

The potential influence of morphology on the evolutionary divergence of an acoustic signal

W. R. PITCHERS*†, C. P. KLINGENBERG‡, T. TREGENZA†, J. HUNT† & I. DWORKIN*

*Department of Zoology, Program in Ecology Evolutionary Biology and Behavior, Michigan State University, East Lansing, MI, USA

†Centre for Ecology and Conservation, University of Exeter, Penryn Campus, Cornwall, UK

‡Faculty of Life Sciences, The University of Manchester, Manchester, UK

Keywords:

advertisement call;
common garden;
field cricket;
geometric morphometrics;
sexual selection.

Abstract

The evolution of acoustic behaviour and that of the morphological traits mediating its production are often coupled. Lack of variation in the underlying morphology of signalling traits has the potential to constrain signal evolution. This relationship is particularly likely in field crickets, where males produce acoustic advertisement signals to attract females by stridulating with specialized structures on their forewings. In this study, we characterize the size and geometric shape of the forewings of males from six allopatric populations of the black field cricket (*Teleogryllus commodus*) known to have divergent advertisement calls. We sample from each of these populations using both wild-caught and common-garden-reared cohorts, allowing us to test for multivariate relationships between wing morphology and call structure. We show that the allometry of shape has diverged across populations. However, there was a surprisingly small amount of covariation between wing shape and call structure within populations. Given the importance of male size for sexual selection in crickets, the divergence we observe among populations has the potential to influence the evolution of advertisement calls in this species.

Introduction

Behavioural traits, particularly social behaviours, can evolve more rapidly than morphological traits (Moore, 1997; Moore *et al.*, 1998; Puniamoorthy *et al.*, 2009), and this can occur for a number of reasons (Losos, 1990a,b). While the expression of both morphology and behaviour can be 'switched' (plasticity) by appropriate environmental stimuli, the expression of a behaviour can be induced near-immediately. Thus, there may be many more reliable cues available for regulating behaviour and a greater opportunity for behavioural diversity (West-Eberhard, 1989). Yet, the range of behaviours available to an animal will ultimately be constrained by its morphology; an animal without wings cannot fly, regardless of its behavioural motivation to do so. More interestingly, the adaptation

of an animal's morphology to one behaviour may provide the opportunity for the evolution of behaviours that utilize that morphology in other ways; for example, claws used for digging can be useful for defence against predators, or a balancing tail used to signal to conspecifics.

Researchers have also described how behaviour may influence the evolvability of morphology (Wcislo, 1989; West-Eberhard, 2003) because selection on a behaviour will apply indirect selection on morphological features utilized in its performance. In some cases, behaviours and the specialized morphologies that support them are intimately linked, for example phase polyphenisms in migratory locusts (Pener & Yerushalmi, 1998), male horn dimorphism and reproductive tactics in dung beetles (Moczek & Emlen, 2000) and caste polyphenisms in eusocial insects (Nijhout, 1999). In these cases, the alternatives are discrete and intermediates are rare, but variation in most traits is quantitative, making the relationship less easy to elucidate. A further complication is that many morphological traits will be utilized in many (if not all) behaviours expressed by the organism,

Correspondence: William R. Pitchers, Department of Zoology, Program in Ecology Evolutionary Biology and Behavior, Michigan State University, East Lansing, MI 48824, USA. Tel.: +1 51743 26730; fax: +1 51743 22789; e-mail: pitchers@msu.edu

meaning that conflicting indirect selection from different behaviours has the potential to influence the evolution of morphology. For example, wings in many *Drosophila* species are used for flight, the production of courtship song (Bennet-Clark & Ewing, 1969) and visual displays for both courtship and territoriality (Chen *et al.*, 2002; Lasbleiz *et al.*, 2006). Aspects of wing morphology may therefore experience contrasting selection for flight performance and for social behaviours. The evolution of those aspects of morphology likely to have been shaped by indirect selection via the performance of behaviours that are essential to fitness – such as advertising to potential mates – is thus likely to be complex and interesting.

