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The effect of temperature and wing morphology on quantitative genetic variation in the cricket *Gryllus firmus*, with an appendix examining the statistical properties of the Jackknife–MANOVA method of matrix comparison

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Abstract

We investigated the effect of temperature and wing morphology on the quantitative genetic variances and covariances of five size-related traits in the sand cricket, *Gryllus firmus*. Micropterous and macropterous crickets were reared in the laboratory at 24, 28 and 32 °C. Quantitative genetic parameters were estimated using a nested full-sib family design, and (co)variance matrices were compared using the *T* method, Flury hierarchy and Jackknife–MANOVA method. The results revealed that the mean phenotypic value of each trait varied significantly among temperatures and wing morphs, but temperature reaction norms were not similar across all traits. Micropterous individuals were always smaller than macropterous individuals while expressing more phenotypic variation, a finding discussed in terms of canalization and life-history trade-offs. We observed little variation between the matrices of among-family (co)variation corresponding to each combination of temperature and wing morphology, with only one matrix of six differing in structure from the others. The implications of this result are discussed with respect to the prediction of evolutionary trajectories.

Introduction

Genetic variation is central to evolutionary biology theory because it represents the material on which evolutionary forces act. Phenotypic evolution can be modelled using a family of multivariate models that are a function of the **G** matrix, the matrix of additive genetic variances and covariances corresponding to a suite of quantitative traits (Lande, 1979; Arnold *et al.*, 2001). These models typically assume that the structure of **G** remains constant during phenotypic evolution (Lande, 1979; Turelli, 1988). However, laboratory experiments have shown that factors such as directional selection (Beniwal *et al.*, 1992; Shaw *et al.*, 1995; Blows & Higgie, 2003), random genetic drift (Phillips *et al.*, 2001), and mutation (Camara & Pigliucci, 1999; Camara *et al.*, 2000), can potentially alter **G** matrices, thereby modifying the evolutionary potential of a population through time. Additionally, the expression of genetic variation may be environment-dependent (e.g. Mazer & Schick, 1991; Bennington & McGraw, 1996; Donohue *et al.*, 2000), thus leading to different evolutionary outcomes across environments for a given population and a given selection regime. The impact and relative importance of these factors for long-term **G** matrix variation in nature are not well understood (reviewed in Steppan *et al.*, 2002). It is therefore crucial to study the extent to which **G** matrices vary, so that knowledge of this variation can be used to improve the current models of phenotypic evolution. This paper focuses on two factors that may induce **G** matrix variation within a population of field crickets: temperature and wing dimorphism.

Thus far, investigations of the variation of quantitative genetic parameters within a population have mainly looked at the effect of a stressful environment, and have typically studied the additive genetic variance

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of individual traits. The results have been equivocal, with increases, decreases and no changes being observed, and several hypotheses being advanced to explain each type of result (reviewed in Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999; Imasheva, 1999). The lability of genetic covariances has been studied in the same context and, as with variances, no consistent pattern has emerged (Stearns *et al.*, 1991), although relatively few studies are available for review (e.g. Service & Rose, 1985; Loeschcke *et al.*, 1999; Karan *et al.*, 2000). Very little theoretical work exists on the change of quantitative genetic parameters along an environmental gradient. First, the genetic correlation's potential for change across environments has been verbally modelled in the context of the evolution of life history traits. It has been proposed, although reliable data on the matter are scarce, that genetic correlations between life history traits are highly plastic along an environmental gradient (Clark, 1987; Stearns, 1989). Secondly, only a few mathematical models of the environmental sensitivity of quantitative genetic parameters have been proposed (de Jong, 1990; Gavrilets & Scheiner, 1993; de Jong & Imasheva, 2001). These showed that changes across environments may be expected as a generality. However, because the link from genetic details to phenotypic variation is mostly unknown, the assumptions of these models are extremely difficulty to verify. Additional investigations are therefore needed to better understand the effect of the environment on genetic parameters.

In addition to this general lack of conclusive information, an important limitation is that many studies have used a univariate approach to the investigation of the effect of the environment on genetic variation (e.g. Imasheva *et al.*, 1998; Bubliy *et al.*, 2000). Describing a change in the genetic variance of a trait across environments does provide useful information on the variation in the magnitude of this parameter, but not on the variation of the structural relationship of this trait to the rest of the organism (i.e. genetic covariances). Phenotypic evolution is inherently multivariate, because natural selection targets fitness, and thus the response to selection is dictated by the additive genetic variances and covariances of all the traits correlated with fitness. It is therefore important to use a more inclusive approach and to look at the lability of **G** matrices across environments using statistical tools that allow whole matrix comparisons. Very few studies have taken this approach so far (Holloway *et al.*, 1990; Guntrip *et al.*, 1997; Donohue *et al.*, 2000; Bégin & Roff, 2001).

Variation in temperature is ubiquitous in nature and affects many aspects of the life of any organism. One such aspect is morphological development, often studied using adult body size in animals. Body size is an evolutionarily important trait because of its crucial role in the life history and ecology of animals (e.g. Peters, 1983; Calder, 1984; Roff, 2002a). The effect of temperature on body size is relatively well characterized, especially in arthropods (Atkinson, 1994), but much less is known about the effect of temperature on the quantitative genetic variation of size-related traits. Such studies have most frequently looked at thorax length and wing length in a few species of the fruit fly genus *Drosophila* (e.g. Tantawy, 1961; Scheiner *et al.*, 1991; Bubliy & Loeschcke, 2001). A frequent conclusion is that the magnitude of the genetic variances or covariances of these traits vary across the thermal tolerance range of these species, but patterns tend to be population- or species-specific (e.g. Barker & Krebs, 1995; Noach *et al.*, 1996; Imasheva *et al.*, 2000). The present study is the first to investigate the environmental sensitivity of a **G** matrix (as opposed to individual variances or covariances) along a temperature gradient. Our study organism is a population of *Gryllus firmus*, a wing dimorphic field cricket collected in Florida. We used growth chambers to rear these crickets at three temperatures (24, 28 and 32 °C) that cover an important part of the range under which *G. firmus* can develop without diapausing (personal observation). None of these temperatures can be considered extreme for *G. firmus*, as inferred from the temperatures they encounter in the field (Veazey *et al.*, 1976) and from a temperature preference experiment (Roff & Shannon, 1993).

