ORIGINAL ARTICLE

QUANTITATIVE GENETICS OF SHAPE IN CRICKET WINGS: DEVELOPMENTAL INTEGRATION IN A FUNCTIONAL STRUCTURE

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The role of developmental and genetic integration for evolution is contentious. One hypothesis states that integration acts as a constraint on evolution, whereas an alternative is that developmental and genetic systems evolve to match the functional modularity of organisms. This study examined a morphological structure, the cricket wing, where developmental and functional modules are discordant, making it possible to distinguish the two alternatives. Wing shape was characterized with geometric morphometrics, quantitative genetic information was extracted using a full-sibling breeding design, and patterns of developmental integration were inferred from fluctuating asymmetry of wing shape. The patterns of genetic, phenotypic, and developmental integration were clearly similar, but not identical. Heritabilities for different shape variables varied widely, but no shape variables were devoid of genetic variation. Simulated selection for specific shape changes produced predicted responses with marked deflections due to the genetic covariance structure. Three hypotheses of modularity according to the wing structures involved in sound production were inconsistent with the genetic, phenotypic, or developmental covariance structure. Instead, there appears to be strong integration throughout the wing. The hypothesis that genetic and developmental integration evolve to match functional modularity can therefore be rejected for this example.

KEY WORDS: Developmental constraint, geometric morphometrics, *Gryllus firmus*, modularity, Procrustes superimposition, selection.

Adaptive evolution of morphological structures requires genetic variation of parts that are involved in one or more fitness-related functions. In turn, the available variation is molded by the developmental system that expresses variation and can manifest itself as a constraint on adaptive evolution. This reciprocal relationship between functional and developmental factors has long been a focus of extensive debate in evolutionary biology (e.g., Whyte 1965; Arthur 1997; Gould 2002; Laubichler and Maienschein 2009). We use the term function to designate the biomechanical or physiological effects of a structure; "basically the function of a feature is its action or how it works" (Bock and von Wahlert 1965, p. 274). Further, we define effects of development to include both the patterning of variation that arises in the developmental system as well as its possible modification by processes such as internal selection (Whyte 1965; Riedl 1975; Cheverud 1984; Arthur 1997; Fusco 2001). It is generally recognized that the evolution

Evolution

of morphological structures is influenced both by the processes involved in the development of a structure and its function in the organism's interactions with the environment, but different investigators have tended to emphasize one or the other (for recent reviews, see Breuker et al. 2006a; Laubichler and Maienschein 2009). Viewpoints differ on the relative roles that function and developmental factors play in influencing the available morphological variation and evolutionary change. Some authors have hypothesized that the genetic and developmental structure should evolve to match functional modules (Riedl 1975; Cheverud 1984; Wagner and Altenberg 1996), whereas others have emphasized that developmental modularity may affect adaptive evolution as a constraint or bias (Raff 1996; Arthur 2001). As a possible strategy to address the relative importance of developmental and functional factors for evolutionary processes, Breuker et al. (2006a) suggested an analysis of modularity in a structure where it can be expected that functional and developmental modules are not congruent.

An example of such a structure is the forewing of male crickets, which contains several morphologically differentiated regions that perform different roles in sound production (Fig. 1A; Nocke 1971; Bennet-Clark 1999, 2003), although the wing develops from a single sheet of cells that is folded over to form a double layer of cells inside an external pocket of cuticle of the nymph, the wing pad (Heming 2003). Moreover, because crickets only start to use their forewings after they are fully formed and hardened, there is no effect of use on the development of the wing. Crickets produce their song by moving one wing over the other (usually the right over the left wing). The teeth of the file on the ventral side of the upper wing rub against the plectrum, or scraper, of the opposite wing and thus cause both wings to vibrate. The area of modified veins called the harp is the main resonator in the wing (Nocke 1971; Bennet-Clark 1999, 2003; Montealegre-Z et al. 2009), whereas the mirror area is likely to be a subsidiary resonator (Bennet-Clark 2003). How fast the file and plectrum move against each other is determined by a "clockwork" mechanism in which the resonance of the wing controls the speed at which the teeth of the file are caught and released by the plectrum (Elliott and Koch 1985; Koch et al. 1988; Prestwich et al. 2000; Bennet-Clark and Bailey 2002). The harp, as the main resonator of the wing, therefore is expected to be of key importance for the frequency of the song. A relationship between the area of the harp and the dominant frequency was indeed found in the field cricket *Gryllus campestris* (Simmons 1995; Simmons and Ritchie 1996). Moreover, Webb and Roff (1992) reported several other relationships between song characteristics and morphological traits in the wing of *Gryllus firmus*. At a larger scale, comparative studies in katydids showed that evolutionary changes in the morphology of sound-producing structures and acoustic signals are phylogenetically associated (Montealegre-Z 2009). Overall, it is reasonable
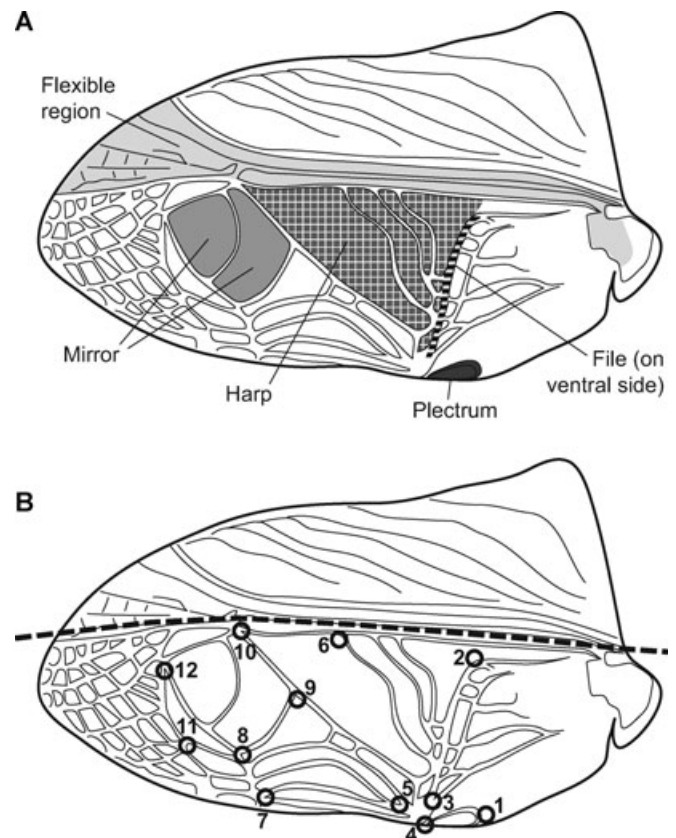
Figure 1. Regions of the wing and landmarks used in this study. (A) The main regions of a forewing that are relevant for sound production (after Bennet-Clark 1999, 2003). The plectrum, a modified wing vein, engages with the file on the ventral side of the opposite wing to produce vibrations of both wings. The harp is the main resonator in the wing, and the mirror is a subsidiary resonator (Bennet-Clark 1999). (B) Landmarks digitized on each wing. Wings were cut along the bold dashed line to flatten them. The diagrams of shape changes in the subsequent figures show only the posterior part of the wing that was retained for digitizing (below the dashed line in the diagram).

to expect that the precise geometry of the different wing parts and their arrangement to each other are under selection related to acoustic performance. Moreover, because the parts of the sound-producing structure perform distinct functions, it is likely that selection on specific song features will favor changes in those parts individually or in different combinations.

Here, we analyze the shape of wings in male sand crickets (*G. firmus*) to examine morphological integration and modularity at the genetic and phenotypic levels. Genetic variation of wing measurements and song characteristics in this species has been documented previously (Webb and Roff 1992). To examine the structure of genetic variation of shape in the cricket wing, we combine the methods of geometric morphometrics with those of evolutionary quantitative genetics (Roff 1997, 2007; Lynch and Walsh 1998; Klingenberg and Leamy 2001; Myers et al. 2006).

This approach makes it possible to search for genetic constraints, whether from development or other origins, and to examine genetic integration and modularity of wing shape. To infer integration at the developmental level, we analyze the patterns of fluctuating asymmetry (Klingenberg and McIntyre 1998; Debat et al. 2000; Klingenberg 2003b, 2008b). Because fluctuating asymmetry stems from random perturbations that occurred in the development of the wings of the individuals under study, it provides an opportunity to quantify the pattern of variation from spontaneous changes in the developmental processes and therefore allows us to analyze directly the intrinsic, developmental component of integration and modularity. In combination, these analyses constitute an integrated approach to explore the functional and developmental aspects of morphological variation in the cricket wing and its evolutionary potential (Breuker et al. 2006a).

Materials and Methods
BREEDING DESIGN AND DATA COLLECTION

This study considers the wing shape of male crickets raised in a breeding experiment from which measurements of females were considered in a previous study, where experimental design, mating procedures, and rearing conditions are described in detail (Bégin et al. 2004). In brief, the breeding experiment is based on a full-sibling design, where the genetic component of variation is estimated from the variation among families of full-siblings. Although the crickets were reared at three different temperatures (24°C, 28°C, and 32°C), we pooled all families in this study to maximize the sample size (genetic covariance matrices for other morphological measurements were stable across temperatures; Bégin et al. 2004). An additional factor is that *G. firmus* is a wing dimorphic species, where some individuals have fully developed hindwings (long-winged morph) and others have reduced, nonfunctional hindwings (short-winged morph; Veazey et al. 1976; Roff 1986). We treated temperature and wing morph as fixed factors in the genetic analysis. The offspring of each family were reared in two separate buckets, which makes it possible to estimate the effect of the common rearing environment. Complete data were available for a total of 1279 male offspring from 112 families.

The cricket wing is a three-dimensional structure folded in the flexible zone anterior to the Cu1 vein (Fig. 1A; Bennet-Clark 2003). We cut the wings along the Cu1 vein (Fig. 1B, dashed line), which made it possible to flatten them, and we focus on the posterior part that is primarily responsible for sound production (Nocke 1971; Bennet-Clark 2003). This posterior part was mounted on a slide under a cover slip in a polyvinyl lactophenol semi-permanent medium.

For each wing, 12 landmarks were digitized, which cover the principal parts of the wing that are involved in sound pro-

duction (Fig. 1B). To assess the measurement error, each wing was digitized twice. The landmarks were digitized using the TPSdig software (v1.07; freely available at http://life.bio.sunysb.edu/morph/).

SHAPE ANALYSIS

Shape information was extracted with a generalized full Procrustes fit and a projection to shape tangent space (Dryden and Mardia 1998). The Procrustes fit included appropriate reflections to take into account that the data contained landmark configurations from both left and right wings (Klingenberg and McIntyre 1998). As a measure of wing size, we computed centroid size (Dryden and Mardia 1998). These and the subsequent steps of the morphometric analysis were performed with the MorphoJ software (Klingenberg 2008a).

