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Stability of genetic variance and covariance for reproductive characters in the face of climate change in a wild bird population

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Abstract

Global warming has had numerous effects on populations of animals and plants, with many species in temperate regions experiencing environmental change at unprecedented rates. Populations with low potential for adaptive evolutionary change and plasticity will have little chance of persistence in the face of environmental change. Assessment of the potential for adaptive evolution requires the estimation of quantitative genetic parameters, but it is as yet unclear what impact, if any, global warming will have on the expression of genetic variances and covariances. Here we assess the impact of a changing climate on the genetic architecture underlying three reproductive traits in a wild bird population. We use a large, long-term, data set collected on great tits (*Parus major*) in Wytham Woods, Oxford, and an 'animal model' approach to quantify the heritability of, and genetic correlations among, laying date, clutch size and egg mass during two periods with contrasting temperature conditions over a 40-year period (1965–1988 [cooler] vs. 1989–2004 [warmer]). We found significant additive genetic variance and heritability for all traits under both temperature regimes. We also found significant negative genetic covariances and correlations between clutch size and egg weight during both periods, and among laying date and clutch size in the colder years only. The overall G matrix comparison among periods, however, showed only a minor difference among periods, thus suggesting that genotype by environment interactions are negligible in this context. Our results therefore suggest that despite substantial changes in temperature and in mean laying date phenotype over the last decades, and despite the large sample sizes available, we are unable to detect any significant change in the genetic architecture of the reproductive traits studied.

Keywords: animal model, climate change, genetic correlation, heritability, *Parus major*, quantitative genetics

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Introduction

The increasing human population, as well as the expansion and nature of its activities, are causing environmental changes at an unprecedented speed at many levels (IPCC 2001, 2007). Among the most important changes identified recently is global warming, which has had numerous effects on populations of animals and plants, especially in temperate regions (Parmesan & Yohe 2003; Root *et al*. 2003; Walther *et al*. 2005; reviewed in Parmesan 2006). However, while the effects on populations and ecosystems resulting from climate change are frequently assessed (for examples, see O'Reilly *et al*. 2003 and Post & Forchhammer 2004), its impacts on the evolutionary processes that generate and maintain biodiversity have received relatively little attention (but see Etterson & Shaw 2001 and Davis *et al*. 2005). This is surprising given that extinction risk is tightly linked to a species' evolutionary potential and plasticity, and that species with low adaptive potential may therefore have little chance of persistence in the face of rapid environmental changes (Burger & Lynch 1995; Lande & Shannon 1996; Etterson 2004).

The evolutionary potential of a given trait is dependent on the selection acting on it and on its underlying genetic variation, but also depends to a large degree on its genetic

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relationships with other selected traits (Lande & Arnold 1983; Roff 1997). For example, genetic covariance with another trait, due either to pleiotropy or linkage disequilibrium, can affect the expected response to directional selection acting on a trait either by constraining, or even accelerating, its potential rate of response (see Roff 1997). One of the first assessments of the importance of such a process in wild populations was made by Schluter (1996) who confirmed, using an empirical framework, that the direction of evolution could be modified and potentially constrained by the patterns of genetic covariance (see also Blows & Higgie 2003). Hence measuring **G**, the matrix of additive genetic variances and covariances corresponding to a set of diverse traits (Lande 1979), is central to our understanding of evolution, because it summarizes the pattern and strength of genetic constraints that act on a population's evolutionary potential (Cheverud 1984). However, despite its importance for predicting the evolutionary potential of a population, little is known about the stability of the **G** matrix in natural populations (Steppan *et al*. 2002).

There is evidence that the expression of genetic variance (V_A) might vary depending on environmental conditions (reviewed in Hoffmann & Merila 1999; Merilä & Sheldon 2001; Charmantier & Garant 2005). Although in general there seems to be a trend for a reduction in magnitude of V_A under poorer conditions, in many individual cases the support for such a difference is unclear because of a lack of reported standard errors and required statistical tests (see Charmantier & Garant 2005). In addition, the environment also seems to influence genetic covariances and correlations, although most of the evidence that this is the case comes from artificial (laboratory) conditions (see Sgrò & Hoffmann 2004 for a review). Although these studies show that the sign and magnitude of genetic correlations is potentially environment-dependent (for examples, see Kause *et al*. 2001 and Messina & Fry 2003), the possible lack of concordances between estimates obtained in controlled and wild habitats (for an example, see Conner *et al*. 2003) make generalizations about the direction of the change in covariance difficult.