In field crickets (Orthoptera: Gryllidae), males display to potential mates acoustically (Alexander, 1962; Zuk, 1987; Andersson, 1994; Gerhardt & Huber, 2002). Male field crickets produce these calls using specialized stridulatory adaptations of the forewings (tegmina). As the forewings of most field crickets are not important for flight, their morphology may be principally the result of adaptation in response to sexual selection for the call characteristics they produce (Nocke, 1971; West-Eberhard, 1989; Bennet-Clark & Bailey, 2002; Bennet-Clark, 2003; Montealegre-Z, 2009). These acoustic signals convey information about species identity and male condition that females can use to evaluate potential mates from a distance (Simmons & Zuk, 1992; Simmons, 1995; Ritchie *et al.*, 1995; Scheuber *et al.*, 2003a). When raised, a plectrum on the (typically) left forewing engages with a toothed file on the ventral surface of the right forewing, although the functional role of the wing varies within (temporally) and between individuals. The movement of the plectrum over the file as the forewings are closed sets up a vibration in the resonant structures of both wings – principally the ‘harp’ and ‘file’, but also the ‘anal area’ (Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003). As the plectrum is moved along the file, the two opposed structures function as an escapement (analogous to the devices that regulate the speed of clockwork mechanisms), linking the catch/release of the plectrum to the resonant frequency of the forewings (Koch *et al.*, 1988; Prestwich *et al.*, 2000; Bennet-Clark and Bailey, 2002).

In the Australian black field crickets (*Teleogryllus commodus*), the structure of the advertisement call begins with a single chirp sequence, which is followed by a variable number of trill sequences (Bentley & Hoy, 1972; Hill *et al.*, 1972, see Fig. 1). Female *T. commodus* show preferences for both temporal (Pollack & Hoy, 1979) and spectral (Hennig & Weber, 1997) properties of this call, resulting in a regime of multivariate stabilizing sexual selection (Brooks *et al.*, 2005; Bentsen *et al.*, 2006) on a suite of covarying call traits. Under such selection, mutations that lead to greater functional or developmental covariation between traits are predicted to be favoured, which can lead to an increase in linkage disequilibrium and pleiotropy (Phillips & Arnold, 1989; Arnold, 1992; Sinervo & Svensson, 2002; Phillips & McGuigan, 2006). If this process were to persist over many generations, it would be expected to facilitate trait integration (Lande, 1980; Cheverud, 1984; McGlothlin *et al.*, 2005), whereby the multivariate pattern and magnitude of correlations between traits may exert an influence on the direction and rate at which those traits are able to evolve (Schluter, 1996).

Previously, we have shown that both temporal and spectral call measures vary between geographically distinct *T. commodus* populations, and this variation has a genetic basis (Pitchers *et al.*, 2013b). Despite these robust inter-population differences in the individual call measures, the intra-population pattern of covariance among call measures (**P** matrix) is relatively stable between populations, despite population divergence (Pitchers *et al.*, 2013b). The degree to which this has been maintained is due to the similarities of multivariate stabilizing selection (Bentsen *et al.*, 2006) on call traits vs. other factors (conflicting selective forces or insufficient genetic variation for wing morphology), is unclear. In the light of research showing a link between the morphology of stridulatory organs and the nature of the calls produced (Simmons & Ritchie, 1996; Montealegre-Z, 2009), it is reasonable to predict that the shape of cricket forewings has been influenced by sexual selection on acoustic performance; though, this is poorly understood. The best-supported relationship between gross morphology and acoustic structure in crickets is the negative correlation between frequency