To quantify the components of variation due to the different factors of the experimental design (temperature, wing morphs, individual variation, asymmetry, and measurement error), we ran a preliminary Procrustes analysis of variance (ANOVA) (Klingenberg and McIntyre 1998). We used the method of summing up effects over the *x* and *y* coordinates of all landmarks (Klingenberg and McIntyre 1998), which is an extension of Goodall's (1991) ANOVA approach and produces intuitive estimates of the magnitudes of the different effects. In addition, we used the multivariate analysis of variance (MANOVA) approach, which also takes into consideration the directionality of variation in the shape tangent space for the statistical tests, but does not provide intuitive statistics on the amounts of variation for which the different factors account (Klingenberg et al. 2002; Klingenberg and Monteiro 2005). The two methods are therefore complementary.

Principal component (PC) analysis, based on the covariance matrix of the landmark coordinates after the Procrustes fit (Dryden and Mardia 1998; Jolliffe 2002), was used to examine the dominant features and dimensionality of shape variation and to extract shape variables for the quantitative genetic analysis.

QUANTITATIVE GENETIC ANALYSIS

To accommodate the variable number of offspring in the different families, we estimated quantitative genetic parameters using REML methods, as implemented in the Wombat software (Meyer 2007). Alternative analyses run with the VCE6 software (Groeneveld et al. 2008) produced very similar results. We present the results obtained with Wombat.

The wing shape data were entered into the analyses as PC scores, including all 20 PCs that had eigenvalues greater than zero, so that the entire dimensionality of the shape tangent space was preserved in the analysis. The genetic model included the rearing temperature and wing morph as fixed effects, centroid size as a covariate, and families and buckets (common environment) as

random effects. The resulting covariance matrices for the random effects were transformed from the PCs back to the original coordinate system for all further analyses. The use of centroid size as a covariate corrects for the allometric effects of size on shape, which are known as a common integrating factor that tends to produce covariation throughout the entire configuration of landmarks (e.g., Klingenberg 2009). Including a size correction such as this therefore tends to make any modularity more apparent.

The matrix $\mathbf{GP}^{-1}$, where $\mathbf{G}$ is the genetic and $\mathbf{P}$ the phenotypic covariance matrix, is a multivariate analogue of the univariate heritability (Roff 2000). The eigenvectors of $\mathbf{GP}^{-1}$ with the largest and smallest eigenvalues define the shape variables with the maximal and minimal heritabilities, and these variables are therefore useful for the study of the potential for evolutionary change of shape variables (Klingenberg and Leamy 2001). Because $\mathbf{P}$ is not of full rank due to the degrees of freedom for size, position, and orientation lost in the Procrustes fit, we used the Moore–Penrose generalized inverse of $\mathbf{P}$ (which we write $\mathbf{P}^{-}$). Heritability has been criticized as a statistic for evaluating the potential for response to selection or constraints (Houle 1992), but is still a useful measure of the evolutionary potential of scalar traits (Falconer and Mackay 1996; Roff 1997; Visscher et al. 2008). Alternatives such as Houle's (1992) evolvability statistic also have their limitations (Roff 1997, p. 122 f.; Lynch and Walsh 1998, p. 175 f.) and the standardization using the mean is not applicable to shape variables derived from shape tangent space, because the origin of the coordinate system is the mean shape (Dryden and Mardia 1998). Shape variables derived from shape tangent space are on an interval scale and usually have zero means (because they are often computed as deviations from the mean shape). For variables on an interval scale, even authors usually critical of heritability agree that it is an appropriate measure of evolutionary potential (Hansen and Houle 2008, p. 1204). As an alternative, in the context of geometric morphometrics, Hansen and Houle (2008) used untransformed shape distances (Procrustes distances) in their example calculations because they felt that the transformation from the original landmark coordinates to shape data by the Procrustes fit "is similar to mean standardization" (p. 1208). This is unsatisfactory because these distances, in units of Procrustes distance, are specific to the dataset under question and cannot be compared among datasets, let alone with variables derived from different types of data. Such comparisons are a key benefit of statistics such as heritability (Mousseau and Roff 1987; Roff and Mousseau 1987; Visscher et al. 2008) or evolvability (Houle 1992). For these reasons, we prefer to use heritability to gauge the potential for evolutionary change of shape variables.

Because of the known bias in the estimation of $\mathbf{G}$, which tends to overestimate large eigenvalues and to underestimate small ones (Hill and Thompson 1978; Hayes and Hill 1981), the multivariate analysis of $\mathbf{GP}^{-}$ may exaggerate extreme heritabilities, both large and small. To address the effect of this bias, we conducted univariate REML analyses to compute the heritabilities of the shape variables corresponding to the eigenvectors of $\mathbf{GP}^{-}$ and the respective standard errors. Even if these variables may still be affected in part by the bias and thus may not optimally estimate the shape variables with extreme heritabilities, their heritabilities are likely to be close to those extremes. The values of these shape variables were computed by projecting datapoints onto the direction of the respective eigenvector in shape tangent space. The genetic model for these univariate analyses was the same as for the multivariate analysis, with wing morph and temperature as fixed effects, centroid size as a covariate, and families and buckets as random effects. Because a single shape variable is used in each of these analyses, univariate statistics such as heritabilities and their standard errors are appropriate (Klingenberg and Leamy 2001).

For the analyses of fluctuating asymmetry, a separate genetic analysis was run to account for possible genetic effects on asymmetry, which may produce differences in the average asymmetry in different families and thus may bias the analyses of fluctuating asymmetry (Stige et al. 2006). The REML analysis was run with PC scores of the individual asymmetries of wing shape, and all 20 PCs with nonzero eigenvalues were included in the analysis. The genetic model included the rearing temperature and wing morph as fixed effects, and families and buckets as random effects. The residual covariance matrix from this analysis was transformed back from the coordinate system of PC scores to that of the Procrustes-aligned landmark coordinates. This residual covariance matrix characterized the patterns of covariation in the asymmetry of the wings after correction for genetic effects and the effects of the common environment in the buckets, and was used in the analyses of fluctuating asymmetry to study integration and modularity in the variation produced by the developmental system (Klingenberg 2003b, 2005, 2008b).

To quantify the similarity of covariance matrices, we computed the matrix correlations between them. Matrix correlation is a measure of the overall similarity of covariance matrices and has been widely used in geometric morphometrics (e.g., Klingenberg and McIntyre 1998; Debat et al. 2000, 2006, 2008, 2009; Breuker et al. 2006b). We computed matrix correlations with and without the diagonal 2×2 blocks corresponding to the variances and covariances of x and y coordinates of single landmarks (Klingenberg and McIntyre 1998). Matrix correlation of the complete covariance matrices considers the total patterns of variation, whereas the analysis without the diagonal blocks focuses just on covariation between different landmarks. Tests of similarity against the null hypothesis of no relationship were based on a matrix permutation procedure, as modified for geometric morphometrics. To simulate the null hypothesis of unrelated patterns of covariation, these tests

permuted the landmarks in the matrices rather than the individual *x* and *y* coordinates (Klingenberg and McIntyre 1998).
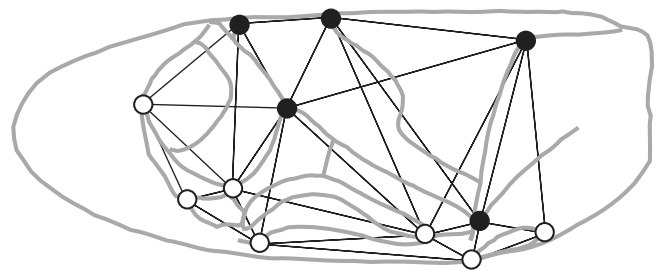
ANALYSIS OF MODULARITY

Because different regions of the forewing have different functions in sound production, we examined whether these regions behave as morphological modules (Klingenberg 2008b, 2009). The harp has been identified as the main resonator in the wing of crickets (Nocke 1971; Simmons and Ritchie 1996; Bennet-Clark 1999, 2003; Montealegre-Z et al. 2009). According to the "clockwork cricket" model of sound production, the resonance of the harp determines the dominant frequency of the sound, not only its volume (Elliott and Koch 1985; Koch et al. 1988; Prestwich et al. 2000; Bennet-Clark and Bailey 2002). Moreover, the ventral side of the vein that forms the proximal border of the harp also bears the file used in stridulation, which is directly integrated into the harp. The mirror also resonates, but at a higher frequency and with more damping than the harp (Nocke 1971; Bennet-Clark 2003), and may therefore play a subsidiary role in sound production that is distinct from the harp. Finally, the plectrum has a key role in sound production as it interacts with the file of the opposite wing, but it is coupled to the ipsilateral harp via a flexible linkage that achieves a phase shift in the excitation of the harp (Bennet-Clark 2003; Montealegre-Z et al. 2009). The plectrum and the surrounding structures may therefore be a module that is distinct from the other parts of the wing. Because all these regions perform distinct functions in the production of sound, they can be considered to be functional modules and it is plausible that selection on different aspects of song may affect these regions differentially.
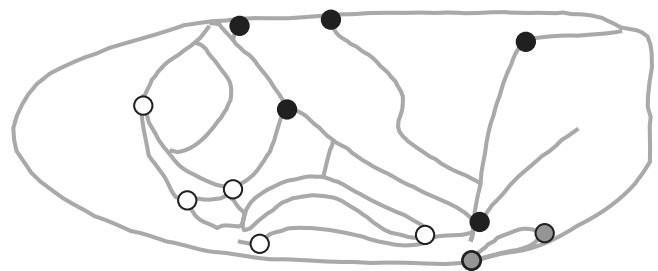
To examine whether the functional modules are reflected in the variation of shape, we formulated three hypotheses of modularity (Fig. 2): a hypothesis contrasting the harp with the other parts of the wing (Fig. 2A), a hypothesis in which the harp and plectrum region are separate from the remainder of the wing (Fig. 2B), and a hypothesis in which the harp, mirror, and the rest of the wing form separate modules (Fig. 2C). For the latter partition of the wing, it is not clear to which hypothetical module landmark 9, at the boundary of harp and mirror, should be allocated. Thus, we performed the analysis with an alternative allocation of this landmark as well.