In addition to environmental variation, some biological factors may be associated with variation in **G** matrices within a population. For example, the **G** matrices of the two sexes, or of the various classes of any other polymorphism, may differ and hence entail different evolutionary potentials for the two morphs. A common polymorphism in field crickets is wing dimorphism (Alexander, 1968; Walker & Sivinski, 1986). Micropterous individuals (hereafter also referred to as SW for short-winged) possess short hind wings and a nonfunctional flight apparatus, whereas macropterous individuals (or LW for long-winged) possess longer wings and can typically fly. Wing morphology is a threshold trait under polygenic control in *G. firmus* (Roff, 1986a, 1990a) and in many other insects (Roff, 1986b), and has been abundantly studied because of its connection with other life-history traits (Harrison, 1980; Roff & Fairbairn, 1991, 2001; Zera & Denno, 1997; Bégin & Roff, 2002). Because of the fecundity advantage of the micropterous morph (see references above), the proportion of the two wing morphs in a population is expected to change with time; macropterous individuals are likely to be the founders of new populations because of their ability to fly, whereas micropterous individuals become progressively more numerous with time once the population is established. If the **G** matrices of the two morphs are not identical, evolution occurring at different stages of the colonization process might produce different outcomes. In this study, we investigated the effect of wing morphology, temperature and their interaction on the **G** matrix variation

corresponding to five size-related traits in the sand cricket, *G. firmus*.

Materials and methods

Experimental protocol and measurements

The sand cricket *G. firmus* is distributed along the south-eastern coast of the USA (Alexander, 1968). The population used in the current study was sampled near Gainesville, FL, USA, in 1998. Wild individuals were brought into the laboratory and kept as a free mating stock of approximately 100–300 individuals at room temperature (approximately 22 °C), for five to seven generations. Because of various constraints, families could not be reared simultaneously under all three temperatures (24, 28 and 32 °C), and hence the crickets used in the three temperature treatments come from three consecutive stock generations. For each temperature treatment, a random sample of emerging nymphs was taken from the stock population to be used as the parental generation of full-sib families. Parents were reared in buckets of 40 individuals with unlimited access to food (rabbit chow) and water. Buckets were kept in a growth chamber at 28 °C and at a photoperiod of 15 h of light and 9 h of darkness (15L : 9D). After the emergence of most adults, virgin male–female pairs were randomly formed and isolated in plastic containers with food, water, and a moist earth dish for oviposition. Upon hatching, 80 nymphs were collected from each parental pair and divided between two 4-L plastic buckets, at a density of 40 nymphs per bucket. Full-sib families were reared at either 24, 28 or 32 °C and at a photoperiod of 15L : 9D (sample sizes are given in Table 1). Adults were preserved in alcohol within 2 days after eclosion. Because *G. firmus* is a sexually dimorphic species, and to facilitate comparison with previous studies, only females were measured.

Five morphological measurements were made on each female offspring: femur length (FEMUR), head width (HEAD), prothorax length (PTHL), prothorax width (PTHW), and ovipositor length (OVIP). These five traits are related to overall size. An analysis of measurement error using a subsample of each trait revealed that the repeatability of each trait was over 98% (measured as the proportion of the total variance explained by the among-individual component; Falconer & Mackay, 1996). All measurements were transformed using the natural logarithm (ln), which was successful in removing the correlation between trait mean and phenotypic variance. Deviations of the trait distributions from normality were minimal, and multivariate outliers were rare and not very distant from the centroids.

Table 1 Sample sizes corresponding to each combination of temperature and wing morphology. 'LW' refers to long-winged individuals (macropters) and 'SW' to short-winged individuals (micropters).

	No. of families	No. of individuals
24 LW	56	533
24 SW	45	262
28 LW	62	862
28 SW	41	315
32 LW	60	715
32 SW	32	133

The estimation of quantitative genetic parameters for the five morphological traits was based on a nested ANOVA/ANCOVA, with family and cage-nested within family as independent variables (having two cages nested within each family allows to correct for common family environmental effects; Roff, 1997, pp. 41–43). For a discussion of the assumptions underlying this quantitative genetic model, see next paragraph. A Jackknife procedure (Manly, 1997, pp. 24–33), in which each family was deleted once to produce a population of samples, was implemented to estimate parameters; a (co)variance was estimated as the average of the corresponding Jackknife pseudovalues, and the standard error (SE) was estimated as the SE of these pseudovalues. The number of Jackknife iterations was equal to the number of families. The Jackknife has been shown through simulations to produce accurate estimates of mean and SEs for heritabilities (Simons & Roff, 1994) and genetic correlations (Roff & Preziosi, 1994). We compared the estimates of the Jackknife with estimates from a Bootstrap procedure (Efron & Tibshirani, 1986) and found that estimates obtained from the two re-sampling approaches were similar (results not shown).

Quantitative genetic assumptions

Predictive models in quantitative genetics are based on additive genetic variances and covariances (the **G** matrix; Lande, 1979; Arnold *et al.*, 2001). By definition (Falconer & Mackay, 1996; Roff, 1997), full-sib estimates of quantitative genetic parameters include the additive genetic component of variance, but are also contaminated mainly by a part of the dominance and epistasis variance and by maternal effects (family environmental effects are here corrected for by our use of two cages per family). Among-group differences in our full-sib estimates of **G** could therefore reflect variation in any of these components. However, we have several lines of evidence that indicate that the effects of dominance and maternal effects are low for these traits in field crickets. First, Crnokrak & Roff (1995) have shown that morphological traits typically express little dominance variance. In addition, Roff (1998) has shown that, in the case of the trait femur length in *G. firmus*, estimates of heritability from a full-sib design (0.37), a half-sib design (0.34) and a parent–offspring regression (0.45) were very similar. This suggests that the dominance and maternal effects are

low because, if they were not, the full-sib estimate would be expected to be larger than the other two. Similarly Roff (1998) and Réale & Roff (2003) showed that head width in *G. firmus* suffered very little inbreeding depression, which implies low levels of dominance variance (Falconer & Mackay, 1996; Roff, 1997; Lynch & Walsh, 1998). Moreover, a diallel analysis of femur length and other leg measurements in inbred lines of *G. firmus* showed that dominance variance was significant but accounted on average for only 5% (restricted maximum likelihood) or 11% (Griffing model) of the phenotypic variance (Roff & Réale, 2004). This range of value is not very large considering that our full-sib variance components account for 28–86% of the total variation, with a median of 40%. Maternal effects have also been shown to be of minor importance in adult morphological traits in inbred lines of *G. firmus*. Roff & Réale (2004) found that maternal effects in leg measurements explained on average 12% of the phenotypic variance but were mostly nonsignificant and Roff & Sokolovska (2004), using a larger sample size, found that the contribution of maternal effects in head width was on the order of 1% and was nonsignificant. Taken together, these results suggest that only a small portion of our full-sib estimates of variance is attributable to dominance or maternal effects. However, the importance of these other sources of variation could not be investigated in the current study and we will therefore conservatively refer to our estimates as among-family (co)variances instead of additive genetic (co)variances (**G**).