There have been even fewer assessments of the influence of the environment on the **G** matrix in a single free-ranging population (though see Cano *et al*. 2004 for an experiment under controlled conditions). As a result, the extent to which one could detect a change in **G** matrices in a single wild population, or the degree to which it could potentially vary with the environment, remains largely unknown, although it is likely to require large sample sizes to achieve any statistical power (Steppan *et al*. 2002; McGuigan 2006). This is an important gap in our understanding, since laboratory-based and environment–specific estimates of genetic variance and covariances will generate predictions for a trait's evolution that are only valid for particular environments. Consequently, we need a more detailed assessment of the potential to detect changes in **G** in the wild and across different environments before we can draw conclusions about the utility of estimating **G** for measuring evolutionary potential (Pigliucci 2006).

This study aims to improve our understanding of the potential changes in genetic architecture of traits in wild populations in the face of changing environmental conditions. We use an animal model approach to directly assess quantitative genetics parameters in the wild. We believe that this method offers an interesting alternative to controlled experiments, especially when aiming to assess the effects of environmental changes on natural populations. We report an extensive analysis of the patterns of quantitative genetic variation in three female reproductive traits (laying date, clutch size and egg weight) in a large population of great tits (*Parus major*) breeding in Wytham Woods, Oxford, UK. Reproductive traits are ideal for study in this context because: (i) they are environmentally responsive (for examples, see Crick *et al*. 1997; McCleery & Perrins 1998; Nussey *et al*. 2005), and (ii) they are often under strong natural selection (for examples, see Sheldon *et al*. 2003; Garant *et al*. 2007). Over the last few decades, breeding individuals in this population have experienced changing temperature regimes, with the average mean spring temperature during the period before and during egg-laying now being around 2 °C warmer than it was 40 years ago (Fig. 1A). Such warming has resulted in the phenological advancement of vegetation (Menzel *et al*. 2001), which in turn affects timing of the caterpillar larvae that are important for these birds as food for their offspring (Visser *et al*. 1998; Buse *et al*. 1999; Visser & Holleman 2001). The reproductive timing of the birds is closely linked to the temperature (Fig. 1B) and, as a result, great tits in Wytham are now on average laying their first egg more than 12 days earlier than four decades ago (see Fig. 1C; see also McCleery & Perrins 1998). This change in the environment and the corresponding change in the phenotype, combined with a large pedigree and extensive reproductive data, thus provide an excellent opportunity to assess the potential changes in the genetic architecture of reproductive traits under contrasting environments.

Materials and methods

Study species and data collection

We used data obtained from the long-term study of the great tit population in Wytham Woods, Oxford, UK (for more details, see Perrins 1965; Perrins & McCleery 1989; McCleery *et al*. 2004). All breeding attempts in nest boxes are monitored from the date of first egg laid until all nestlings have fledged, by regular visits to each nest box. All nestlings (on day 15 after hatching) and adults (during