Hypotheses of modularity were evaluated by computing the strength of covariation between the hypothesized modules with alternative partitions of the landmarks into subsets of the corresponding sizes (Klingenberg 2009). If a hypothesis of modularity is correct, the covariation between those subsets of landmarks should be weaker than the covariation between different partitions of the landmarks into subsets. To evaluate this prediction, we computed the *RV* coefficient (Escoufier 1973) or multiset *RV* coefficient (Klingenberg 2009) as a measure of the strength of covariation between the sets of landmarks. The *RV* coefficient is

A Harp versus all other landmarks

B Harp, plectrum, and remaining landmarks

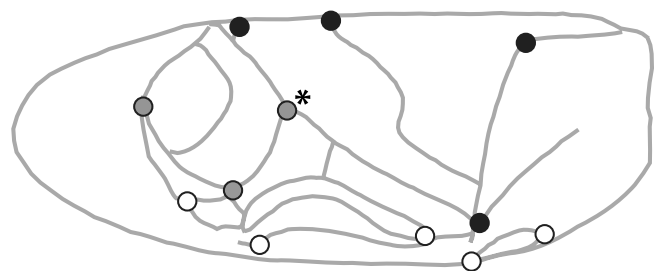**C** Harp, mirror, and remaining landmarks

Figure 2. Hypotheses of modularity in the cricket wing (diagrams show the posterior part of the wing only, see Fig. 1B). (A) The hypothesis that contrasts the landmarks of the harp (solid black circles) and the remainder of the wing (open circles) as two separate modules. The thin black lines represent the adjacency graph used to define spatially contiguous subsets of landmarks (Klingenberg 2009). (B) The hypothesis in which the harp (black circles), the landmarks at the ends of the plectrum (gray circles), and the remaining landmarks (open circles) form three modules. (C) The hypothesis where the harp (black circles), the mirror (gray circles), and the remainder of the wing (open circles) are distinct modules. Because landmark 9 (marked with an asterisk) is at the boundary of the harp and mirror, we also considered an alternative subdivision for which this landmark was allocated to the harp.

a multivariate measure of covariation between two sets of landmarks and can be computed as follows:

$$RV = \frac{\text{trace}\,(\mathbf{S}_{12}\mathbf{S}_{21})}{\sqrt{\text{trace}\,(\mathbf{S}_1\mathbf{S}_1)\,\text{trace}\,(\mathbf{S}_2\mathbf{S}_2)}}.$$

In this equation, the matrices $\mathbf{S}_{12}$ and $\mathbf{S}_{21}$ contain the covariances between the coordinates of the two sets of landmarks ($\mathbf{S}_{12}$ is

the transpose of $\mathbf{S}_{21}$), whereas the matrices $\mathbf{S}_1$ and $\mathbf{S}_2$ contain the variances and covariances of the landmarks within sets 1 and 2, respectively. The trace of a matrix is the sum of its diagonal elements. The *RV* coefficient can be interpreted as a multivariate analogue of the squared correlation coefficient (Escoufier 1973; Klingenberg 2009). The multiset *RV* coefficient is a generalization for more than two sets of variables and is computed as the average of the *RV* coefficients between all pairs of sets (Klingenberg 2009).

For each hypothesis, the *RV* coefficient or multiset *RV* coefficient obtained from the partition of landmarks according to modules was compared to the corresponding statistics for the full enumeration of all alternative partitions of the landmarks into subsets with the corresponding numbers of landmarks. If the *RV* coefficient for the partition according to hypothesized modules is not at or near the lower extreme of the distribution of *RV* coefficients from the alternative partitions, the expectation that the modules are relatively independent from each other is not met, and the hypothesis of modularity can therefore be rejected (Klingenberg 2009).

Because the developmental interactions that produce the morphological integration in the wings are tissue-bound processes such as intercellular signaling (Heming 2003; Blair 2007) and because sound production involves mechanical transmission of oscillations through the wing, it may be desirable to restrict comparisons to just those subsets of landmarks that are spatially contiguous to qualify as potential developmental or functional modules. Accordingly, in addition to analyses without restrictions, we also conducted comparisons that were limited to just those partitions that separated the landmarks into spatially contiguous subsets (Klingenberg 2009). Spatial contiguity was defined with an adjacency graph (fine black lines in Fig. 2A): a partition of landmarks is spatially contiguous if all resulting subsets of landmarks are connected through the edges of the adjacency graph (Klingenberg 2009).

HYPOTHETICAL SELECTION

To examine genetic integration in the wing further, we defined hypothetical selection gradients focusing on specific, localized shape features of the wing and examined the shape change corresponding to the expected response to selection (Klingenberg and Leamy 2001). The influence of relative constraints on adaptation can be gauged by assessing how much the direction of the predicted response is deflected from the direction of selection. For instance, an effect of constraints is manifest if selection for a localized shape feature yields a predicted response with global shape changes throughout the entire landmark configuration (Klingenberg and Leamy 2001). The selection response can be predicted from the selection gradient and the genetic and phe-

notypic covariance matrices using the multivariate version of the breeders' equation $\Delta\boldsymbol{\mu} = \mathbf{G}\boldsymbol{\beta} = \mathbf{G}\mathbf{P}^{-1}\mathbf{s}$ (Lande 1979), where $\Delta\boldsymbol{\mu}$ is the change in the average phenotype in response to selection, $\mathbf{G}$ is the additive genetic covariance matrix, $\mathbf{P}$ is the phenotypic covariance matrix, $\boldsymbol{\beta}$ is the selection gradient, and $\mathbf{s}$ is the selection differential.

Selection gradients are not shape changes, and previous analyses have therefore characterized selection on geometric shape with selection differentials, which are shape changes and can be directly visualized and interpreted (Klingenberg and Leamy 2001; Klingenberg and Monteiro 2005). The disadvantage of selection differentials is that they do not separate direct selection from indirect selection due to phenotypic covariation between traits (Lande and Arnold 1983). To avoid this difficulty, it is possible to represent the selection gradient as the product of a shape vector indicating the direction of selection in shape tangent space, which is a shape variable, and a scale factor that represents the amount of change in relative fitness per unit of change in shape.

Selection gradients were chosen to reflect shape changes relating to the biomechanics of sound production in the cricket wing: an increase in the relative size of the harp, an increase in the relative size of the mirror, and a change in the relative position of the plectrum. Note that these selection gradients are purely hypothetical, and that we do not imply that they reflect patterns of selection in real populations (no information about selection on wing shape in cricket populations is currently available). These changes of relative landmark positions may have altered the overall centroid size, position, and orientation of the landmark configuration, and therefore may not have been pure shape changes. To ensure that the directions of the selection gradients were shape changes, the vectors of these landmark shifts were projected onto the shape tangent space (Dryden and Mardia 1998). This projection resulted in small shifts of landmarks other than those that were targeted directly. The magnitude of the scale factor was arbitrarily set so that the standardized selection gradient (Lande and Arnold 1983) of the corresponding shape variable was 1.0 (i.e., one unit of change in relative fitness per standard deviation of change of the shape variable corresponding to the selection gradient). This standardized selection gradient is large, but not unrealistic by comparison to values found in natural populations of various animals and plants (Hoekstra et al. 2001; Kingsolver et al. 2001). In all other details, the analyses of hypothetical selection were conducted as described by Klingenberg and Leamy (2001).

The total response to selection, $\Delta\boldsymbol{\mu}$, was separated into the direct response, the component in the direction of the selection gradient, and the correlated response, perpendicular to it (Klingenberg and Leamy 2001). The direct and correlated responses add up to the total response by vector addition. This decomposition was chosen to match the meanings of the terms

Table 1. Procrustes ANOVA and MANOVA for the contributions of the various experimental factors to shape variation.

	Procrustes ANOVA					MANOVA	
	SS	MS	df	*F*	*P*	Pillai's trace	*P*
Wing morph	0.066	0.003316	20	11.03	<0.0001	0.33	<0.0001
Temperature	0.770	0.019251	40	64.03	<0.0001	1.23	<0.0001
Individual	7.667	0.000300	25,500	5.89	<0.0001	17.90	<0.0001
Side	0.826	0.041304	20	809.20	<0.0001	0.95	<0.0001
Individual × side	1.305	0.000051	25,560	5.66	<0.0001	14.27	<0.0001
Measurement error	0.454	0.000009	50,320				

SS, sums of squares; MS, mean squares; df, degrees of freedom.

direct and correlated response in traditional quantitative genetics (e.g., Falconer and Mackay 1996; Roff 1997): the direct response is the component of the response that is in the direction of the shape feature under selection and the correlated response is the change in the remaining shape features, which were not selected for (hence the orthogonality of the two components). This decomposition is also identical to the use of the terms direct and correlated response in the multivariate context by Arnold and Wade (1984, p. 711). The magnitudes of the direct and correlated response were computed in units of Procrustes distance. The analyses of hypothetical selection were conducted as implemented in the MorphoJ software (Klingenberg 2008a).

Results

SHAPE VARIATION

The Procrustes ANOVA indicated that both wing morph and rearing temperature had substantial effects on wing shape (significant main effects and substantial mean squares; Table 1). There was also significant directional asymmetry (main effect of side). Moreover, fluctuating asymmetry (individual × side interaction) exceeded measurement error more than fivefold, so that the estimates of fluctuating asymmetry are not compromised by measurement error. All other effects are much larger, and the effects of measurement error are therefore negligible for them.

A principal component (PC) analysis showed that much of the phenotypic variation was concentrated in the first few dimensions and that the eigenvalues gradually decreased, with a slight step after the ninth PC (Fig. 3A). The shape changes for the first three PCs (Fig. 4A) consisted of relative shifts and deformations of structures in multiple regions of the wing. For instance, the PC1 was associated with a large shift of the central vein of the harp (landmark 6), with smaller changes in the distal part of the mirror and the landmarks posterior to the mirror and in the proximal part of the harp (landmark 2). The PC2 and PC3 are similarly composed of changes in multiple regions of the wing, and not localized to a single part.
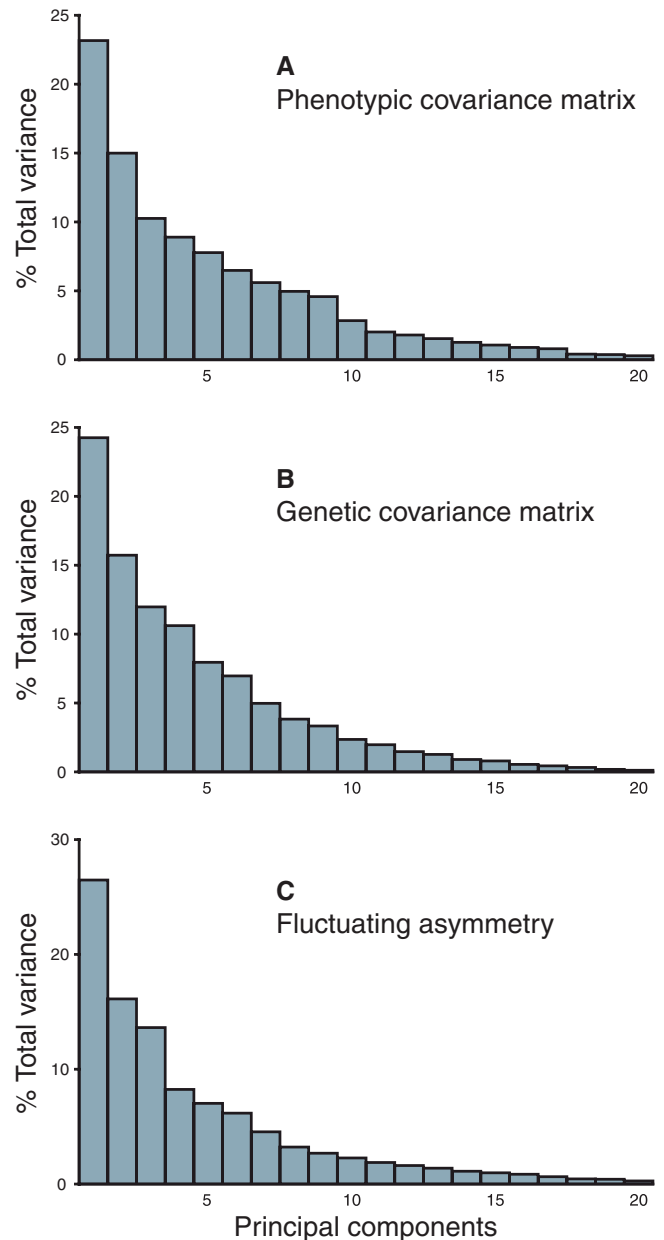