A second important assumption is that our laboratory estimates of among-family (co)variances are good surrogates for **G** matrices in nature, or at least that the laboratory environment does not systematically deform **G** matrices. This has been previously addressed in a study of *G. pennsylvanicus*, a sister species of *G. firmus* (Bégin & Roff, 2001). This study of morphological traits consisted in a comparison of laboratory and field estimates of among-family (co)variances, and showed no important differences between the two types of environment.

Matrix comparison

Several different matrix comparison methods exist, but little is known about their properties and merits (Steppan *et al.*, 2002). In this study, we used and compared the results of three different methods.

The T method

The *T* method, developed by Roff *et al.* (1999), uses matrix disparity as an index of difference between two matrices. It is similar to the method suggested by Willis *et al.* (1991) and discussed by Steppan (1997). The method is based on the sum of element-by-element absolute differences between two matrices and tests the hypothesis that two matrices are equal, by calculating $T_{12} = \sum_{i=1}^{c} |M_{i1} - M_{i2}|$, where M_{i1} and M_{i2} are the estimates of the *i*th element of each of the two matrices and *c* is the number of nonredundant elements in the matrix (sum of the number of diagonal elements plus the number of elements above the diagonal). The probability that the two matrices come from the same statistical population is estimated by a randomization procedure (4999 iterations) in which families are randomly assigned to the groups being compared, and quantitative genetic parameters estimated for each iteration. The probability is estimated as $P = (n + 1)/(N + 1)$, where *n* is the number of iterations in which the *T* from the randomized data set is greater than or equal to that obtained from the original data set and *N* is the total number of iterations (the '+ 1' is to account for the original estimate). The randomization procedure sets the mean and SD to 0 and 1, respectively, for each trait in each randomized data set. To provide a more intuitively interpretable statistic, we present the *T*% statistic which estimates the average difference between the elements of two matrices as a percentage of the average size of the elements in these matrices:

$$T\%_{12} = \frac{T_{12}/c}{(\bar{M}_1 + \bar{M}_2)/2} \times 100,$$

where $\bar{M}_1$ and $\bar{M}_2$ are the averages of the elements of the two matrices. However, all statistical tests used *T*, not *T*%. Note that the *T*% statistic is unreliable when covariances of both signs are present (Steppan, 1997), but this was not the case in this data set.

The *T* method can be modified to provide information on the difference in magnitude between two matrices (i.e. testing whether the (co)variances of one matrix are larger overall than the (co)variances of the other matrix). For this, a signed version of the *T* method can be used: Signed $T_{12} = \sum_{i=1}^{c} (M_{i1} - M_{i2})$. A randomization procedure is then implemented as described above. Note that this procedure is only useful when all matrix elements are of the same sign, which is the case here.

The Flury hierarchy

The Flury hierarchy is a principal components approach to the comparison of matrices (Flury, 1988), whose application to **G** matrices was discussed by Cowley & Atchley (1992) and developed by Phillips & Arnold (1999). This method, based on maximum likelihood, determines which model is the best descriptor of the structural differences between two or more matrices. The hierarchically nested models are (i) 'Unrelated Structure': matrices have no eigenvector in common, (ii) 'Partial Common Principal Components': matrices share some eigenvectors, (iii) 'Common Principal Components': matrices share all eigenvectors, but not eigenvalues, (iv) 'Proportionality': matrices share all eigenvectors, and eigenvalues all differ by the same constant between matrices and (v) 'Equality': matrices share eigenvectors and eigenvalues. For each model, the Flury hierarchy calculates a log-likelihood statistic to

quantify the fit of that model to the observed matrices. A likelihood ratio is then calculated for each model against the model of 'Unrelated Structure' ('jump up' procedure, Phillips & Arnold, 1999). To avoid the assumption of multivariate normality in hypothesis testing and because the degrees of freedom are unknown under the null hypothesis, randomization is used to determine the probability that a model fits the data significantly better than the 'Unrelated Structure' model. In this analysis, 4999 randomized data sets were created, each iteration randomly assigning whole families to the groups being compared. The best fitting model (called verdict in the Results section) is determined as the model immediately under the first significant probability, going from the bottom ('Unrelated Structure' model) to the top ('Equality' model) of the hierarchy ('jump up' procedure, Phillips & Arnold, 1999). The randomization procedure sets the mean and SD to 0 and 1, respectively, for each trait in each randomized data set. This analysis was performed using the program CPCrand (Phillips, 1998). This program does not allow the nesting of cages within families and thus comparisons of matrices by the Flury hierarchy are potentially biased by common family environmental effects, unlike the results of the two other methods. However, comparisons of matrices using the *T* method suggested that there is no large difference between the results corresponding to the nested and non-nested designs (results not shown). Two studies have so far explored the properties of the Flury hierarchy (Houle *et al.*, 2002; Mezey & Houle, 2003).

*The Jackknife–*MANOVA *method*

This method, recently developed by Roff (2002b), makes use of the Jackknife procedure (Manly, 1997, pp. 24–33) and of the multivariate analysis of variance (MANOVA). The Jackknife is first used to produce a distribution of pseudovalues of matrix elements within each group. A pseudovalue is calculated by estimating a matrix element after deleting all individuals of one family, using the formula $\phi_{ij} = nM_i - (n-1)M_{i-j}$, where ϕ_{ij} is the pseudovalue of the ith matrix element corresponding to the deletion of family j, n is the number of families, M_i is the ith matrix element estimated for the observed data and M_{i-j} is the matrix element estimated for the data set minus the jth family. The number of pseudovalues calculated for a group is equal to the number of families. For a given family that has been removed, the pseudovalues corresponding to each matrix elements (15 elements in this case: five variances and 10 covariances) can be arranged in a row which will then constitute the pseudovalues of the whole matrix. Two or more matrices can then be compared using the pseudovalues as data in a MANOVA. The advantage of the Jackknife–MANOVA method is that it allows the investigation of the effect of any number of independent variables on **G** matrix variation.