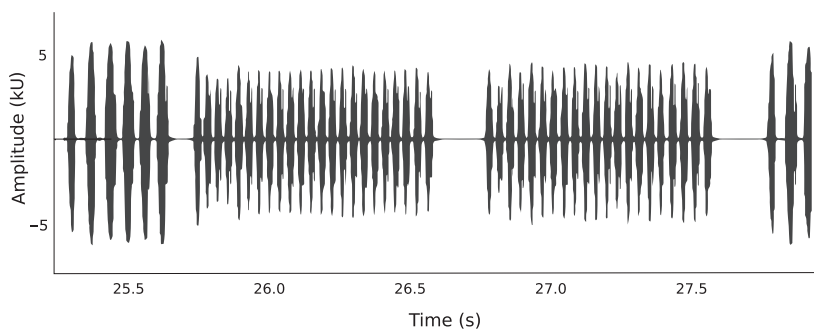


Fig. 1 The five structural traits measured on the advertisement call of a male *Teleogryllus commodus*; chirp inter-pulse interval (CIPD) and inter-call duration (ICD) measured in seconds, dominant frequency (DF) measured in kHz, chirp pulse number (CPN) and trill number (TN) were counted (e.g. 6 chirp pulses and 2 trills for this call).

and body size (Gerhardt & Huber, 2002; Scheuber *et al.*, 2003a,b). This correlation (also seen in anurans; Wagner & Sullivan, 1995, and particularly strongly in katydids Montealegre-Z, 2009) is assumed to be informative to females where large males are preferred, a mechanistic relationship whereby larger males bear larger resonant structures that produce lower frequencies. However, a review of the literature found no clear relationship between call frequency and body size in crickets (Verburgt & Ferguson, 2009). Indeed, the same study presented experimental evidence that female *Gryllus bimaculatus* cannot reliably detect male body size acoustically. Additionally, in *Gryllus campestris*, dominant frequency has been shown to change with male age; with older males calling with lower (i.e. more attractive) frequencies (Jacot *et al.*, 2007). In fact, despite the assumption in the literature that body size predicts call frequency (e.g. Gerhardt & Huber, 2002; Jacot *et al.*, 2005), a surprising number of studies report no such association (Simmons, 1988; Simmons & Zuk, 1992; Webb & Roff, 1992; Simmons, 1995; Gray, 1997; Ryder & Siva-Jothy, 2000; Ferreira & Ferguson, 2002; Bateman *et al.*, 2004; Hunt *et al.*, 2004). Clearly, the relationship between morphology and acoustic performance is not well understood (See Supplement for summary).

Most of the studies that have tested for a relationship between male wing morphology and acoustic performance focus on the length and/or area of wing structures, and how they correlate with one or a few call parameters. Neither linear measurements nor measures of area calculated from them are particularly useful for describing the complex shape of the stridulatory organs on the wing, which may also be an important determinant of call structure. In fact, recent findings suggest that the geometry of the wing may be crucially important in understanding the resonance of the wing (Mhatre *et al.*, 2012). In the light of this, geometric morphometrics is the appropriate tool – enabling us to describe shape in a multivariate fashion, and permitting statistical comparison of different forms, and covariation between shape and other variables such as fitness (Klingenberg & Ekau, 1996; Adams & Rohlf, 2000; Gómez *et al.*, 2006).

Here, we present an analysis of covariation between forewing size and shape with call structure in male *T. commodus*. We measured crickets from six populations known to be genetically divergent for call parameters (Pitchers *et al.*, 2013a,b). Moreover, we measured call parameters that are known to be under strong sexual selection in this species in both the laboratory (Brooks *et al.*, 2005; Hunt *et al.*, 2007) and field (Bentsen *et al.*, 2006). From each population, we measured two cohorts: one wild-caught and one reared under common-garden conditions. In addition to a multivariate characterization of advertisement call, we employ geometric morphometric methods (Dryden & Mardia,

1998; Klingenberg, 2010) to quantify the size and shape of the entire forewing. As the primary function of the forewing (tegmina) is to produce acoustic signals, characterizing the relationship between morphology and acoustic structure should reveal the extent to which the evolution of calls may be influenced by integration between shape, size and acoustic performance. We find that forewing shape and size vary among populations, and more importantly, the form of forewing shape allometry also varies. This means that the relationship between wing size and wing shape has diverged among populations. Given that the call is presumed to carry information about size, this pattern has the potential to influence the divergence of these populations.