Figure 3. Relative contributions of the eigenvalues of the phenotypic (A) and genetic (B) covariance matrices and of the covariance matrix for fluctuating asymmetry (C).
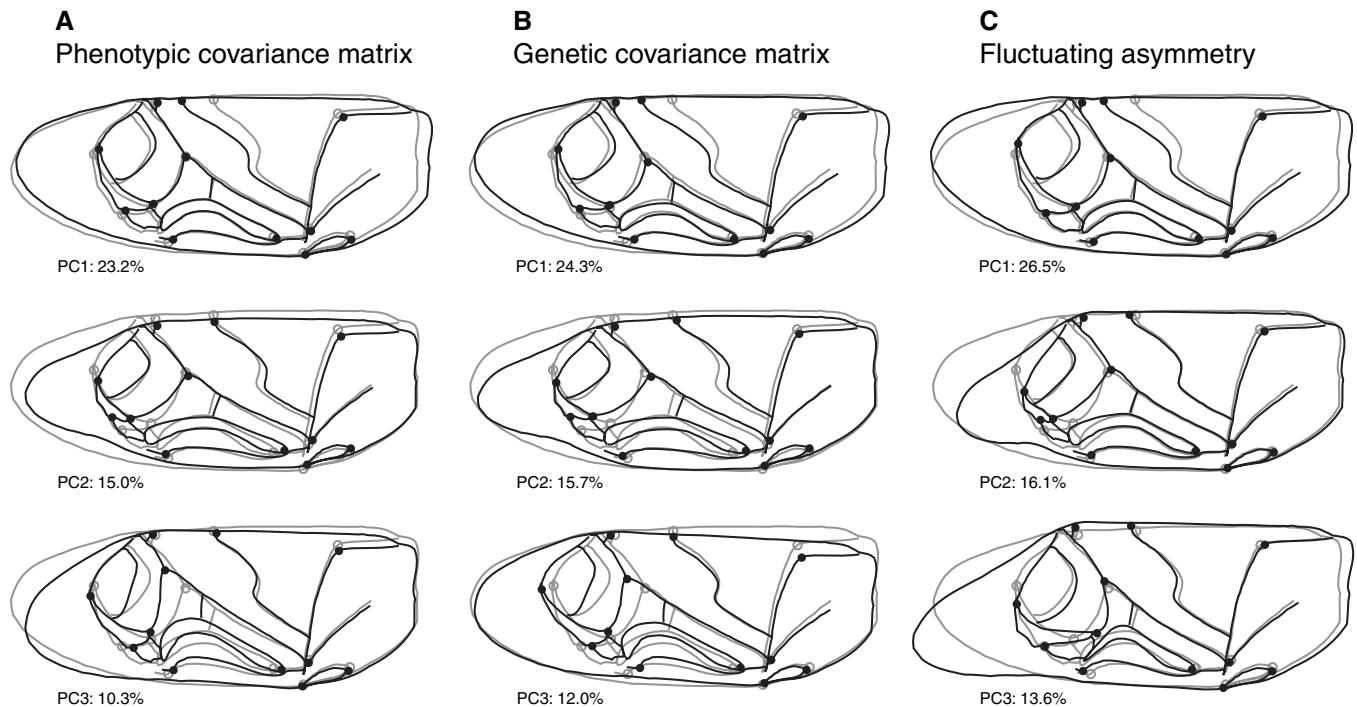
A
Phenotypic covariance matrix

B
Genetic covariance matrix

C
Fluctuating asymmetry

PC1: 23.2%   PC1: 24.3%   PC1: 26.5%

PC2: 15.0%   PC2: 15.7%   PC2: 16.1%

PC3: 10.3%   PC3: 12.0%   PC3: 13.6%

Figure 4. Shape changes associated with the principal components. The shape changes from the gray outlines and empty circles (average shape) to the black outlines and solid circles represent a shift along the respective PC in positive direction by 0.1 units of Procrustes distance (this is a large change relative to the variation in the data). Note that the directions of the shape changes are arbitrary, the opposite shape change is therefore equally associated with each PC. Outline drawings were warped according to the shape change for the landmarks with the thin-plate spline technique (Bookstein 1989). Note that the warped outline diagrams indicate interpolated changes even in regions without nearby landmarks (e.g., in the wing base and tip), which should be interpreted with caution.

INHERITANCE OF WING SHAPE

The genetic variation of wing shape was distributed over the dimensions of shape space (Fig. 3B) in a similar manner as it was found for the phenotypic covariance matrix (Fig. 3A). Moreover, the PCs of the genetic covariance matrix were similar to those of the phenotypic covariance matrix (cf. Fig. 4A,B). In line with these observations, the matrix correlation between the phenotypic and genetic covariance matrices was very high (0.97 if the diagonal 2×2 blocks were included, 0.91 if the diagonal blocks were excluded from the computation of matrix correlations; the matrix permutation test against the null hypothesis of no similarity whatsoever yielded a $P < 0.0001$ in both cases).

The analysis of the matrix $\mathbf{GP}^-$ produced a range of eigenvalues from 0.95 to 0.083, with a fairly smooth scale of values between these two extremes (Fig. 5A). These two extreme values also indicate the range of possible heritabilities of the shape variables that can be computed as linear combinations of the landmark coordinates. The shape variable that has the maximal heritability (Fig. 5B; the eigenvector corresponding to the maximum eigenvalue) is fairly similar to the PC1 of the phenotypic covariance matrix (Fig. 4A; the angle between the two vectors in shape tangent space is 52.9°, an angle substantially smaller than expected for random vectors in 20-dimensional space).

To evaluate these expected heritabilities, we conducted univariate analyses with the shape variables corresponding to the eigenvectors of the matrix $\mathbf{GP}^-$. None of these variables had an extremely small variance (each accounted for more than 5% of the total variance), suggesting that no numerical problems were to be suspected. The estimated heritabilities for these variables ranged from 0.33 to 0.82 and standard errors were fairly small (Table 2). The heritabilities of these shape variables followed the ordering of the corresponding eigenvalues of $\mathbf{GP}^-$ mostly, but not strictly (e.g., the smallest heritability was obtained for eigenvector 18, not 20). That the range of these heritabilities was narrower than the spectrum of eigenvalues of $\mathbf{GP}^-$ reflects the bias expected in multivariate analyses (Hill and Thompson 1978; Hayes and Hill 1981). Nevertheless, even these heritabilities from univariate analyses extended over a considerable range.

FLUCTUATING ASYMMETRY AND DEVELOPMENTAL INTEGRATION

To investigate variation spontaneously produced by the developmental system of the wings, we used the covariance matrix for fluctuating asymmetry after removing the small genetic component of variation (accounting for 5.5% of the phenotypic component of the total variance for shape asymmetry).
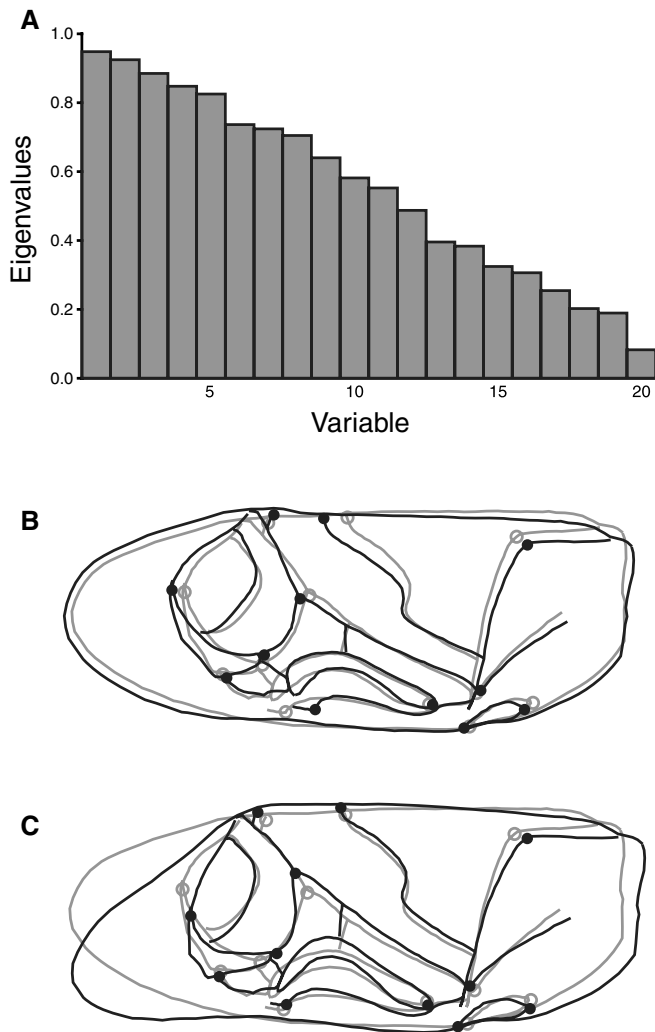
Figure 5. Analysis of the matrix GP⁻. (A) Eigenvalues of the matrix GP⁻, which correspond to the heritabilities of the shape variables associated with the respective eigenvectors. (B) The shape change associated with the largest eigenvalue. This is the shape variable with the highest heritability. (C) The shape change associated with the smallest eigenvalue. This is the shape variable associated with the lowest heritability, or with the most stringent genetic constraint. Conventions for the warped outline drawings are the same as for Figure 4.