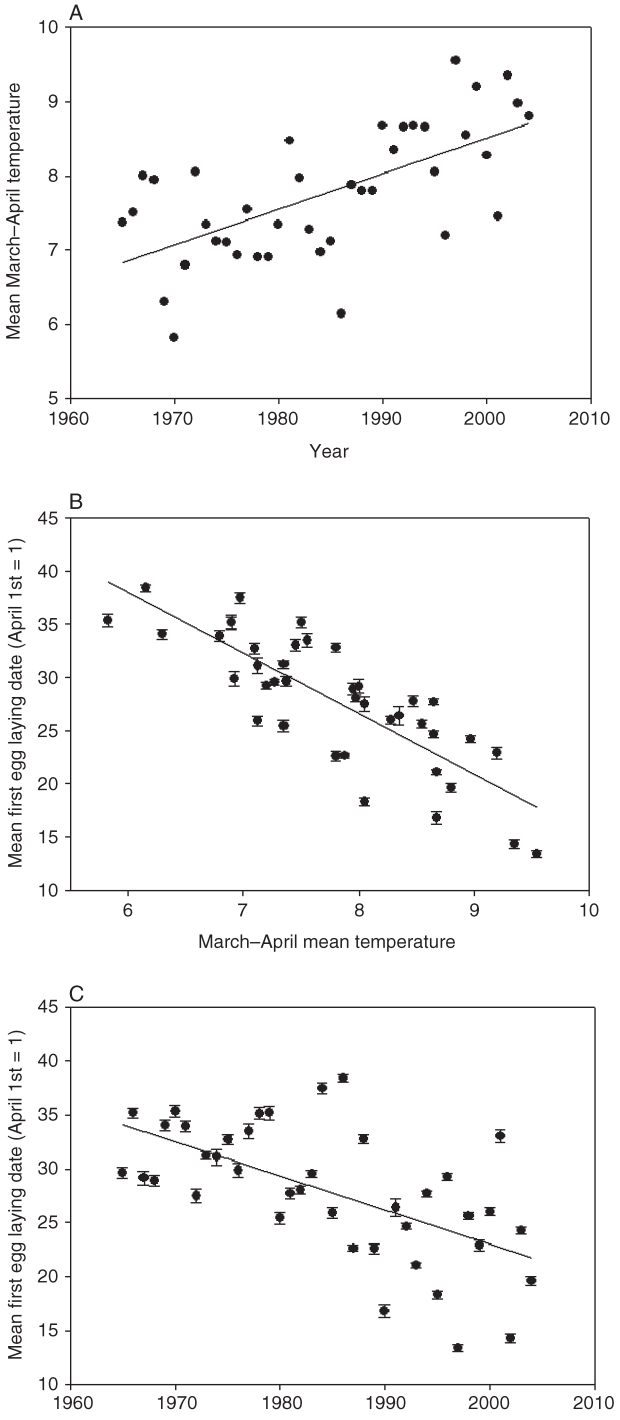

Fig. 1 Climate change and reproductive timing. (A) Mean spring (March–April) temperature increased by almost 2 °C from 1965 to 2004 (effect: 0.0479 ± 0.0095 °C/year, $t_{38} = 5.06$, $P < 0.001$); (B) The reproductive timing (mean first egg laying date) of female great tits in Wytham is largely dependent on the spring temperature (r^2: 0.66, effect: –5.70 ± 0.66 day/°C, $t_{38} = 8.70$, $P < 0.001$); (C) The observed trend of the mean first egg laid in Wytham over the last four decades: females are now laying on average more than 12 days early (day/year: –0.3167 ± 0.0684, $t_{38} = 4.63$, $P < 0.001$).

the breeding season or in the winter) are marked with individually numbered aluminium rings. This allowed us to build pedigrees for the quantitative genetic analysis (for more details, see Garant *et al*. 2004, 2005). Here we specifically used data for (i) laying date (expressed as number of days since 31 March, so that 1 April = 1); (ii) clutch size (number of eggs); (iii) mean egg weight (total egg mass in grams divided by the number of eggs in the clutch, for a sample of at least three eggs laid at the start of the clutch) for each female in each year from 1965 to 2004. Possible second clutches and repeat clutches laid after failure of the first clutch were removed from the data set (following procedures outlined in Garant *et al*. 2004, 2005, 2007). In total, data for laying date and clutch size were available for 6932 breeding attempts involving 4642 females and data for egg weight were obtained for 5945 breeding attempts and 4110 females. Our pedigree consisted of 10 332 adults (biparental links) spanning 32 generations (number of generations per lineage ± SD: 6.6 ± 7.3).

To assess the change in temperature over time and its effect on the genetic architecture of reproductive traits, we used spring temperature, defined as the March–April mean air temperature (°C). Temperature data for Oxford (51°45′N, 1°15′W) were obtained from a station of the Met Office at the following website: http://www.met-office.gov.uk/climate/uk/stationdata/oxforddata.txt. We choose to analyse temperature effects on **G** under two contrasting sets of conditions while aiming to use similar sample sizes in both environments. We therefore analysed data collected during the following two periods: (i) 1965–1988 and (ii) 1989–2004. The mean spring temperature (as defined here) during the latter period (8.5 °C ± 0.2 SE) was warmer than the spring temperature of every year forming part of the former period, and the mean spring temperature of the former period (7.3 °C ± 0.1 SE) was colder than the spring temperature of every year that was part of the latter period (except for 1996, mean = 7.2 °C) (Fig. 2A). Thus, the difference between the average spring temperature in the two periods is both highly statistically significant ($t_{38} = 5.95$, $P < 0.001$, Fig. 2A), but also represents a quite marked shift in the mean environment over a few decades. The mean annual temperature (calculated over 12 months and also obtained from the Oxford Met Office weather station) was also significantly warmer in the 1989–2004 (10.7 °C ± 0.1) than in the 1965–1988 period (9.7 °C ± 0.1) ($t_{38} = 6.63$, $P < 0.001$) (see Fig. 2B). Finally, the mean winter North Atlantic oscillation (NAO) index (see Hurrell 1995) was significantly higher in recent years (1965–1988 mean: –0.039 ± 0.39, 1989–2004: 1.45 ± 0.58, $t_{38} = 2.21$, $P = 0.034$), which is indicative of warm moist weather. The mean spring (1 March–30 April) temperature as defined here differs slightly from the measure used previously to describe spring temperature environment with respect