The Jackknife approach to the estimation of heritabilities and genetic correlations has been verified by simulation (Roff & Preziosi, 1994; Simons & Roff, 1994) and thus it is reasonable to suppose that the Jackknife–MANOVA approach is valid. However, to verify that this is so we performed a simulation analysis that explored the type 1 error rate of the method. The results of this analysis are presented in the Appendix: they confirm that the statistical behaviour of the Jackknife–MANOVA method is appropriate. Additionally, Roff (2002b) showed that the results of the Jackknife–MANOVA method were similar to those of the Flury hierarchy with respect to the equality model.

Results

Mean trait values

We investigated the effects of wing morphology and temperature on the five phenotypic traits using a two-way MANOVA. This analysis revealed that wing morphology (Wilk's $\lambda = 0.87$, $F_{5,2810} = 81$, $P < 0.001$), temperature (Wilk's $\lambda = 0.55$, $F_{10,5620} = 193$, $P < 0.001$) and their interaction (Wilk's $\lambda = 0.94$, $F_{10,5620} = 17$, $P < 0.001$) all had a highly significant effect on morphology, and five independent ANOVAS indicated that this was also the case for each trait individually ($P < 0.001$ in all cases). These results revealed that there was significant variation in size across groups (the term group is here used to refer to a combination of temperature and wing morph). Maximum differences in mean phenotypic trait values between the smallest and largest groups were approximately 7% for FEMUR, HEAD, PTHL and PTHW, and 12% for OVIP. Temperature reaction norms generally differed across traits within a wing morph, and across wing morphs within a trait. However, the traits HEAD, PTHL and PTHW appeared to have relatively similar reaction norms, different from both FEMUR and OVIP (Fig. 1). The effect of wing morphology within a temperature was consistent across the five traits; LW individuals were almost always larger than SW individuals (Fig. 1).

Variances

We tested the effect of family and cage-nested within family on the five morphological traits using a nested ANOVA for each trait within each group. The family effect was significant in all cases (results not shown) and revealed that genetic variation was present in all combinations of temperature and wing morphology. The cage effect was statistically significant in approximately half of the cases, but never achieved more than half the predictive power of the family effect (results not shown). Phenotypic (co)variance matrices (**P**) and among-family (co)variance matrices are shown in Table 2. We plotted the elements of **P** matrices against

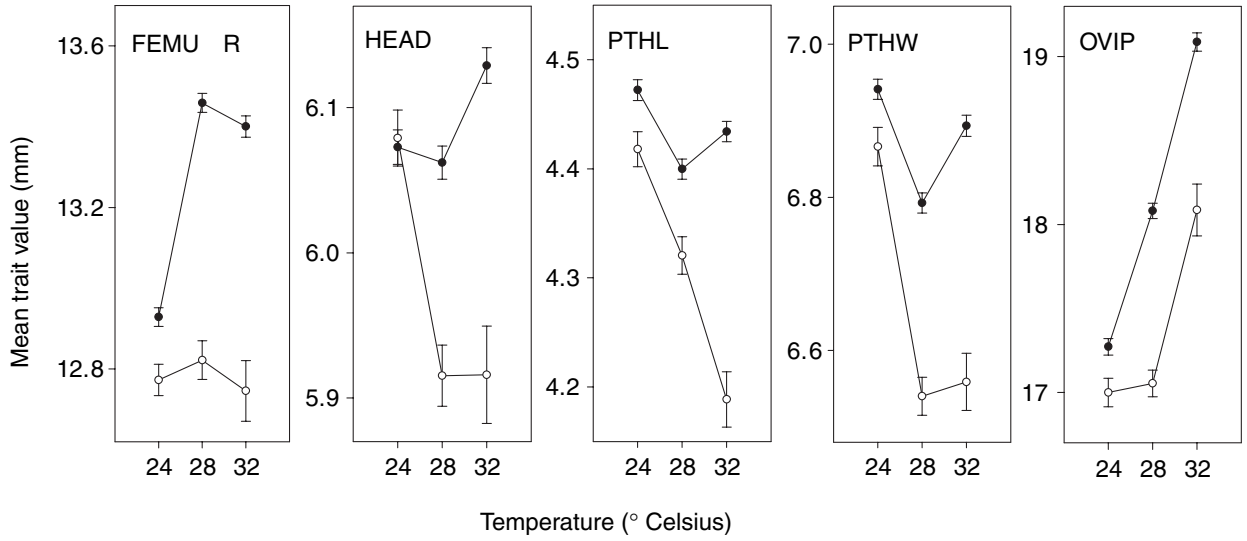

Fig. 1 Temperature reaction norms, ±1 SE. Black circles (●) represent macropterous individuals (LW), and white circles (○) represent micropterous individuals (SW). Data are not ln-transformed.

Table 2 Matrices of phenotypic (above) and genetic (below) variances/covariances corresponding to each of the six combinations of temperature and wing morphology. Matrix elements are followed by their SE and are multiplied by 1000. Calculations are based on ln-transformed data.