Table 2. Heritabilities of the shape variables corresponding to the eigenvectors of the matrix GP⁻. These heritabilities and their standard errors were computed in univariate REML analyses.

Eigenvector	Heritability	Standard error
1	0.82	0.082
2	0.81	0.079
3	0.80	0.080
4	0.70	0.077
5	0.72	0.080
6	0.66	0.077
7	0.68	0.077
8	0.55	0.075
9	0.59	0.074
10	0.63	0.076
11	0.63	0.078
12	0.58	0.073
13	0.60	0.075
14	0.51	0.071
15	0.50	0.071
16	0.46	0.070
17	0.54	0.074
18	0.33	0.064
19	0.52	0.071
20	0.37	0.071

G matrices for shape (Fig. 4A,B). Overall, this indicates that the contribution of new variation produced by the developmental system is patterned in a similar way as the phenotypic and genetic variation in the population under study.

MODULARITY OF WING SHAPE

The *RV* coefficients or multiset *RV* coefficients between subsets of landmarks corresponding to hypothesized modules indicated that there was a low-to-moderate degree of covariation between the different regions of the wing (Table 3). Covariation for fluctuating asymmetry was consistently weaker than for the same partitions of the **P** and **G** matrices of shape.

For all the hypotheses of modularity that were examined (Fig. 2), the comparisons of the strength of covariation between the hypothesized modules with the strength of covariation between subsets of landmarks for alternative partitions showed that there were many partitions that produced weaker covariation than the hypothesized modules (Table 3). This was true regardless of whether all partitions or just spatially contiguous partitions were considered. Accordingly, the covariance structures did not match the predictions from any of the hypotheses of modularity.

HYPOTHETICAL SELECTION

The analysis of hypothetical selection for a relative expansion of the harp area (Fig. 6A) used a selection gradient with coordinated

The covariance matrix for fluctuating asymmetry was similar to the **P** matrix for shape (matrix correlation 0.94 if the diagonal 2×2 blocks were included, 0.81 without the diagonal blocks; $P <$ 0.0001 for both) and to the **G** matrix for shape (matrix correlation 0.88 with the diagonal blocks, 0.66 without the diagonal blocks; $P < 0.0001$ for both). PCA indicated that variation for fluctuating asymmetry was slightly more concentrated in the first three PCs (Fig. 3C) than it was observed for the phenotypic and genetic components of shape (Fig 3A,B). The shape changes for the first three PCs (Fig. 4C) were very similar to those of the **P** and

Table 3. Evaluation of the hypotheses of modularity (Fig. 2) in the P and G matrices for wing shape and in the covariance matrix for fluctuating asymmetry. Each cell of the table contains the *RV* coefficient or multiset *RV* coefficient (*RV*$_M$) and the counts of the alternative partitions that yielded lower *RV* coefficients or multiset *RV* coefficients and the number of partitions, for all partitions into subsets with the corresponding numbers of landmarks ("All:") and for only the contiguous partitions ("Cont.:").

Hypothesis	**P** matrix	**G** matrix	Fluctuating asymmetry
Harp versus remainder (Fig. 2A)	$RV=0.252$	$RV=0.247$	$RV=0.149$
	All: 337/792	All: 131/792	All: 50/792
	Cont.: 130/290	Cont.: 48/290	Cont.: 41/290
Harp, plectrum, remainder (Fig. 2B)	$RV_M=0.268$	$RV_M=0.208$	$RV_M=0.123$
	All: 8,084/8,316	All: 4,160/8,316	All: 2,523/8,316
	Cont.: 635/722	Cont.: 282/722	Cont.: 475/722
Harp, mirror, remainder (Fig. 2C)	$RV_M=0.184$	$RV_M=0.213$	$RV_M=0.138$
	All: 7,286/27,720	All: 12,847/27,720	All: 3,438/27,720
	Cont.: 439/1,422	Cont.: 738/1,422	Cont.: 377/1,422
Harp, mirror, remainder, alternative partition[1]	$RV_M=0.189$	$RV_M=0.212$	$RV_M=0.142$
	All: 3,899/8,316	All: 4,593/8,316	All: 2,443/8,316
	Cont.: 281/722	Cont.: 316/722	Cont.: 264/722

[1]In this partition, landmark 9 is assigned to the harp and not to the mirror.

relative shifts of the landmarks at the anterior edge of the harp (landmarks 2, 6, and 10). The total response included changes not only in the landmarks of the harp, but also in other regions of the wing. Those changes were clearly visible in the correlated response to selection. The magnitude of the direct response, 0.0060, was slightly less than that of the correlated response, 0.0061 (both in units of Procrustes distance). This means that the total response deviated from the direction of the selection gradient by an angle of just over 45°.

The selection gradient for a relatively bigger mirror consisted of relative movements of landmarks 8, 9, 10, 11, and 12 away from the center of the mirror area (Fig. 6B). The total response, again, combined changes in these landmarks as well as the landmarks in other regions of the wing. The direct response, 0.0062, was considerably greater than the correlated response of 0.0042. The direct response differed from the direction of the selection gradient by an angle of 34°.

The third selection gradient represented a localized change of the relative position and orientation of the two landmarks near the ends of the plectrum (landmarks 1 and 4; Fig. 6C). Although this selection gradient was highly localized, it caused shape changes in the whole wing, for instance in the anterior boundary of the harp and in the distal and posterior region of the wing. The magnitude of the direct response to selection was 0.0033 and that of the correlated response was 0.0045. Accordingly, the total response to selection diverged by an angle of 57° from the direction of the selection gradient.

Discussion
GENETIC VARIATION AND CONSTRAINTS

The quantitative genetic analyses indicated that univariate heritabilities for shape variables were moderate to high, ranging from 0.33 to 0.82 (Table 2). These results of the quantitative genetic analysis of shape are comparable with the results from an earlier half-siblings experiment, where heritabilities of wing characteristics ranged from 0.42 to 0.73 (combined estimates from dam and sire components, the range of heritabilities was larger for estimates from only the sire or dam component; Webb and Roff 1992). The observed range of heritabilities of shape variables, although they were derived from this single landmark configuration, covered much of the range observed for morphological traits in general (Roff 1986; Mousseau and Roff 1987). Accordingly, the potential for evolutionary change in response to selection depends substantially on the specific shape variable of interest (or, equivalently, on the direction of selection in shape space).

There is little evidence for any absolute genetic constraints, that is, the absence of genetic variation from some directions of shape space (Lande 1979; Klingenberg 2005; Kirkpatrick 2009). Even the smallest eigenvalue of the **GP**$^-$ matrix, which is an estimate of the lowest heritability in the entire shape data, was 0.082. In a similar study in mouse mandibles, the eigenvalues of the **GP**$^-$ matrix declined from high values to very nearly zero (Klingenberg and Leamy 2001). It should be noted, however, that the eigenvalues of **GP**$^-$ are likely to be biased toward the extremes (Hill and Thompson 1978; Hayes and Hill 1981). The

A
Bigger harp

B
Bigger mirror

C
Shift of the plectrum

Selection gradient

Total response

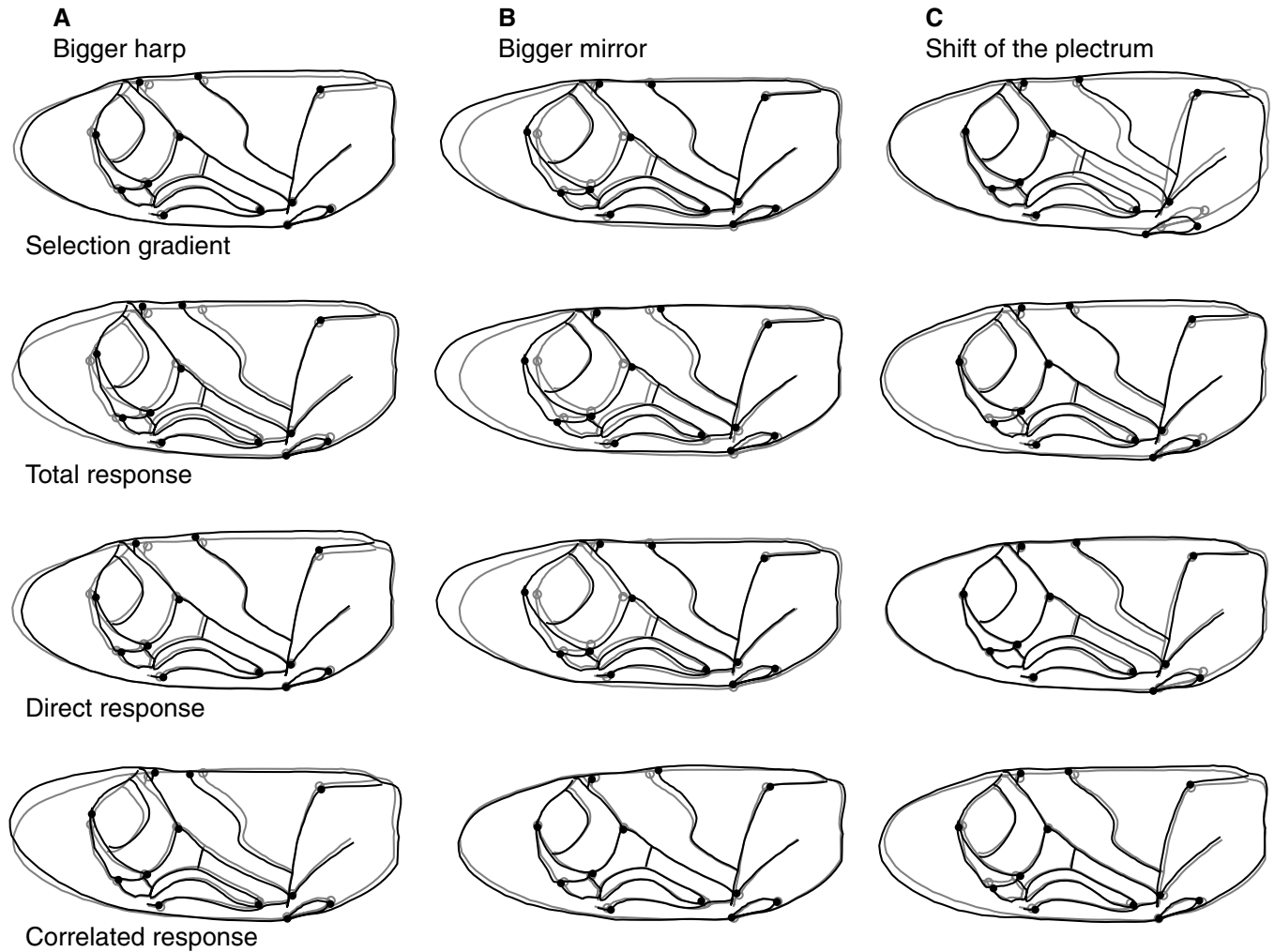Direct response

Correlated response

Figure 6. Analysis of hypothetical selection. (A) Selection for a relative expansion of the harp. (B) Selection for a relative expansion of the mirror. (C) Selection for a relative shift and rotation of the plectrum. For each hypothetical selection regime, the first row indicates the shape variable associated with the selection gradient, the second row shows the total response to selection, and the third and fourth rows show the direct and correlated responses to selection (in the direction of the selection gradient and perpendicular to it). Because the selection gradients per se are not shape changes, they are drawn with an arbitrary scaling factor (0.0005), chosen to provide a convenient visualization of the associated shape variable. The total, direct and correlated responses are drawn as the shape change resulting from the simulations, exaggerated 10-fold to make the shape changes easily visible (changes from the gray outlines and empty circles to the black outlines and solid circles).