Materials and methods

Experimental animals

Adult *Teleogryllus commodus* were collected from six widely separated locations across the species' range (see Fig. 2 – populations are referred to subsequently as Australian Capital Territory (ACT), Kioloa (KL), South Australia (SA), Smith's Lakes (SL), Tasmania (TAS) and Western Australia (WA)). The males' advertisement calls were recorded, and their wings were removed and mounted (see protocol later). Females were provided with cotton wool egg-pads upon which to oviposit, and the resulting offspring were used to establish laboratory colonies representing each population [for further details, see (Pitchers *et al.*, 2013b)]. Laboratory-reared animals were kept at a constant temperature of 28 °C, with a 16:8 h light/dark cycle. They were kept in large (100 L) plastic storage containers, supplied with water and fed *ad libitum* on 'Go-Cat senior' cat-food pellets (Nestlé Purina PetCare). Stocks were replenished by rearing the offspring of 100 randomly selected adult pairs per generation. After three generations of captive rearing, adult males' calls were again recorded and their wings removed and mounted for morphometric measurement.

Call recording and analysis

Male calling song was recorded between 8 and 10 days post-eclosion to adulthood. The call-recording chamber was maintained at the same environmental settings as the rearing chamber (28 °C and 16:8 h light/dark). Inside the recording chamber, males were housed in individual sonically insulated boxes, each with a microphone built into the lid. These microphones were sampled throughout the night by connecting them in turn to a digital cassette recorder, which was then activated if the male in question was calling. These recordings were quantified using 'Raven' software version 1.1 (Bioacoustics Research Group: Cornell Lab of Ornithol-

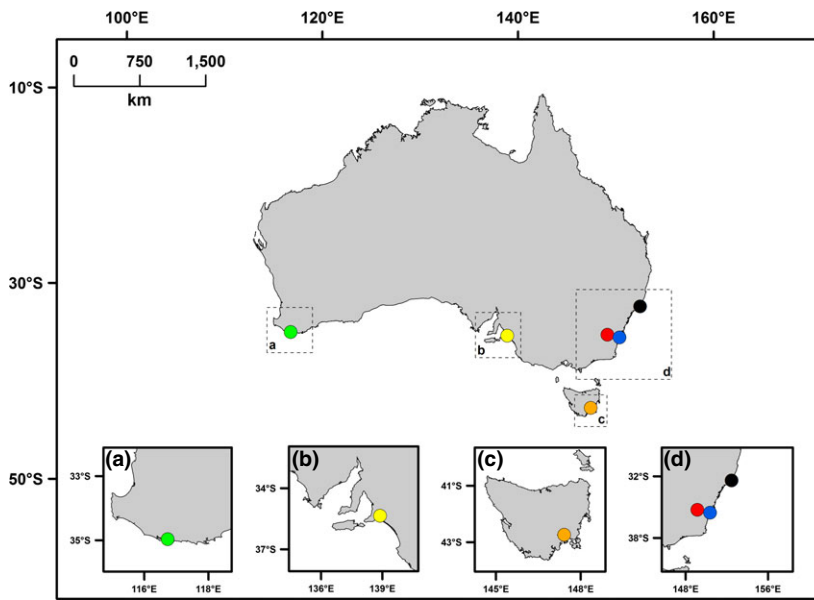


Fig. 2 The sampling locations for our six field populations: Australian Capital Territory (ACT, red symbol, Canberra: 35.2°S, 149.1°E), Kioloa (KL, blue symbol, 35.5°S, 150.3°E), South Australia (SA, yellow symbol, McLaren Vale: 35.2°S, 138.5°E), Smith's Lakes (SL, black symbol, 32.2°S, 149.1°E), Tasmania (TAS, orange symbol, Richmond: 42.7°S, 147.5°E) and Western Australia (WA, green symbol, Walpole: 34.9°S, 116.7°E).