	24 LW	24 SW	28 LW	28 SW	32 LW	32 SW
FEMUR	1.76 (0.12)	2.51 (0.26)	2.70 (0.17)	4.58 (0.63)	2.90 (0.30)	4.97 (1.04)
	0.51 (0.18)	1.00 (0.28)	1.29 (0.27)	1.50 (0.86)	0.98 (0.35)	4.32 (1.46)
FEMUR-HEAD	1.53 (0.12)	2.19 (0.19)	2.46 (0.16)	3.38 (0.39)	2.39 (0.23)	4.21 (0.93)
	0.46 (0.17)	1.23 (0.30)	1.13 (0.27)	1.02 (0.73)	0.67 (0.36)	3.70 (1.35)
FEMUR-PTHL	1.53 (0.13)	2.13 (0.20)	2.60 (0.19)	3.71 (0.44)	2.42 (0.22)	4.49 (0.94)
	0.47 (0.19)	0.87 (0.48)	1.42 (0.35)	1.16 (0.80)	0.84 (0.35)	4.10 (1.27)
FEMUR-PTHW	1.45 (0.09)	1.66 (0.22)	2.54 (0.19)	3.62 (0.43)	2.36 (0.24)	4.24 (0.93)
	0.41 (0.18)	0.35 (0.44)	1.09 (0.28)	1.30 (0.84)	0.65 (0.36)	3.85 (1.32)
FEMUR-OVIP	1.88 (0.17)	2.94 (0.38)	2.57 (0.22)	3.59 (0.46)	2.87 (0.28)	5.48 (1.21)
	0.44 (0.25)	1.44 (0.47)	1.12 (0.43)	0.41 (0.85)	1.12 (0.46)	3.70 (2.20)
HEAD	2.08 (0.15)	2.71 (0.21)	3.11 (0.20)	4.07 (0.43)	3.00 (0.25)	4.61 (0.95)
	0.72 (0.19)	1.64 (0.39)	1.50 (0.33)	1.15 (0.73)	0.93 (0.42)	3.87 (1.51)
HEAD-PTHL	1.70 (0.13)	2.43 (0.26)	2.83 (0.21)	3.78 (0.49)	2.52 (0.23)	4.38 (0.91)
	0.57 (0.19)	1.47 (0.53)	1.47 (0.40)	1.18 (0.80)	0.73 (0.37)	3.71 (1.28)
HEAD-PTHW	1.67 (0.12)	2.01 (0.29)	2.88 (0.20)	3.93 (0.45)	2.63 (0.25)	4.22 (0.92)
	0.55 (0.18)	0.84 (0.46)	1.37 (0.33)	1.32 (0.84)	0.75 (0.41)	3.51 (1.41)
HEAD-OVIP	2.18 (0.20)	3.19 (0.41)	2.96 (0.24)	4.07 (0.57)	3.06 (0.28)	5.57 (1.19)
	0.66 (0.27)	2.13 (0.54)	1.28 (0.49)	0.83 (0.83)	1.05 (0.54)	3.99 (2.32)
PTHL	2.48 (0.15)	3.57 (0.38)	3.87 (0.28)	5.10 (0.56)	3.28 (0.24)	5.22 (0.89)
	0.83 (0.24)	1.84 (0.72)	2.27 (0.54)	1.80 (0.89)	1.15 (0.36)	4.28 (1.22)
PTHL-PTHW	1.58 (0.12)	2.35 (0.29)	2.98 (0.22)	4.24 (0.50)	2.50 (0.23)	4.38 (0.88)
	0.46 (0.19)	1.22 (0.48)	1.59 (0.41)	1.59 (0.91)	0.67 (0.36)	3.74 (1.21)
PTHL-OVIP	2.10 (0.22)	3.09 (0.46)	2.95 (0.27)	4.38 (0.67)	2.87 (0.27)	5.39 (1.16)
	0.59 (0.30)	1.95 (0.79)	1.49 (0.52)	0.88 (0.87)	1.11 (0.49)	3.39 (2.10)
PTHW	1.97 (0.13)	3.39 (0.64)	3.27 (0.22)	4.61 (0.50)	2.96 (0.25)	4.64 (0.90)
	0.57 (0.22)	1.40 (0.65)	1.51 (0.35)	1.87 (0.96)	0.86 (0.40)	3.98 (1.30)
PTHW-OVIP	1.95 (0.18)	2.75 (0.43)	2.86 (0.25)	4.31 (0.60)	2.76 (0.29)	5.39 (1.17)
	0.41 (0.28)	0.93 (0.65)	1.10 (0.50)	0.83 (0.99)	0.78 (0.52)	3.76 (2.21)
OVIP	4.61 (0.43)	7.03 (1.07)	5.54 (0.43)	7.03 (0.88)	6.04 (0.37)	11.01 (1.84)
	1.53 (0.48)	3.59 (1.27)	3.09 (0.85)	2.24 (1.04)	2.31 (0.69)	4.02 (4.88)

the corresponding elements of among-family (co)variance matrices, pooling the estimates from all six groups (Fig. 2). This graph revealed that, with the exception of the group 32SW, the two were linearly related despite a substantial scatter (note that the significance of this regression cannot be tested because of the part/whole

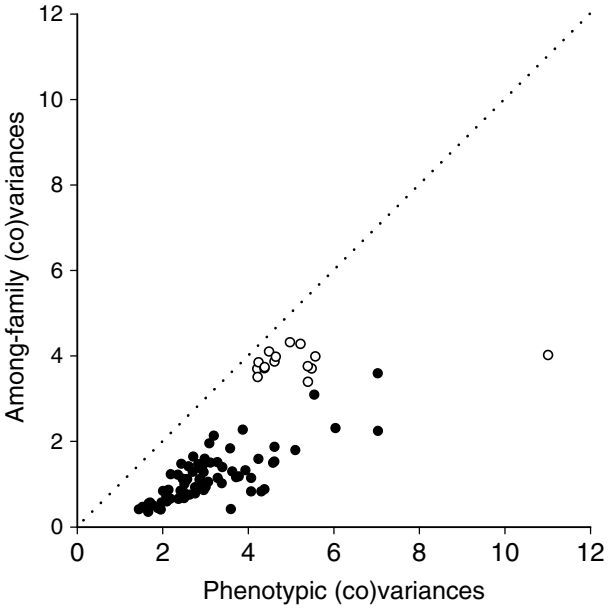

Fig. 2 Plot of among-family variances and covariances against their corresponding phenotypic (co)variances. This graph contains the estimates from all six combinations of temperature and wing morphology. White circles (○) refer to the matrix 32SW. The outlier is the variance of OVIP for the group 32SW. All (co)variances were multiplied by 1000. The dotted line is the 1 : 1 line.

relationship of the two variables). The important scatter and the peculiarity of the group 32SW therefore suggest caution with respect to the use of the **P** matrix as a surrogate for the among-family (co)variance matrix in this data set.