lowest of the univariate heritability estimates computed from the shape variables corresponding to the eigenvectors of $\mathbf{GP}^-$ was 0.33, with a standard error of 0.064, and appears to be clearly greater than zero (Table 2). This value also needs to be interpreted with caution, however, as it may overestimate the minimal heritability because of the problems with estimating the eigenvectors of the matrix $\mathbf{GP}^-$. Despite these caveats, there appears to be no evidence for absolute genetic constraints on wing shape in this population of crickets, which would require at least one shape variable with a heritability of zero (or equivalently, a singular $\mathbf{G}$ matrix with at least one dimension devoid of any variation). Similarly, Mezey and Houle (2005) found genetic variation in all dimensions of the shape space in their study of wing shape

in *Drosophila melanogaster*. In contrast, McGuigan and Blows (2007) reported absolute constraints in $\mathbf{G}$ matrices for distance measurements of *D. bunnanda* wings.

It is important to note that different studies use different statistical approaches to identify absolute genetic constraints. For instance, Hine and Blows (2006) discuss tests that use models with absolute constraints as the null hypothesis (the same approach was applied by McGuigan and Blows 2007). The purpose of those tests is to demonstrate the presence of genetic variation in all or at least some dimensions of the phenotypic space, and therefore the null hypothesis of the absence of genetic variation needs to be rejected. If statistical power is limited, this inevitably leads to cases in which the null hypothesis of no genetic variation

in some dimensions cannot be rejected (but note that this does not imply the truth of the null hypothesis, i.e., the existence of an absolute genetic constraint). Similarly, Kirkpatrick (2009) defined a measure of the "effective number of dimensions" as the reciprocal of the proportion of total variance accounted for by the dominant eigenvalue of a covariance matrix. Because the bulk of the total variance is often concentrated in relatively few dimensions of the phenotypic space (e.g., Fig. 3), this number may frequently be much smaller than the total dimensionality (e.g., for the **G** matrix in our study, it would be 4.1 out of a total dimensionality of 20). In these approaches, the primary focus is on ascertaining and the genetic variation that is present in the **G** matrix, not absolute constraints.

The observation that variation is concentrated mostly in some dimensions of phenotypic space or the failure to reject the null hypothesis of dimensions without genetic variation, however, do not provide strong evidence for the existence of absolute genetic constraints. Because most morphological traits have moderate-to-high heritabilities (Mousseau and Roff 1987; Roff 1997), it is to be expected that there will be at least a small amount of genetic variation even in many instances in which the null hypothesis of no genetic variation cannot be rejected. And small amounts of variation can be sufficient for producing an evolutionary response (e.g., Beldade et al. 2002). If the focus of interest is the search for absolute genetic constraints, it may be more effective to identify variables with the lowest heritabilities and to examine those. We used this approach for the example of the cricket wings, but found no evidence for absolute genetic constraints.

The further analyses, however, suggested that even the relative genetic constraints can have a large influence on the evolution of wing shape. Our analyses of hypothetical selection on specific, localized features of shape consistently showed marked deflections of the phenotypic response from the direction of selection, with angles between the directions of selection and the total response ranging from 34° to 57°. As a result, the shape changes in the response were distinctly different from the shape features favored by selection and there was a noticeable correlated response to selection (Fig. 6). This deflection is toward the directions of the dominant eigenvectors of the **G** matrix (Schluter 1996; Walsh and Blows 2009), which, in our dataset, represent changes that are distributed throughout the wing (Fig. 4). In other words, localized selection can be transformed into an integrated response by the relative genetic constraints inherent in the structure of the **G** matrix. Similar results were found in previous studies using hypothetical selection based on data from mice and humans (Klingenberg and Leamy 2001; Martínez-Abadías et al. 2009).

Whether relative constraints from genetic integration hinder or facilitate the evolution of wing shape depends on the direction of selection. In a general survey of the effect of genetic correlations on the rates of adaptive evolution Agrawal and Stinchcombe (2009) found both positive and negative effects (i.e., they can act as both negative and positive evolutionary constraints; Gould 1989; Arthur 2001). For crickets, very rapid evolution of wing morphology has been observed, but the example involves an almost complete loss of the sound-producing structures under selection by a parasitoid fly that kills calling males (Zuk et al. 2006). The basis of this evolutionary change appears to be a single sex-linked mutation (Tinghitella 2008), which is likely to affect a gene that participates in a regulatory switch. For more subtle modifications of the sound-producing structures of the wing, selective forces and the dynamics of evolutionary responses remain to be investigated.

PATTERNS OF INTEGRATION AND MODULARITY IN THE WING

The patterns of integration appeared to be similar at the phenotypic and genetic levels as well as for fluctuating asymmetry. The matrix correlations indicated a high degree of overall similarity among the respective covariance matrices, the principal component analyses yielded similar results as well (Figs. 3 and 4), and the tests of the different hypotheses of modularity produced comparable results for all three covariance matrices (Table 3).

The close similarity of the **G** and **P** matrices reflects the part-whole relationship of these matrices as well as the long-known observation that genetic and phenotypic correlations tend to be similar (e.g., Cheverud 1988; Roff 1995, 1996, 1997; Waitt and Levin 1998). Our results also underscore, however, that this similarity of the **G** and **P** matrices cannot be taken to mean that they are necessarily proportional, as is implicitly assumed by the use of **P** as a substitute for **G** (Cheverud 1988; Ackermann and Cheverud 2004; Weaver et al. 2007; Marroig et al. 2009; Perez and Monteiro 2009). If the proportionality of **G** and **P** matrices is only approximate, that is, if the two matrices are similar but not proportional, the results from computations substituting **P** for **G** will also be only approximate. Proportionality of the **G** and **P** matrices entails that the heritabilities of all shape variables are identical (this is true for any variable formed as a linear combination of the shape variables, including those computed as a projection onto a particular direction in shape tangent space; Klingenberg 2003c; Klingenberg and Monteiro 2005). The large differences found between the estimated heritabilities for different shape variables, combined with their fairly small standard errors, are therefore inconsistent with proportional **G** and **P** matrices (Table 2). Proportionality of the **G** and **P** matrices also implies that the $\mathbf{GP}^-$ matrix is an identity matrix multiplied by a constant and thus that its eigenvalues are all equal (Klingenberg 2003c; Klingenberg and Monteiro 2005), which is also not consistent with our results (Fig. 5). Statistical interpretations are further complicated, however, by the biases in estimating these parameters: as noted above, univariate estimates are likely to underestimate the range of heritabilities because the

eigenvectors of $\mathbf{GP}^-$ may not correctly indicate the shape variables with extreme heritabilities, whereas the estimated eigenvalues of $\mathbf{GP}^-$ are expected to be too extreme due to the known bias (Hill and Thompson 1978; Hayes and Hill 1981). Because these biases are in opposite directions, they permit at least an informal assessment of the effects. The ranges of eigenvalues of the $\mathbf{GP}^-$ and of the heritabilities of scores for different shape variables might be used to gauge the degree of uncertainty introduced by the substitution of $\mathbf{P}$ for $\mathbf{G}$, for instance, in retrospective analyses of selection and drift (e.g., Ackermann and Cheverud 2004; Weaver et al. 2007; Perez and Monteiro 2009). In our example, this uncertainty would be considerable. These observations add to earlier caveats, based on theoretical arguments or empirical evidence, against using $\mathbf{P}$ as a surrogate for $\mathbf{G}$ (e.g., Willis et al. 1991; Klingenberg and Leamy 2001; Bégin and Roff 2004; Kruuk et al. 2008).

The results also indicate a clear similarity between the covariance matrix of fluctuating asymmetry and the $\mathbf{G}$ and $\mathbf{P}$ matrices computed from the averages of the left and right wings. This result is consistent with previous findings from studies of wing shape in other insects that found similarity on the patterns of variation for fluctuating asymmetry and individual variation (Klingenberg and McIntyre 1998; Klingenberg and Zaklan 2000; Klingenberg et al. 2001; Breuker et al. 2006b), but there are also studies with mixed results (Debat et al. 2006) and others that did not find such a similarity (Santos et al. 2005). Likewise, for mice and other mammals, some studies found a correspondence (Leamy 1993; Klingenberg et al. 2003; Badyaev and Foresman 2004; Zeldhitch et al. 2008), whereas others found discrepancies (Debat et al. 2000; Hallgrímsson et al. 2004; Willmore et al. 2005) or different results depending on the context of the comparison (Drake and Klingenberg 2010). It is not clear how these patterns, as well as the agreement or discrepancies among them, relate to the underlying processes of developmental buffering (i.e., canalization and developmental stability; Debat et al. 2000, 2008, 2009; Debat and David 2001; Breuker et al. 2006b).

The comparison of patterns at the three levels of variation can be used to make inferences on the origins of variation. Fluctuating asymmetry results from small random differences in the development of the left and right wings of the individuals in the sample (Klingenberg 2003a). It therefore reflects the intrinsic tendency of the developmental system to generate new variation. Because wing development occurs relatively late in the ontogeny of hemimetabolous insects such as crickets (Heming 2003), small developmental aberrations in the left or right wings are unlikely to have effects on viability, and the patterns of fluctuating asymmetry of wing shape are unaffected by processes such as internal selection (Whyte 1965; Arthur 1997; Fusco 2001). Moreover, because insect wings are not used before they are fully formed, effects of external function on the development can also be ruled out.

Similarity of the $\mathbf{G}$ and $\mathbf{P}$ matrices to the covariance matrix for fluctuating asymmetry therefore indicates that the developmental system is a primary factor molding the expression of variation at the genetic and phenotypic levels. Differences, by contrast, can indicate the effect of selection and similar processes that influence the composition of populations. Our data indicate a high degree of similarity between the three covariance matrices, confirming the strong influence of developmental factors on the structure of shape variation.