ogy, Ithaca, NY, USA). We measured five call traits: dominant frequency (DF), chirp pulse number (CPN), chirp inter-pulse interval (CIPD), trill number (TN) and inter-call duration (ICD; see Fig. 1). These traits were chosen because they have been shown to vary among populations (Pitchers *et al.*, 2013b) and to be subject to sexual selection (Brooks *et al.*, 2005; Bentsen *et al.*, 2006). The expectation that spectral traits (i.e. DF) should covary with wing morphology is well documented (Gerhardt & Huber, 2002; Scheuber *et al.*, 2003a,b), but in the case of temporal traits (i.e. CPN, CIPD, TN and ICD), the rationale is that the shape of the forewings may influence their rigidity, which in turn may act on the cycling rate of the file-plectrum escapement (Elliott & Koch, 1985; Koch *et al.*, 1988; Prestwich *et al.*, 2000; Bennet-Clark and Bailey, 2002). Each character was measured five times for each call, and the means of these measures were taken. As these traits have different units of measurement, individual means were standardized (converted to *z*-scores) before analysis. We then used a MANOVA to test for differences between populations and between generations.

Morphometric analyses

We used landmark-based geometric morphometrics to quantify variation in size and shape. We selected a suite of 19 features on the male forewing to serve as landmarks for morphometric analysis. These points were selected so as to define the margins of the known call-related structures and also to capture the outline shape of the wing (see Fig. 3). After successful call recording, males were killed by freezing at -20°C . Forewings were raised and held with lightweight forceps and then removed by cutting through the articular sclerites at

the attachment point to the thorax using iris-dissecting scissors (just above landmark 2 on Fig. 3). Cricket forewings have a flexible zone anterior to the cubitus 1 vein. When held at rest, this zone flexes almost to a right angle such that the two parts of the wing lie along the dorsal and lateral surfaces of the animal's body, respectively. We found that in *T. commodus* this zone was flexible enough that we could mount the wings whole (using transparent tape to secure them to a standard microscope slide), rather than cutting the wings into two as was necessary in *Gryllus firmus* (Klingenberg *et al.*, 2010). After mounting, each slide was photo-

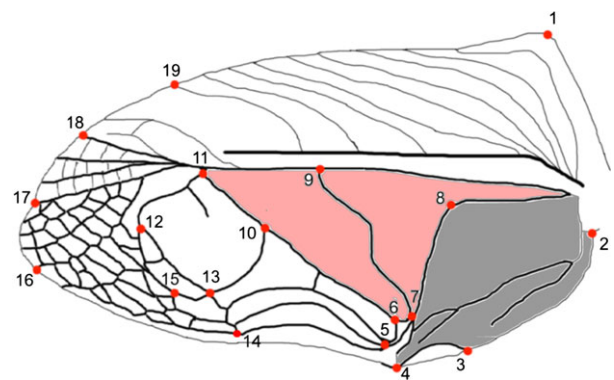


Fig. 3 A simplified outline drawing of the venation of male forewings. Landmark points are indicated in red. The cubitus 1 vein connects landmarks 17, 11 and 9 before terminating at the proximal wing boundary. The 'harp' is the pink-shaded (light grey in print) structure subtended by landmarks 6, 7, 8, 9, 10 and 11. The 'file' lies along the vein between 7 and 8 and the plectrum between 3 and 4. The grey-shaded area subtended by landmarks 2, 8, 7, 4 and 3 is the 'anal area'.