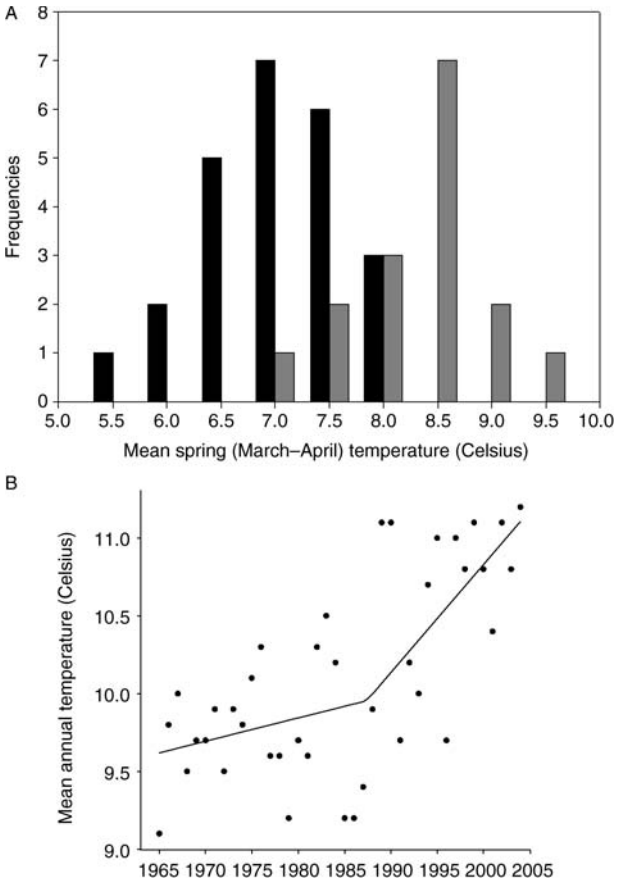

Fig. 2 Two contrasted temperature regimes. (A) Mean spring temperature (March–April daily average) for the 1965–1988 period (black) and the 1989–2004 period (grey). Values on the *X*-axis indicate the lower bound of the given temperature category. (B) Mean annual temperature change over time illustrated using a split-line regression. The breakpoint is 1988. The slope before the breakpoint is nonsignificantly different from zero (0.0150 ± 0.0151 °C/year) while the slope after the breakpoint is significantly positive (0.0693 ± 0.0235 °C/year).

to this population (see McCleery & Perrins 1998), which is the 'warmth sum': the sum of daily maxima from 1 March to 25 April in each year. However, these two values are very closely correlated (*r*: 0.91), and the separation into contrasting blocks of years ('environments') — the main focus of this study — would be the same for both measures. Although we are aware that other variables might have potentially changed over the study period, here we specifically assume a functional link between the change in phenology and the observed change in temperature.

Quantitative genetic analysis

Previous work in this population has showed that all three traits are heritable (McCleery *et al*. 2004). In this study, we extend this work by including more than twice as many individuals in our analyses, and more specifically by focusing on the changes in the variance–covariance matrix **G** with respect to the temperature regimes (environments) as defined above. We used our pedigree to fit animal models (Lynch & Walsh 1998; as reviewed in Kruuk 2004) with the software ASREML (version 1.1, Gilmour *et al*. 2002). For each temperature regime, we decomposed phenotypic (co)variances into their additive genetic (V_A) and other fixed and random components. Animal ID, which account for permanent environmental variance (V_{PE}) via the repeated measures on individual birds across successive years, and year (V_Y) were included as additional random effects, and age (categorical: 1 year old or older) was included as a fixed effect. The presence of maternal effects was also assessed by including the maternal identity in the model, but as this component of variance was very small, and never statistically significant, results are presented excluding maternal effects. The narrow-sense heritability (h^2) for each environment was estimated as the ratio of the additive genetic variance (V_A) to the total phenotypic variance (V_P): $h^2 = V_A/V_P$. Genetic correlations were calculated as: $r_A = COV_{Aij}/(V_{Ai}V_{Aj})^{0.5}$. Standard errors for variance and covariance components, as well as for heritabilities and genetic correlations, were computed by ASREML (Gilmour *et al*. 2002). The statistical significance of each additive genetic component was tested by rerunning a constrained model where either the variance or the covariance were set to be equal to zero, and then comparing the difference in log-likelihood ratio between the original and the constrained model, against the chi-square distribution, where $\chi^2 = -2$ *difference in log likelihood. Finally, we estimated the genetic covariances (and correlations) across temperature regimes by conducting bivariate analyses in which the trait value under cold temperatures was considered a different trait from that measured in the warm temperature period. We tested each covariance for significance using the method described above.