This common pattern appears to include a considerable degree of morphological integration throughout the whole wing. The tests of the various hypotheses of modularity did not conform to the predictions derived from those hypotheses, because the covariation observed between the subsets of landmarks corresponding to the hypothesized modules was not clearly less than the covariation between randomly assembled subsets of the corresponding numbers of landmarks (Table 2; Klingenberg 2009). This result is inconsistent with what is expected under the hypotheses of modularity, but is consistent with the alternative of strong integration throughout the entire wing, as it was found for other insects (e.g., Klingenberg and Zaklan 2000; Klingenberg et al. 2001; Klingenberg 2009).

Because this pattern of covariation throughout the wing applies to fluctuating asymmetry, which derives from spontaneous variation in developmental processes, there must be developmental interactions that transmit the effects of this variation throughout the entire developing wing (Klingenberg 2003b, 2005, 2008b). This indicates that morphological integration in the wing has a strong developmental basis, which may relate to the important role of signaling that has been demonstrated for *Drosophila* (Blair 2007) and may also apply to other insects (Heming 2003). It is to be expected that such strong developmental integration enables the developmental system to impose a common pattern on the morphological expression of variation from different sources. Congruence of patterns of integration at different levels, such as the $\mathbf{G}$ and $\mathbf{P}$ matrices and fluctuating asymmetry, should therefore be more likely if developmental integration in a structure is high. In contrast, if a structure consists of separate modules with only little developmental integration, greater discrepancies between the patterns of integration at different levels are to be expected. This hypothesis remains to be tested.

The overall similarities of the three covariance matrices, in spite of the differences in the underlying processes giving rise to variation, suggest that the developmental system producing the wings imparts a common pattern of integration to the expression of variation from all those processes. As is evident from the differences between the $\mathbf{P}$ and $\mathbf{G}$ matrices, however, the resulting patterns of variation need not be completely congruent because particular sources of variation may differentially affect particular developmental pathways in the overall system.

INTEGRATION AND FUNCTIONAL ADAPTATION

The influence of morphological integration in the wing on the potential for evolutionary change is also evident from the analyses of hypothetical selection (Fig. 6). We used localized shape changes in the mirror, harp, or plectrum as the selection gradients, and consistently obtained correlated responses in different regions of the wing. These correlated responses were substantial, with landmark shifts of similar magnitudes as those of the landmarks targeted by the selection gradients.

As a consequence, selection on individual functional features of the cricket wing will produce correlated responses in other parts. The mechanism for sound production depends on the geometry of the wing. For instance, the area of the harp has been shown to correlate with the dominant frequency (Simmons 1995; Simmons and Ritchie 1996), which is in agreement with expectations from the "clockwork cricket" model in which resonance in the harp determines the frequency of sound (Elliott and Koch 1985; Koch et al. 1988; Bennet-Clark and Bailey 2002). In contrast, Webb and Roff (1992) found no correlation between mirror area and the dominant frequency of song, which conforms with the role of the mirror as a subsidiary, but not the main resonator in the wing (Bennet-Clark 2003). Moreover, it is likely that the arrangement of parts also is important (e.g., the various flexible regions and the phase-shifting mechanism between plectrum and harp; Bennet-Clark 2003; Montealegre-Z et al. 2009). These considerations suggest that selection on specific song characteristics makes specific demands for changes in wing shape. Deflections due to the correlated response of shape in turn are likely to have effects on acoustic properties.

Selection for changes of specific song characteristics does not necessarily lead to changes of specific parts of the sound-producing structures of the wing, because there may also be different morphological alterations that can bring about the same change of song. For instance, the dominant frequency is not only correlated to the area of the harp in the wing (Simmons 1995; Simmons and Ritchie 1996), but has also been shown to correlate with measures of overall body size such as femur length or pronotum width (Simmons and Zuk 1992; Webb and Roff 1992; Simmons 1995). Whether selection on an acoustic feature such as frequency leads to a response in wing shape or a change in overall body size depends on other selective pressures on morphological and life-history traits (Roff 1992). It is not clear whether song attributes other than frequency also have such alternative evolutionary pathways for achieving the same functional change may be achieved by a specific change in the sound-producing structures of the wing or by a general morphological or physiological change.

The study of structures where developmental and functional modularity are not congruent are particularly informative for studies of the evolution of modularity (Breuker et al. 2006a). Our study

considered an example in which parts of a single developmental module are differentiated for special functions. Other studies have considered cases in which developmentally separate structures are integrated to form a single functional unit. For instance, Laffont et al. (2009) examined shape variation of the three lower molar teeth in voles, which are coordinated to form a single grinding surface. The results of their analyses indicated both modularity at the level of individual teeth and integration within the entire molar row. Similarly, the jaw structures of cichlid fish are composed of multiple parts and show complex patterns of functional and developmental integration (Albertson et al. 2005; Albertson and Kocher 2006). The relationship between developmental and functional modularity, and whether such modularity indeed exists, still remains to be explored. The relationship between development, form, and function may be key to understanding diversification in systems like these (Alfaro et al. 2005; Wainwright 2009).

Conclusion

Two hypotheses have been proposed about the role of developmental and genetic modules in relation to functional modules. One of them, the matching hypothesis, states that the developmental system and genetic architecture evolve to match the subdivision of organismal structures into functional modules (Cheverud 1984; Wagner and Altenberg 1996). The alternative hypothesis posits that developmental modules are evolutionarily conservative and act primarily as constraints or biases on adaptive evolution (Raff 1996; Arthur 2001, 2002). To distinguish between the two hypotheses, Breuker et al. (2006a) proposed a research program focusing particularly on structures in which functional and developmental modules are discordant. This study in the cricket wing is an example of such an analysis, because the wing, which is a single developmental module, contains several functionally distinct parts such as the harp, mirror, and surrounding structures involved in sound production (Fig. 1A). We found that these functionally distinct areas of the wing were not matched by corresponding modules in the genetic or phenotypic covariance structure. Instead, the structure of the **G** and **P** matrices reflected the developmental integration throughout the entire wing. In this case, therefore, the matching hypothesis is rejected.

LITERATURE CITED

Ackermann, R. R., and J. M. Cheverud. 2004. Detecting genetic drift versus selection in human evolution. Proc. Natl. Acad. Sci. USA 101:17946–17951.

Agrawal, A. F., and J. R. Stinchcombe. 2009. How much do genetic covariances alter the rate of adaptation? Proc. R. Soc. Lond. B 276:1183–1191.

Albertson, R. C., and T. D. Kocher. 2006. Genetic and developmental basis of cichlid trophic diversity. Heredity 97:211–221.

Albertson, R. C., J. T. Streelman, T. D. Kocher, and P. C. Yelick. 2005. Integration and evolution of the cichlid mandible: the molecular basis of alternate feeding strategies. Proc. Natl. Acad. Sci. USA 102:16287–16292.

Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. Am. Nat. 165:E140–E154.

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.

Arthur, W. 1997. The origin of animal body plans: a study in evolutionary developmental biology. Cambridge Univ. Press, Cambridge.

———. 2001. Developmental drive: an important determinant of the direction of phenotypic evolution. Evol. Dev. 3:271–278.

———. 2002. The interaction between developmental bias and natural selection: from centipede segments to a general hypothesis. Heredity 89:239–246.

Badyaev, A. V., and K. R. Foresman. 2004. Evolution of morphological integration. I. Functional units channel stress-induced variation in shrew mandibles. Am. Nat. 163:868–879.

Bégin, M., and D. A. Roff. 2004. From micro- to macroevolution through quantitative genetic variation: positive evidence from field crickets. Evolution 58:2287–2304.

Bégin, M., D. A. Roff, and V. Debat. 2004. 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. J. Evol. Biol. 17:1255–1267.

Beldade, P., K. Koops, and P. M. Brakefield. 2002. Developmental constraints versus flexibility in morphological evolution. Nature 416:844–847.

Bennet-Clark, H. C. 1999. Resonators in insect sound production: how insects produce loud pure-tone songs. J. Exp. Biol. 202:3347–3357.

———. 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. J. Exp. Biol. 206:1479–1496.

Bennet-Clark, H. C., and W. J. Bailey. 2002. Ticking of the clockwork cricket: the role of the escapement mechanism. J. Exp. Biol. 205:613–625.

Blair, S. S. 2007. Wing vein patterning in *Drosophila* and the analysis of intercellular signaling. Annu. Rev. Cell Dev. Biol. 23:293–319.

Bock, W. J., and G. von Wahlert. 1965. Adaptation and the form–function complex. Evolution 19:269–299.

Bookstein, F. L. 1989. Principal warps: thin-plate splines and the decomposition of deformations. IEEE Trans. Pattern Anal. Mach. Intell. 11:567–585.

Breuker, C. J., V. Debat, and C. P. Klingenberg. 2006a. Functional evo-devo. Trends Ecol. Evol. 21:488–492.

Breuker, C. J., J. S. Patterson, and C. P. Klingenberg. 2006b. A single basis for developmental buffering of *Drosophila* wing shape. PLoS ONE 1:e7.

Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. 110:155–171.

———. 1988. A comparison of genetic and phenotypic correlations. Evolution 42:958–968.

Debat, V., and P. David. 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol. Evol. 16:555–561.

Debat, V., P. Alibert, P. David, E. Paradis, and J.-C. Auffray. 2000. Independence between developmental stability and canalization in the skull of the house mouse. Proc. R. Soc. Lond. B Biol. Sci. 267:423–430.

Debat, V., C. C. Milton, S. Rutherford, C. P. Klingenberg, and A. A. Hoffmann.

2006. Hsp90 and the quantitative variation of wing shape in *Drosophila melanogaster*. Evolution 60:2529–2538.

Debat, V., R. Cornette, A. B. Korol, E. Nevo, D. Soulet, and J. R. David. 2008. Multidimensional analysis of *Drosophila* wing variation in Evolution Canyon. J. Genet. 87:407–419.

Debat, V., A. Debelle, and I. Dworkin. 2009. Plasticity, canalization, and developmental stability of the *Drosophila* wing: joint effects of mutations and developmental temperature. Evolution 63:2864–2876.

Drake, A. G., and C. P. Klingenberg. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. Am. Nat. 175:289–301.

Dryden, I. L., and K. V. Mardia. 1998. Statistical shape analysis. Wiley, Chichester.

Elliott, C. J. H., and U. T. Koch. 1985. The clockwork cricket. Naturwissenschaften 72:150–153.

Escoufier, Y. 1973. Le traitement des variables vectorielles. Biometrics 29:751–760.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, Essex.

Fusco, G. 2001. How many processes are responsible for phenotypic evolution? Evol. Dev. 3:279–286.

Goodall, C. R. 1991. Procrustes methods in the statistical analysis of shape. J. R. Statist. Soc. B 53:285–339.

Gould, S. J. 1989. A developmental constraint in *Cerion*, with comments on the definition and interpretation of constraint in evolution. Evolution 43:516–539.