graphed using a firewire digital camera (Pixelink PL-A662, BFi OPTiLAS[®]; Acal BFI, Wokingham, UK) mounted on a binocular microscope (Leica MZ6, 0.8x objective, Buffalo Grove, IL, USA) with an external light source (Leica CLS 100X, Buffalo Grove, IL, USA). Before we recorded data, each photograph was reflected so that each wing appeared to be a right wing. This was performed to control for error resulting from any perceptual or mechanical difference in digitizing the same landmark from different parts of the image (Klingenberg & McIntyre, 1998). Coordinates for the landmark points on each wing were then digitized from these photographs using the ImageJ application (Rasband, 2008) and a macro written by CPK. We performed a Procrustes superimposition (and projection into tangent space) of these landmark coordinates using 'gpagen' from the 'geomorph' package (version 1.1-1; Adams & Otárola-Castillo, 2013) for **R** (version 3.0.1, R Core Development Team, 2014).

After superimposition, we tested for effects of directional asymmetry (left forewing vs. right) on wing size and shape by modelling each as a response to side (left or right) (Klingenberg *et al.*, 1998). Our models for size and shape, respectively, were as follows:

$$y_i = S_i + \varepsilon_i \quad (1)$$

$$\mathbf{Y}_i = \mathbf{S}_i + \varepsilon_i \quad (2)$$

where y_i is the centroid size of the i th wing, \mathbf{Y}_i is the shape configuration of the i th wing, and S_i is left or right wing. We found no statistical support for directional asymmetry in wing size, but there was a modest effect on wing shape ($R^2 = 0.07$). As asymmetry is not the focus of this study, we used the average of the left and right forewing configurations and sizes for each individual for further analyses.

We used an ANOVA to test for wing size differences between populations and between generations. We then used a MANOVA to test for differences in wing shape; we included size as a covariate to account for any allometric shape effects. Our models, respectively, were as follows:

$$y_{ijk} = P_i + G_j + S_k + PG_{ij} + PS_{ik} + GS_{jk} + \varepsilon_i \quad (3)$$

$$\mathbf{Y}_{ijk} = \mathbf{P}_i + \mathbf{G}_j + \mathbf{S}_k + \mathbf{PG}_{ij} + \mathbf{PS}_{ik} + \mathbf{GS}_{jk} + \varepsilon_i \quad (4)$$

where y_{ijk} is the mean centroid size of the wings of the i th individual from the j th population and the k th generation, and \mathbf{Y}_{ijk} is the mean shape configuration of the wings of the i th individual from the j th population and the k th generation. The terms 'P', 'G' and 'S' represent the population, generation and wing centroid size, respectively, and 'PG', 'PS' and 'GS' are the interactions between them. As *F*-tests indicated that the interactions were important in both cases, we evaluated both models using type III sums of squares using the 'ANOVA' and 'MANOVA' functions from the 'car' package (Fox & Weis-

berg, 2009) in **R**. It is worth noting that because we sampled from a small number of populations, we treated these as fixed effects. For the multivariate tests, we confirmed results using permutation tests to assign *P*-values (1000 permutations each). In addition to reporting R^2 value from our univariate (size) model, we also calculated the partial R^2 coefficients (Kutner *et al.*, 2003; Pitchers *et al.*, 2013a) and a multivariate extension of the R^2 coefficient from our shape model. This is equivalent to the established 'Procrustes variance' metric (e.g. Breuker *et al.*, 2006).

To visualize the pattern of effects found with the MANOVA, we used a linear discriminant analysis ('lda' function from the 'MASS' package) to find the rotation that best separated the principal components of shape by both population and generation. As the MANOVA revealed statistical support for the interactions between size and both population and generation, we structured the data by population and generation for further analyses.