Matrix comparisons

To test whether the (co)variance matrices differed generally between the two time periods, we fitted two models to the data: one in which the (co)variances were free to vary across the time periods, and one in which the (co)variance estimates were consistent between time periods. This was done in turn for each random effect (genetic, permanent environment and year), and in all cases the (co)variances for the remaining random effects were pooled across time periods but the error residual (co)variances were free to vary between environments. The comparison is similar to the maximum-likelihood method of Shaw (1991), although the two matrices are not treated as independent, as pedigree links exist between the two time periods. In addition to this general test, we also fitted a model in which the

Table 1 Summary of animal model analyses on reproductive traits depending on the temperature period analysed: (A) 1965–1988: data for 2450 females, 3575 breeding events (only 3111 for EGGW); (B) 1989–2004: data for 2285 females, 3357 breeding events (only 2834 for EGGW). Clutch size (CS), laying date (LD) and egg weight (EGGW). Variance components: additive (V_A), permanent environment (V_{PE}), year (V_Y), residual (V_R) and total phenotypic variance (V_P), heritability (h^2). Standard errors in parentheses. Means are trait means for the period and are given in number of eggs (CS), days (LD) and grams (EGGW) and provided with their standard deviation (SD)

	Means (SD)	V_A	V_{PE}	V_Y	V_R	V_P	h^2
(A) 1965–1988							
CS	8.8 ± 1.7	0.79 (0.12)	0.30 (0.11)	0.48 (0.15)	1.16 (0.047)	2.73 (0.16)	0.29 (0.05)
LD	31.0 ± 7.1	7.57 (1.48)	3.74 (1.50)	15.29 (4.56)	21.42 (0.86)	48.02 (4.64)	0.16 (0.03)
EGGW	1.69 ± 0.13	0.0082 (0.0010)	0.0025 (0.0009)	0.0005 (0.0002)	0.0070 (0.0003)	0.0182 (0.0006)	0.45 (0.06)
(B) 1989–2004							
CS	8.6 ± 1.6	0.68 (0.12)	0.53 (0.11)	0.23 (0.09)	1.13 (0.05)	2.58 (0.11)	0.26 (0.05)
LD	22.8 ± 7.6	5.43 (1.36)	8.89 (1.43)	28.09 (10.29)	15.68 (0.66)	58.10 (10.34)	0.09 (0.03)
EGGW	1.67 ± 0.13	0.0082 (0.0009)	0.0006 (0.0008)	0.0002 (0.0001)	0.0069 (0.0003)	0.0159 (0.0005)	0.51 (0.06)

correlational structures of the matrices are identical but the variances differ by a constant. This is equivalent to the common principal component test of proportionality (Phillips & Arnold 1999). Using likelihood ratio tests, each model was compared to a model in which the residual covariance matrices were free to vary between the time periods, but the random covariance matrices were estimated across time periods. The model with varying residual covariance matrices was tested against a model with a single common residual covariance matrix.

Although these methods give *P* values associated with the null hypotheses, they provide little indication of effect sizes, and it is hard to say whether a failure to reject the null hypothesis is because effect sizes are small, or whether power is low, or both. To address this, we also calculated the angle between the dominant eigenvectors of the (co)variance matrices estimated for each population, and also the proportional difference in the sum of the eigenvalues. For three traits, comparing the angle between the dominant eigenvectors is equivalent to Krzanowski's comparison of subspaces (Krzanowski 1979) and is a measure of eigenstructure differences. The sum of the eigenvalues is a measure of the overall genetic variance for the three traits. For both tests, the phenotypic data were scaled to unit variance to avoid the problem of scale effects (Hadfield *et al*. 2007). Standard errors for the angles and sum of the eigenvalues are not available as appropriate bootstrap procedures for data connected through an arbitrary pedigree are hard to define (Shao 1996).

Results

Variance components and heritability of reproductive traits

All traits showed significant additive genetic variance and heritability under both temperature regimes (Table 1). Element-by-element comparisons, using *t*-tests, of the additive variance components revealed no significant differences among temperature regimes for any of the traits (Table 1; all $P > 0.29$). There was a significantly higher permanent environment component of variance for laying date in the warm period than in the cold ($t_{6930} = 2.49$, $P = 0.01$); the effect is quite large, with the size of this variance component more than doubling. The overall phenotypic variance was rather similar for egg weight and clutch size in the two environments, but was slightly (although not significantly) increased for laying date under warmer years due to larger permanent environment and year components of variance (Table 1).