Effect of wing morphology on variances

The effect of wing morphology on the magnitude of phenotypic (co)variances (**P**) was investigated by comparing the matrices of the two morphs in each temperature separately, using the signed version of the *T* method. A strong pattern was observed in each temperature (Fig. 3a); SW individuals were more phenotypically variable than LW individuals ($P < 0.001$, <0.01 and <0.001 at 24, 28 and 32 °C respectively). To better understand this effect, we also compared the two wing morphs with respect to the genetic (among-family) and environmental ($\mathbf{E} = \mathbf{P} - \mathbf{G}$) components of phenotypic (co)variance. At the genetic level (Fig. 3b), SW individuals were more variable than LW individuals at 24 and 32 °C ($P < 0.05$ and <0.01, respectively), but did not differ at 28 °C (n.s.). As for the environmental (co)variations, no significant differences between the two morphs were detected (n.s., n.s. and $P = 0.07$ at 24, 28 and 32 °C respectively).

While the analysis described in the previous paragraph was restricted to a single temperature at a time, we now

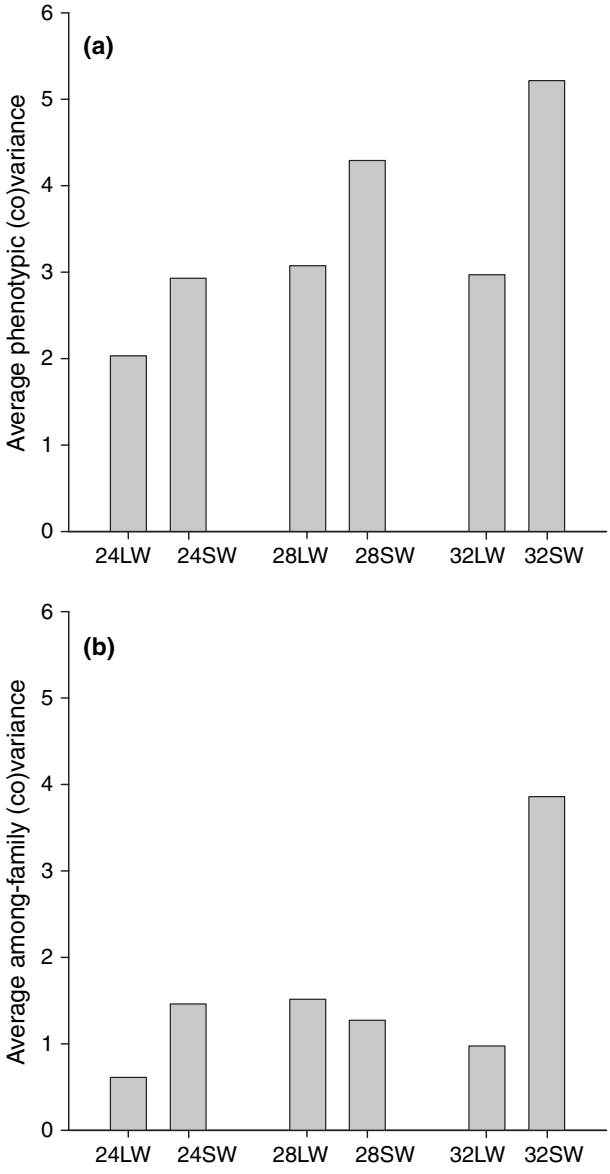

Fig. 3 Average values of the (a) phenotypic and (b) among-family (co)variances for each of the six combinations of temperature and wing morphology. The plotted values are averages of the (co)variances shown in Table 2. Note that this figure aims only at presenting general patterns, and no statistical tests are based on it.

ask whether SW crickets differed from LW crickets with respect to the overall sensitivity to temperature of their phenotypic (co)variances. For each wing morph, we calculated the variance over the three temperatures of each of the phenotypic (co)variances separately. A high variance meant that a specific phenotypic (co)variance has wide-ranging values across temperatures. We then used these variances as data points and compared the distribution of the two morphs using a nonparametric analogue to the paired *t*-test: the Wilcoxon signed rank

Table 3 *T*% statistic (above diagonal) and verdict of the Flury hierarchy (below diagonal) for all pair-wise comparisons of **G** matrices. The *T*% statistic is an index of distance between two matrices, and the associated probability corresponds to the test of the null hypothesis that the two matrices are equal (*T* statistic). The Flury hierarchy verdict is the model that best describes the difference between two matrices.

	24 LW	24 SW	28 LW	28 SW	32 LW	32 SW
24 LW	–	83*	85	71	46	145***
24 SW	Equal	–	25	44	43	90*
28 LW	Equal	PCPC2	–	25	43	87**
28 SW	Equal	Equal	Equal	–	41	101
32 LW	Equal	CPC	Equal	CPC	–	119**
32 SW	PCPC3	Unrelated	Unrelated	CPC	PCPC3	–

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Equal: the two matrices share their principal components structure.

CPC: the two matrices share all of their eigenvectors but not their eigenvalues.

PCPC2 or PCPC3: the two matrices only share 2 or 3 eigenvectors, respectively.

Unrelated: the two matrices do not share their principal components structure.

test. The results showed that the variances of the phenotypic (co)variances of the SW morph were always larger than the ones of LW individuals ($Z_{0.05(2),15} = 3.41$, $P < 0.001$). Very similar results were found when analysing among-family (co)variance data ($Z_{0.05(2),15} = 3.41$, $P < 0.001$) or environmental (co)variance data ($Z_{0.05(2),15} = 3.41$, $P < 0.001$).

Comparisons of among-family (co)variance matrices

We made all pair-wise comparisons of among-family (co)variance matrices using the *T* method, Flury hierarchy and Jackknife–MANOVA method (all methods but the Flury hierarchy corrected for cage effect, see Materials and methods). The only consistently significant result across the three methods was that the matrix 32SW was different from the other matrices. This can be seen from the results of the *T* method (Table 3) which indicated that the *T*% values that correspond to the matrix 32SW ranged from 87 to 145%, whereas all other matrices did not differ by more than 85% amongst themselves (note that the statistical tests corresponded to the *T* statistic, not the *T*%). This pattern can be easily visualized from Fig. 3b. Similarly, the results of the Flury hierarchy (Table 3) revealed that nine of the 10 pair-wise comparisons of matrices that did not include the group 32SW shared all of their principal components (models 'CPC' or 'Equality'), whereas the group 32SW was associated with most of the verdicts that were hierarchically lower than the 'CPC' model (note that the matrix 32SW had to be bent; Phillips & Arnold, 1999). These results therefore suggested that all matrices, except 32SW, were similar to each other. Note however that the group 24SW tended to express a lower magnitude of genetic variation than the other groups (Table 3), but this did not systematically result in differences according to the three methods.