We then used two-block partial least-squares (PLS) analyses to model the covariance between wing form and the structure of the individual's call. PLS calculates vectors that describe the directions of covariance between two 'blocks' of data, each with a singular value that describes the covariance between the scores on the two PLS axes of the respective pair. We also used the RV coefficient – a multivariate generalization of the Pearson correlation coefficient – which quantifies the amount of covariance accounted for (Dryden & Mardia, 1998; Rohlf & Corti, 2000). Within each population and rearing environment, we ran one PLS to examine the covariation between wing size and call structure and a second PLS to examine the covariation between wing shape and call structure (Rohlf & Corti, 2000). In both cases, we expressed shape as the residuals from a regression against size to control for covariation between size and shape (allometry). As PLS analyses return as many PLS vectors as there are dimensions in the smaller block, the analyses for calls and size return a single vector (because size is a univariate measure) and the analyses for calls and shape returned five vectors (because we have five call parameters). For each PLS analysis, we then performed both a bootstrap to provide confidence intervals on our estimates (1000 iterations in each case) and a permutation to allow for the estimation of a *P*-value for each vector (1000 iterations in each case, one-tailed *P*-value estimated as the proportion of permutations returning a larger RV coefficient value than the real data).

To quantify allometry, we fit a multivariate regression of shape on size within each population/rearing environment subset:

$$\mathbf{Y}_{ik} = \beta S_{ik} + \varepsilon_i \quad (5)$$

where 'S' represents size as above, and used the vector of coefficients relating shape to size to describe allometric shape variation within each population.

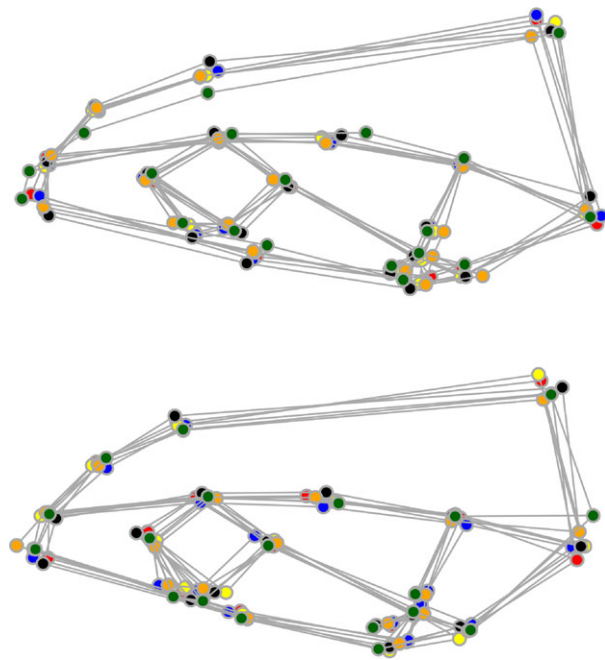


Fig. 4 The wing shape variation among populations and between rearing environments. The configurations for each population are represented by connecting the within-population landmark positions – note that the differences between each population and the overall mean configuration have been multiplied by a factor of 5 to make them easier to see. The upper panel shows configurations from the wild-caught sample, and the lower, from the common-garden-reared sample. Landmarks are colour-coded by population as per the legend of Fig. 2.

We then compared the direction of the PLS and allometry vectors by calculating pairwise vector correlations (r_{VC}) between them as:

$$r_{VC} = \frac{|\mathbf{a} \cdot \mathbf{b}|}{\|\mathbf{a}\| \times \|\mathbf{b}\|} \quad (6)$$

where \mathbf{a} and \mathbf{b} are the vectors in question (Pitchers *et al.*, 2013a).

Lastly, we examined the covariance between call structure and wing shape at the between-population level, using a PLS on the within-population means for both shape and calls. To control for the effect of generation, we performed this analysis separately in the wild-caught and common-garden-reared generations. In

each case, we used permutation tests to evaluate the relative magnitude of the major PLS axis; because there are six populations in this study, the maximum number of permutations possible for these analyses was 720. We then compared the directions of these *inter*-population PLS axes to those of the *intra*-population PLS axes by calculating pairwise vector correlations between them as mentioned earlier.

PLS analyses, resampling and vector correlations were performed using custom **R** scripts, which are provided with our data at Dryad: doi:10.5061/dryad.1kp0s & github: https://github.com/DworkinLab/PitchersJEB2014_cricket_wings.