Genetic covariances and correlations among traits

We found a negative and significant genetic covariance among laying date and clutch size in the cold period (Table 2; $\chi^2_{(1)} = 10.20$, $P = 0.001$). This relationship was much weaker and not statistically significant under warmer conditions (Table 2; $\chi^2_{(1)} = 1.80$, $P = 0.18$). In contrast, the genetic covariance among clutch size and egg weight

Table 2 Genetic correlations (below) and covariances (above) obtained from animal models in (A) 1965–1988 (B) 1989–2004, for clutch size (CS), laying date (LD) and egg weight (EGGW). Values in bold are significant

	CS	LD	EGGW
(A) 1965–1988			
CS	X	**–1.03 (0.32)**	**–0.017 (0.008)**
LD	**–0.42 (0.11)**	X	0.026 (0.028)
EGGW	**–0.22 (0.10)**	0.10 (0.11)	X
(B) 1989–2004			
CS	X	–0.41 (0.29)	**–0.015 (0.008)**
LD	–0.21 (0.14)	X	0.036 (0.026)
EGGW	**–0.21 (0.10)**	0.17 (0.12)	X

Table 3 Tests for matrix equivalence and proportionality for each random effect. Each model was tested against the model in which only the residual effects were free to vary between time periods

	Test for matrix equivalence		Test for matrix proportionality	
Random effect	Log-likelihood	Probability, d.f.	Log-likelihood	Probability, d.f.
	5205.83			
Residual	–5192.03	< 0.001, 6		
Residual + genetic	–5187.49	0.17, 6	–5191.75	0.45, 1
Residual + PE	–5187.50	0.17, 6	–5190.95	0.14, 1
Residual + year	–5186.37	0.08, 6	–5191.46	0.29, 1

d.f., degree of freedom; PE, permanent environment.

was significant during both the cold ($\chi^2_{(1)} = 4.36$, $P = 0.04$) and warm ($\chi^2_{(1)} = 3.84$, $P = 0.05$) periods (see Table 2). Covariances among laying date and egg weight were not significant (both $P > 0.15$). Element-by-element comparisons, using *t*-tests, revealed no significant differences among temperature regimes for any genetic covariances (all $P > 0.15$), even if the genetic covariance between laying date and clutch size during the warm years was only 40% of that during the cold years.

Cross-environment genetic correlations

Genetic correlations across environments were significantly different from zero, but not different from unity, for clutch size ($r_A = 1.20 \pm 0.20$) and also for egg weight ($r_A = 0.85 \pm 0.16$), which suggests that there is no genotype–environment interaction for the covariance between these traits, and hence that the same genes affected these traits under both temperature conditions. Similarly, the laying date cross-environment correlation was also not significantly different from 1 ($r_A = 0.65 \pm 0.39$), but was also not different from zero, perhaps due to the larger standard error associated with this character.

Matrix comparisons among temperatures

There was little evidence that the matrices differed generally, or by a proportional constant, for any random effect ($P > 0.08$), although a model in which the residual (co)variances were allowed to vary over time periods was significantly better ($P < 0.001$) than one in which residual (co)variances remained constant (Table 3). However, the magnitude of this effect was not large and the high significance may be largely attributable to the precision with which error variances can be estimated in such a large data set.

We also calculated the proportional change in dispersion at the level of each random effect for the multivariate phenotype. In all cases the proportional change was less than 15%. The angle between the dominant eigenvectors of **G** estimated for the two time periods was also small (6.6°) suggesting that the large *P* value was associated with a small effect size (Table 4, Fig. 3). The angles between the dominant eigenvectors for the other random effects were larger (21°–56°), suggesting that a failure to find significant differences in these cases may be a consequence of low power (Table 4).

Table 4 Angles between the dominant eigenvectors, and the ratio of the summed eigenvalues of each random covariance matrix estimated for each time period. The angles are in degrees, and the numerator of the ratio is the summed eigenvalues of the matrices estimated for the first (cold period). The matrices were estimated using a model in which all covariances were free to vary across time periods

Random effect	Angle between dominant eigenvectors	Ratio of the sum of eigenvalues
Residual	23.8°	1.09
Genetic	6.6°	1.09
PE	56.0°	0.86
Year	21.1°	0.86

PE, permanent environment.

