amount of standing variation that was reduced during adaptation. More empirical work specifically testing this hypothesis is definitely needed in the future.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Genetic variance–covariance matrices of the four study populations for seven traits (length, width 1, 4 and 7, H, S and V).

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