To avoid making inferences based on multiple statistical tests (as was performed in the previous paragraph), a comparison of all six matrices simultaneously was made using the Flury hierarchy. The results revealed that the model that best described the differences between the six matrices was the 'Unrelated model' (the matrices did not share their principal components structure). By contrast, when the matrix 32SW was removed from the analysis, the five remaining matrices shared all of their principal components and had proportional eigenvalues ('Proportionality' model). This therefore confirmed the pattern observed in the pair-wise comparisons; only the matrix 32SW differed from the others with respect to its eigenvector structure.

The MANOVA method was used to analyse the effects of wing morphology, temperature and their interaction on among-family (co)variance matrix variation. This analysis used matrix pseudovalues as data. The results revealed that the variables wing morphology (Wilk's $\lambda = 0.91$, $F_{15,276} = 1.72$, $P = 0.05$) and temperature (Wilk's $\lambda = 0.84$, $F_{30,552} = 1.65$, $P < 0.05$) had a significant effect on matrix variation, whereas their interaction did not (Wilk's $\lambda = 0.88$, $F_{30,552} = 1.21$, n.s.). As with the other two methods, this result reflected mainly the effect of the treatment 32SW because, once the 32 °C treatment was removed, no significant effects were detected (results not shown).

Discussion

The effect of temperature on body size

The relationship between temperature and body size has been studied in many arthropods (Atkinson, 1994), and is particularly well understood in some species of the fruit fly genus *Drosophila*. In this taxonomic group, wing length and thorax length typically present a very similar reaction norm with respect to temperature; size increases to a maximum around 15 and 19 °C, respectively, and then decreases continuously (e.g. David *et al.*, 1994; Karan *et al.*, 2000). No study had yet investigated the effect of temperature on body size in crickets, but Roe *et al.* (1985) looked at dry weight in the house cricket *Acheta domesticus* and found that individuals reared at 35 °C during their last instar were heavier than crickets reared at 25 °C, which implies that a higher temperature is associated with a larger body. The current study did not support this pattern. The variation in body size of *G. firmus* between 24, 28 and 32 °C was significant but did not follow any consistent pattern across traits and across wing morphologies (Fig. 1). Ovipositor length and femur length appeared to have distinct reaction norms that each contrasted with the

common reaction norm of the traits head width, prothorax length and width (Fig. 1). These different environmental sensitivities suggest that the developmental mechanisms leading to the adult size of various parts of the cricket body may be different, perhaps because of the different functions associated with the ovipositor, legs and trunk. These reaction norms should however be confirmed using more temperatures and by covering the whole thermal range of the species.

Life-history correlates of wing morphology

Wing dimorphism is an important part of the life history of many species of insects, and is known to be associated with variation in several other types of traits (Harrison, 1980; Roff & Fairbairn, 1991; Zera & Denno, 1997; Roff & Fairbairn, 2001; Bégin & Roff, 2002). In *G. firmus*, the evolutionary maintenance of wing dimorphism through life-history trade-offs is relatively well understood. In addition to being unable to fly, micropterous individuals (short-winged or SW) are smaller (Roff, 1984) and have a longer developmental time (Roff, 1995) than macropterous crickets (long-winged or LW). On the other hand, this fitness disadvantage of the micropterous morph is compensated by an increased fecundity (Roff, 1984) and by an earlier onset of reproduction after eclosion (Roff, 1990b). These differences between the two morphs reflect two alternative strategies of energy allocation between reproduction and growth/maintenance. The current results provide additional information on this complex fitness trade-off.

A consistent trend was observed within each of the three temperatures; micropterous crickets expressed significantly more phenotypic variation than macropterous individuals (Fig. 3a) despite being smaller. Such a difference in phenotypic variation between two groups reared in the same environment is often discussed in the context of canalization theory (Schmalhausen, 1949; Waddington, 1957; Zakharov, 1992; Gibson & Wagner, 2000; Debat & David, 2001). Canalization is defined as a set of processes ensuring the production of a consistent phenotype in spite of environmental variation and/or any kind of genetic differences. Applied to the current study, this perspective suggests that within a temperature treatment, the short-winged morph has a lower capacity to maintain a consistent development (this culminates at 32 °C), and is thus less canalized. At 24 and 32 °C (results are inconclusive for 28 °C), the increase in phenotypic (co)variance in the micropterous morph was caused mainly by an increase in the genetic component of (co)variance, while the environmental component remained approximately constant. It therefore appears that, within a temperature, the short-winged morph is less efficient than the long-winged morph in its capacity to buffer genetic variation. Additionally, the heterogeneity of the phenotypic, genetic and environmental (co)variations across the three temperatures were significantly larger in the micropterous morph, thus suggesting that the genetic and environmental buffering systems of this morph are more sensitive to temperature variation than that of the macropterous morph. Additional evidence comes from a comparison across wing morphs of the level of fluctuating asymmetry in wing traits, using the same sample of crickets as here (V. Debat, M. Bégin & D.A. Roff, unpublished). In this study, micropterous individuals were found to be less symmetric than the macropters, with the group 32SW exhibiting the highest asymmetry. Overall, these results support the hypothesis that by allocating a relatively large amount of energy to reproduction instead of growth and maintenance, the micropterous morph impairs its developmental buffering mechanisms, especially at 32 °C. Micropterous crickets therefore trade-off their high fecundity against an inability to fly, a smaller body size, a longer developmental time, a higher level of fluctuating asymmetry and a lower canalization.

There is another, but nonexclusive, interpretation of the increase in (co)variance of the micropterous morph. It has been observed that environmental stresses (Roff, 1990a) and genetic stresses (i.e. inbreeding; Roff, 2002c) are associated with increased proportions of the short-winged morph. Nymphs that were genetically predetermined to macroptery may thus become micropterous adults because of stress. Because the two morphs differ in size, this switch from macroptery to microptery of some portion of the nymphs increases the phenotypic variance of the micropterous morph. Similarly, among-family differences in stress resistance cause among-family variation in the proportion of morph-switching nymphs, which is reflected in an increase in the genetic component of variance of the short-winged morph. This mechanism probably combines with canalization to produce the observed difference in (co)variation between the two morphs.