Discussion

Genetic architecture in a changing environment

We used quantitative genetics analyses and a long-term data set to try to improve our understanding of the stability of the genetic architecture of traits in wild populations in the face of a rapidly changing environment. We found that all traits under investigation showed significant additive genetic variance under both temperature regimes and could thus potentially respond to natural selection. Comparisons of the (co)variance matrices revealed no significant differences among temperature regimes. Although, some element-by-element comparisons of the matrices showed quite large differences, the general magnitude of any difference was small (Fig. 3). Our results therefore suggest that

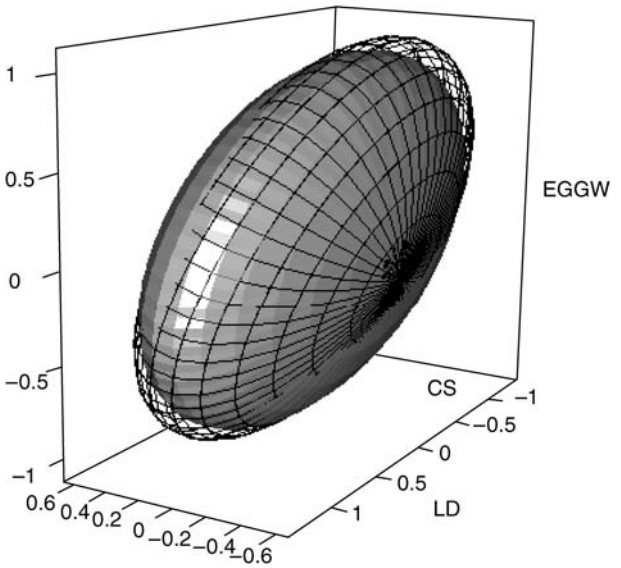

Fig. 3 Matrix comparisons among temperatures. Each ellipsoid represents the genetic variance for the three traits in each time period: filled surface represents warm years and wire-framed surface is for cold years. The ellipsoids can be thought of as bounding a space containing 95% of the expected breeding values in a hypothetical population.

despite the change in temperature and in mean breeding time phenotype, and despite the large sample sizes available, there is no clear evidence for a significant change in the genetic architecture of reproductive traits in this population over the study period.

Previous experiments, under controlled conditions, have suggested that **G** could vary with the environment. For example, Cano *et al*. (2004) assessed the impact of different desiccation regimes (three treatments) on the genetic basis of development traits for *Rana temporaria* tadpoles originating from two populations. While the largest differences were found between populations, presumably due to selection, they also found some evidence for a significant difference in **G** matrices between desiccation treatments within populations, suggesting genotype-by-environment interactions (Cano *et al*. 2004). Previously, Bégin & Roff (2001) compared morphological traits for two species of crickets for which they manipulated breeding conditions (temperature, light). They found changes in **G** among treatments for *Gryllus firmus* but not for *Gryllus pennsylvanicus*. Also, Kraft *et al*. (2006) showed that the amount of genetic variance for body size was increased by almost an order of magnitude in green frogs (*Rana lessonae*) larvae growing in an environment simulating the presence of predators (dragonfly larvae) as compared to a predator-free environment. The study also showed that the first genetic principal component of body size measurements was significantly different among habitats and that the across-environment genetic correlation for this principal component was lower than 1, suggesting different genetic patterns of development and potentially independent evolutionary responses across environment for body size (Kraft *et al*. 2006).

There are several possibilities as to why our results differ from those documented in these previous studies. First, the fact that these studies were all performed in a controlled environment obviously points to the likely lack of resemblance between estimates of **G** obtained with artificial breeding designs vs. **G** obtained by sampling a natural population, which is a problem rarely addressed (Pigliucci 2006). For example, most studies in the laboratory use individuals maintained under much contrasted environments and using few generations, which might generate quite different responses from the more gradual change over many generations (26 in our case) that occurred in our population. Furthermore, it should be noted that since we analysed data obtained in a natural population, other environmental variables than temperature may have changed over the study period, some of which potentially influence any genotype by environment relationships in contrasting ways. Also there is the potential discordance in the accuracy of methods used to assess matrix difference. Common principal component (CPC) is the method usually chosen to perform comparisons among matrices in previous studies (reviewed in Steppan *et al*. 2002). However, as underlined by Houle *et al*. (2002; see also Blows & Higgie 2003), there are many concerns with using CPC for comparing **G** matrices. For example, CPC seems to generally underestimate the degree of structure that matrices share, potentially resulting in the conclusion that data sets are unrelated, even when a relationship does exist between them (see Houle *et al*. 2002).