———. 2002. The structure of evolutionary theory. Harvard Univ. Press, Cambridge, MA.

Groeneveld, E., M. Kovač, and N. Mielenz. 2008. VCE user's guide and reference manual, version 6.0. Institute of Farm Animal Genetics, Neustadt, Germany.

Hallgrímsson, B., K. Willmore, C. Dorval, and D. M. L. Cooper. 2004. Craniofacial variability and modularity in macaques and mice. J. Exp. Zool. 302B:207–225.

Hansen, T. F., and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. J. Evol. Biol. 21:1201–1219.

Hayes, J. F., and W. G. Hill. 1981. Modification of estimates of parameters in the construction of genetic selection indices ('bending'). Biometrics 37:483–493.

Heming, B. S. 2003. Insect development and evolution. Cornell Univ. Press, Ithaca, NY.

Hill, W. G., and R. Thompson. 1978. Probabilities of non-positive definite between-group or genetic covariance matrices. Biometrics 34:429–439.

Hine, E., and M. W. Blows. 2006. Determining the effective dimensionality of the genetic variance-covariance matrix. Genetics 173:1135–1144.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proc. Natl. Acad. Sci. USA 98:9157–9160.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.

Jolliffe, I. T. 2002. Principal component analysis. Springer-Verlag, New York.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Kirkpatrick, M. 2009. Patterns of quantitative genetic variation in multiple dimensions. Genetica (Dordr.) 136:271–284.

Klingenberg, C. P. 2003a. A developmental perspective on developmental instability: theory, models and mechanisms. Pp. 14–34 *in* M. Polak,

ed. Developmental instability: causes and consequences. Oxford Univ. Press, New York.

———. 2003b. Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the developmental origins of morphological integration. Pp. 427–442 *in* M. Polak, ed. Developmental instability: causes and consequences. Oxford Univ. Press, New York.

———. 2003c. Quantitative genetics of geometric shape: heritability and the pitfalls of the univariate approach. Evolution 57:191–195.

———. 2005. Developmental constraints, modules and evolvability. Pp. 219–247 *in* B. Hallgrímsson and B. K. Hall, eds. Variation: a central concept in biology. Elsevier, Burlington, MA.

———. 2008a. MorphoJ. Faculty of Life Sciences, University of Manchester, Manchester. http://www.flywings.org.uk/MorphoJ_page.htm

———. 2008b. Morphological integration and developmental modularity. Annu. Rev. Ecol. Evol. Syst. 39:115–132.

———. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a-priori hypotheses. Evol. Dev. 11:405–421.

Klingenberg, C. P., and L. J. Leamy. 2001. Quantitative genetics of geometric shape in the mouse mandible. Evolution 55:2342–2352.

Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52:1363–1375.

Klingenberg, C. P., and L. R. Monteiro. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. Syst. Biol. 54:678–688.

Klingenberg, C. P., and S. D. Zaklan. 2000. Morphological integration between developmental compartments in the *Drosophila* wing. Evolution 54:1273–1285.

Klingenberg, C. P., A. V. Badyaev, S. M. Sowry, and N. J. Beckwith. 2001. Inferring developmental modularity from morphological integration: analysis of individual variation and asymmetry in bumblebee wings. Am. Nat. 157:11–23.

Klingenberg, C. P., M. Barluenga, and A. Meyer. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56:1909–1920.

Klingenberg, C. P., K. Mebus, and J.-C. Auffray. 2003. Developmental integration in a complex morphological structure: how distinct are the modules in the mouse mandible? Evol. Dev. 5:522–531.

Koch, U. T., C. J. H. Elliott, K.-H. Schäffner, and H.-U. Kleindienst. 1988. The mechanics of stridulation of the field cricket *Gryllus campestris*. J. Comp. Physiol. A Sens. Neural Behav. Physiol. 162:213–223.

Kruuk, L. E. B., J. Slate, and A. J. Wilson. 2008. New answers for old questions: the evolutionary quantitative genetics of wild animal populations. Annu. Rev. Ecol. Evol. Syst. 39:525–548.

Laffont, R., E. Renvoisé, N. Navarro, P. Alibert, and S. Montuire. 2009. Morphological modularity and assessment of developmental processes within the vole dental row (*Microtus arvalis*, Arvicolinae, Rodentia). Evol. Dev. 11:302–311.

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

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Laubichler, M. D., and J. Maienschein, eds. 2009. Form and function in developmental evolution. Cambridge Univ. Press, Cambridge.

Leamy, L. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. Genetica 89:139–153.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Marroig, G., L. T. Shirai, A. Porto, F. B. de Oliveira, and V. De Conto. 2009. The evolution of modularity in the mammalian skull II: evolutionary consequences. Evol. Biol. 36:136–148.

Martínez-Abadías, N., C. Paschetta, S. de Azevedo, M. Esparza, and R. González-José. 2009. Developmental and genetic constraints on neurocranial globularity: insights from analyses of deformed skulls and quantitative genetics. Evol. Biol. 36:37–56.

McGuigan, K., and M. W. Blows. 2007. Phenotypic and genetic covariance structure of drosophilid wings. Evolution 61:902–911.

Meyer, K. 2007. WOMBAT—A tool for mixed model analyses in quantitative genetics by restricted maximum likelihood (REML). J. Zhejiang Univ. Sci. B 8:815–821.

Mezey, J. G., and D. Houle. 2005. The dimensionality of genetic variation for wing shape in *Drosophila melanogaster*. Evolution 59:1027–1038.

Montealegre-Z, F. 2009. Scale effects and constraints for sound production in katydids (Orthoptera: Tettigoniidae): correlated evolution between morphology and signal parameters. J. Evol. Biol. 22:355–366.

Montealegre-Z, F., J. F. C. Windmill, G. K. Morris, and D. Robert. 2009. Mechanical phase shifters for coherent acoustic radiation in the stridulating wings of crickets: the plectrum mechanism. J. Exp. Biol. 212:257–269.

Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness components. Heredity 59:181–197.

Myers, E. M., F. J. Janzen, D. C. Adams, and J. K. Tucker. 2006. Quantitative genetics of plastron shape in slider turtles (*Trachemys scripta*). Evolution 60:563–572.

Nocke, H. 1971. Biophysik der Schallerzeugung durch die Vorderflügel der Grillen. Z. Vgl. Physiol. 74:272–314.

Perez, S. I., and L. R. Monteiro. 2009. Nonrandom factors in modern human morphological diversification: a study of craniofacial variation in southern South American populations. Evolution 63:978–993.

Prestwich, K. N., K. M. Lenihan, and D. M. Martin. 2000. The control of carrier frequency in cricket calls: a refutation of the subalar–tegminal resonance/auditory feedback model. J. Exp. Biol. 203:585–596.

Raff, R. A. 1996. The shape of life: genes, development and the evolution of animal form. Univ. of Chicago Press, Chicago.

Riedl, R. 1975. Die Ordnung des Lebendigen: Systembedingungen der Evolution. Parey, Hamburg.

Roff, D. A. 1986. The genetic basis of wing dimorphism in the sand cricket, *Gryllus firmus* and its relevance to the evolution of wing dimorphisms in insects. Heredity 57:221–231.

———. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.

———. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity 74:481–490.

———. 1996. The evolution of genetic correlations: an analysis of patterns. Evolution 50:1392–1403.

———. 1997. Evolutionary quantitative genetics. Chapman & Hall, New York.

———. 2000. The evolution of the **G** matrix: selection or drift? Heredity 84:135–142.

———. 2007. A centennial celebration for quantitative genetics. Evolution 61:1017–1032.

Roff, D. A., and T. A. Mousseau. 1987. Quantitative genetics and fitness: lessons from *Drosophila*. Heredity 58:103–118.

Santos, M., P. Fernández Iriarte, and W. Céspedes. 2005. Genetics and geometry of canalization and developmental stability in *Drosophila subobscura*. BMC Evol. Biol. 5:7.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Simmons, L. W. 1995. Correlates of male quality in the field cricket, *Gryllus campestris* L.: age, size, and symmetry determine pairing success in field populations. Behav. Ecol. 6:376–381.

Simmons, L. W., and M. G. Ritchie. 1996. Symmetry in the song of crickets. Proc. R. Soc. Lond. B 263:305–311.

Simmons, L. W., and M. Zuk. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: the effects of age, size and parasite load. Anim. Behav. 44:1145–1152.

Stige, L. C., B. David, and P. Alibert. 2006. On hidden heterogeneity in directional asymmetry—can systematic bias be avoided? J. Evol. Biol. 19:492–499.

Tinghitella, R. M. 2008. Rapid evolutionary change in a sexual signal: genetic control of the mutation 'flatwing' that renders male field crickets (*Teleogryllus oceanicus*) mute. Heredity 100:261–267.

Veazey, J. N., C. A. R. Kay, T. J. Walker, and W. H. Whitcomb. 1976. Seasonal abundance, sex ratio, and mactroptery of field crickets in northern Florida. Ann. Entomol. Soc. Am. 69:375–380.

Visscher, P. M., W. G. Hill, and N. R. Wray. 2008. Heritability in the genomics era—concepts and misconceptions. Nat. Rev. Genet. 9:255–266.

Wagner, G. P., and L. Altenberg. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976.

Wainwright, P. C. 2009. Innovation and diversity in functional morphology. Pp. 132–152 *in* M. D. Laubichler and J. Maienschein, eds. Form and function in developmental evolution. Cambridge Univ. Press, Cambridge.

Waitt, D. E., and D. A. Levin. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. Heredity 80:310–319.

Walsh, B., and M. W. Blows. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. Annu. Rev. Ecol. Evol. Syst. 40:41–59.

Weaver, T. D., C. C. Roseman, and C. B. Stringer. 2007. Were neanderthal and modern human cranial differences produced by natural selection or genetic drift? J. Hum. Evol. 53:135–145.

Webb, K. L., and D. A. Roff. 1992. The quantitative genetics of sound production in *Gryllus firmus*. Anim. Behav. 44:823–832.

Whyte, L. L. 1965. Internal factors in evolution. Tavistock Publications, London.

Willis, J. H., J. A. Coyne, and M. Kirkpatrick. 1991. Can one predict the evolution of quantitative characters without genetics? Evolution 45:441–444.

Willmore, K. E., C. P. Klingenberg, and B. Hallgrímsson. 2005. The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. Evolution 59:898–909.

Zelditch, M. L., A. R. Wood, R. M. Bonett, and D. L. Swiderski. 2008. Modularity of the rodent mandible: integrating bones, muscles, and teeth. Evol. Dev. 10:756–768.

Zuk, M., J. T. Rotenberry, and R. M. Tinghitella. 2006. Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. Biol. Lett. 2:521–524.

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