Evolutionary implications

The main objective of this study was to investigate the effects of temperature and wing morphology on the multivariate genetic variation (the **G** matrix, here approximated using a matrix of among-family variances and covariances) of five size-related traits in the sand cricket, *G. firmus*. Evolutionary predictions are theoretically straightforward if the structure of the **G** matrix is conserved, or remains proportional, throughout phenotypic evolution and, thus, across environments (Lande, 1979). However, the degree to which **G** matrices vary in nature is still an unknown parameter (Steppan *et al.*, 2002) that needs to be estimated. Although some variation in the magnitude of among-family (co)variance matrices was evidenced in the current study, the three statistical approaches used here (*T* method, Flury hierarchy and Jackknife–MANOVA method) agreed that all matrices were very similar to one another in structure and generally shared all of their eigenvectors, with the

notable exception of the 32SW group. It therefore appears that most combinations of temperature and wing morphology (five of the six tested here) do not affect the structure of among-family (co)variance matrices. This result corroborates the conclusion of other studies that have shown a similarly low level of matrix variation for the same traits within and among other cricket species (Bégin & Roff, 2001, 2003). We therefore believe that among-family (co)variance matrices corresponding to morphological traits are relatively resistant to change in field crickets, both within and between species. This conclusion is supported by the simulation results of Jones *et al.* (2003) who report that **G** matrices that are composed of highly correlated traits, such as the ones studied here, are expected to be stable over long periods of time.

However, the case of the among-family (co)variance matrix corresponding to micropterous crickets reared at 32 °C deserves some attention. This particular combination of temperature and wing morphology was associated with a different expression of among-family variation. On biological grounds, it is likely that some environmental conditions produce a change in the expression of genetic variation. Studies concerning the effect of thermal stress on the univariate genetic variation of size-related traits in the genus *Drosophila* have sometimes shown that genetic variation increases at the extremes of the temperature range (Noach *et al.*, 1996; de Moed *et al.*, 1997; Imasheva *et al.*, 1998; Karan *et al.*, 1999; Loeschcke *et al.*, 1999; Imasheva *et al.*, 2000; Bubliy & Loeschcke, 2002). The 32 °C treatment of our experiment does not constitute a limit of the cricket temperature tolerance range, but it nonetheless seemed to produce an effect in micropterous individuals. It is however difficult to conclude on the evolutionary implications of this observation for two reasons. First, the peculiarity of the 32SW group may be caused, in part, by a statistical artefact. Indeed, this group had the lowest sample size (less families and less individuals per family) which may have rendered variance component estimation unreliable, a possibility supported by the fact that this matrix had to be bent (Phillips & Arnold, 1999) when using the Flury hierarchy. On the other hand, this group did express the highest amount of phenotypic variation, the estimation of which is not affected by the above-mentioned problem, indicating that finding high levels of genetic variation in 32SW was not unlikely. We therefore believe that the differences between 32SW and the other groups were real, but probably overestimated to some extent. Secondly, it is possible that the difference observed for the 32SW group reflected a change in the expression of components of variation other than additive genetic. For example, Blows & Sokolowski (1995), using life-history traits in *Drosophila*, have reported an increase in dominance and epistasis variance under stressful conditions while the additive genetic component was stable. We cannot preclude the possibility that a high temperature has induced the unmasking of nonadditive genetic variation in the current experiment, but this would likely be accompanied by an increase in additive genetic variation given our solid indirect evidence for the prevalence of additive genetic variation in morphological traits in this species (see Materials and methods section). We therefore believe that the expression of additive genetic (co)variation was somewhat changed in macropterous individuals at 32 °C. This leads to the conclusion that variation in **G** must occur in nature under some circumstances and that complete constancy of **G** is not realistic. We are however left with this open question. Are situation-dependent changes in the **G** matrix (such as the one seen for 32SW) enough proof to reject the possibility of predicting evolutionary trajectories based on classical quantitative genetic models, or is the general stability of among-family (co)variance matrices across most combinations of wing morphologies and temperatures the most promising evidence for this field of investigation?

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Appendix

Testing the type 1 error rate of the Jackknife–MANOVA method

Under the null model of no difference between two **G** matrices, the Jackknife–MANOVA method should erroneously declare the two matrices to be different with a probability of 5%. To test this, we generated populations using the model outlined in Roff & Preziosi (1994). The model generates the variances for two traits and the corresponding covariance. Assuming a full-sib pedigree structure, the values for traits X and Y were given by the equations:

$$X_{i,j} = a_{x,i}\sqrt{\frac{1}{2}h_x^2} + b_{x,i,j}\sqrt{1-\frac{1}{2}h_x^2}$$

and

$$Y_{i,j} = r_A a_{x,i}\sqrt{\frac{1}{2}h_x^2} + a_{y,i}\sqrt{\frac{1}{2}(1-r_A^2)h_y^2} + r_E b_{x,i,j}$$
$$\sqrt{1-\frac{1}{2}h_x^2} + b_{y,i,j}\sqrt{\left(1-\frac{1}{2}h_y^2\right)(1-r_E^2)},$$

where $X_{i,j}$ and $Y_{i,j}$ are the trait values for the jth individual in family i; $a_{x,i}$ and $a_{y,i}$ are random standard normal values $N(0,1)$ common to the ith family; $b_{x,i,j}$ and $b_{y,i,j}$ are random standard normal values $N(0,1)$ of the jth individual from the ith family; h_x^2 and h_y^2 are the heritabilities of traits x and y, respectively; r_A is the additive genetic correlation between the two traits; and r_E is the environmental correlation between the traits calculated from the phenotypic correlation, r_P, as

$$r_E = \left(r_P - \frac{1}{2}r_A h_x h_y\right) \Big/ \sqrt{\left(1-\frac{1}{2}h_x^2\right)\left(1-\frac{1}{2}h_y^2\right)}.$$

Pairs of populations with identical **G** matrices were generated and compared using the Jackknife–MANOVA method of analysis. To roughly match the sample sizes used in the current study, we used populations of 50 full-sib families with 10 offspring per family. The actual values of the heritabilities and correlations should make no difference under the null hypothesis. Indeed, we used a range of parameter values and, as all gave the same result, we combined the data to estimate the probability. Of 2100 simulations, 4.7% were found to be significant: this is not significantly different from the predicted 5% ($\chi_1^2 = 0.49$, n.s.).

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