Genetic relationships among reproductive traits

Laying date and clutch size are very commonly studied traits in birds, yet surprisingly, this is to our knowledge only the fourth time that the genetic correlation between these traits has been reported. First, Sheldon *et al*. (2003) studied a population of collared flycatchers (*Ficedula albicollis*) from Gotland (Sweden) and reported a significant negative correlation among these traits ($r_A = –0.41 \pm 0.09$) which was very similar in magnitude to our estimate under cold temperatures (Table 2). On the other hand, Gienapp *et al*. (2006) found no significant genetic correlation ($r_A = –0.01 \pm 0.12$) between laying date and clutch size in another population of great tits from the Netherlands. Lastly, Charmantier *et al*. (2006a) reported that the genetic correlation between clutch size and laying date in mute swans (*Cygnus olor*) was nonestimable, because of the absence of genetic variance for laying date (for further discussion, see Charmantier *et al*. 2006b). Interestingly, in the light of these variable findings, our results suggest that

this genetic correlation might be the most susceptible to change with the environment and that such change might occur despite little parallel change at the phenotypic level (1965–1988: $r_P = -0.27 \pm 0.02$, 1989–2004: $r_P -0.26 \pm 0.02$).

Here we also report for the first time a negative correlation between egg weight and clutch size in birds. The demonstration of the presence of such negative genetic correlation is somewhat consistent with the phenotypic correlation observed in some studies, although the general pattern at this level is not striking (reviewed in Christians 2002; $r_P \pm$ SE in our study data set: -0.082 ± 0.015). A negative genetic relationship between these traits is also in line with expectation from life-history theory of a trade-off between propagule number and size (Stearns 1992). Such findings help improve our understanding of each trait's evolutionary potential. For example, there is limited evidence for a link between egg weight and fitness, and hence of selection on this trait (reviewed in Williams 1994), but our results suggest that egg weight might influence the multivariate evolutionary response indirectly via its negative link with clutch size, which itself is usually under selection (see Garant *et al*. 2007). We also showed that this genetic correlation remained stable independently of the temperature context. Again, few studies conducted in the wild are available for comparisons, but a previous study of the seed beetle (*Stator limbatus*), conducted in the laboratory, showed that a negative genetic correlation between egg size (measured as length) and fecundity was only observed in habitats where larval survival was lower (Czesak & Fox 2003).

Conclusion

Our analyses suggest that despite the change in temperature observed over the last 40 years, and the impact it has had on mean phenotype in this population, there is no evidence that the genetic architecture of reproductive traits has been affected. This suggests that, all else being equal (for example, similar selection intensity for all traits over the study period and/or no variable genetic correlations with other traits of importance), the evolutionary potential response of these reproductive traits has remained largely unchanged. The currently observed change at the phenotypic level is likely to reflect individual plasticity, which allows individuals to display an adaptive response to climate change in this population. It is however, unclear to which extent phenotypic plasticity alone will allow individuals to cope with the projected increase in temperature (IPCC 2007).

While our study represents the most powerful test for a change in **G** yet performed for a wild population, we call for more studies of this kind, using large data sets collected on wild populations to assess the generality of our findings. Analyses of changes in **G**, for reproductive and other traits, could be performed in other long-term studies for which animal models are applicable such as red squirrels (*Tamiasciurus hudsonicus,* Réale *et al*. 2003) and bighorn sheep (*Ovis canadensis*, see Wilson *et al*. 2005), other bird species such as collared flycatchers (*Ficedula albicollis*, Sheldon *et al*. 2003) or mute swans (*Cygnus olor*, Charmantier *et al*. 2006a, b), and other studies of great tits (see Gienapp *et al*. 2006) for examples. Another potential future improvement to our approach would be to model a continuous change in variance components using random regression (for example, see Wilson *et al*. 2005), rather than a simple test among contrasted categories, as we employed. While this would be interesting to explore further with our data and with the different long-term data sets, it should also be noted that assessing continuous variance components might come at the cost of supposing a particular form of functional relationship between an individual's additive genetic effect and time. The accuracy of the parameter estimates will depend on the validity of this supposed relationship (see Wilson *et al*. 2005). More detailed explorations and comparisons of various relationships need to be performed before we could apply such approach to our data set.

In any case, we hope that our work has partly improved our understanding of potential genetic changes of wild populations and of factors allowing individuals to face rapidly changing conditions. Gathering information of potential genetic changes of wild populations and of factors allowing individuals to face rapidly changing conditions across many species and environments should help us to develop the management practices and policies that are required to maintain the genetic and ecological integrity of natural populations. Such adjustment and integration of information becomes crucial in light of the impact of humans on evolution (see Palumbi 2001). As it seems unlikely that our impact on species and ecosystems will decline in the coming decades, gaining a better knowledge about the process of evolution is a measure we need to take to understand and potentially alleviate the evolutionary changes we inflict on species.

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