Results

A total of 307 crickets were both successfully recorded and had intact wings available for morphometric analysis. The resulting data set comprised 140 males from the field and 167 from the laboratory sample, with a minimum of 45 males per population. The variation present in the two samples is represented in Fig. 4.

Populations are divergent in wing size and shape

While there was no evidence that wing size differed overall between wild-caught and common-garden-reared generations (Table 1), populations do differ in size and the interaction between population and rearing environment terms is clearly the most substantial effect. This indicates that populations responded differently to common-garden rearing, with wing size reducing in four populations but not changing in the other 2 (Fig. 5). The partial R^2 value was moderate (0.18), indicating that almost 20% of variation in wing size was accounted for by population-specific effects of rearing environment, even after accounting for main effects (Table 1).

Populations also differ in wing shape, and there was support for a main effect of rearing environment on wing shape (Table 2). The populations also responded differently to common-garden rearing in terms of shape (Table 2), with the population-by-rearing environment interaction being the largest effect (marginal $R^2 = 0.16$). This pattern is strikingly clear in the visualization of the linear discriminant analysis (Fig. 6), where the populations form obvious clusters by rearing environment.

	Sum of Sq.	d.f.	F-value	P-value	partial R^2	marginal R^2	adjusted R^2
Intercept	0.01	1	0.00	0.95	–	–	0.46
Population	79.27	5	15.36	<0.001	<0.01	0.13	
Generation	0.83	1	0.80	0.37	<0.01	0.26	
Pop. × Gen.	66.98	5	12.98	<0.001	0.18	0.48	
Residuals	304.41	295					

Table 1 ANOVA table for the analysis of population/generation differences in wing centroid size.

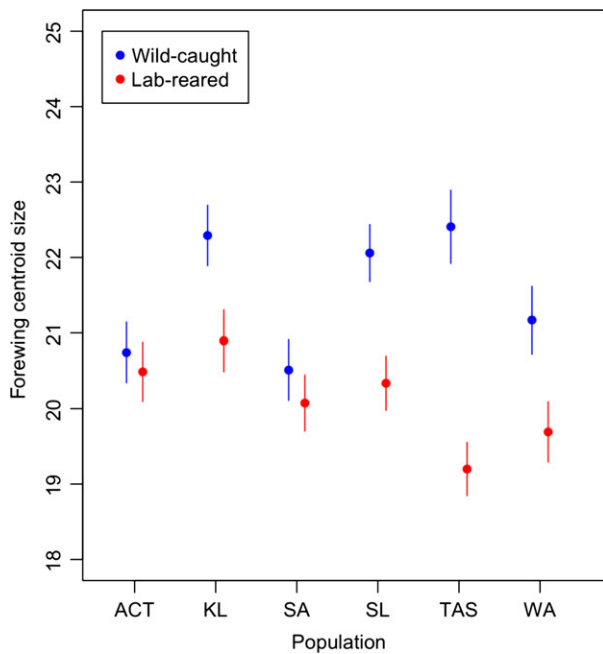


Fig. 5 Centroid sizes (mm) and 95% confidence intervals for the male forewing structured by population and generation.

The main effect of forewing size on shape (which would indicate allometry) was not supported, but there was support for the interactions between size and population and between size and generation; though, these effects were smaller (marginal $R^2 = 0.06$ and 0.05 , respectively, see Fig. 6). This suggests that the allometric component of wing shape variance differs among populations and across generations.

In the light of the statistical support for interactions with wing size from our MANOVA, we modelled allometry separately within each population and rearing environment. To determine whether patterns of size–shape allometry were similar across populations, we calculated pairwise vector correlations between the modelled vectors of allometry coefficients (Table 3). These vector correlations were mostly of moderate magnitude, being on average 0.39 ($SD = 0.1$) within populations across generations, 0.42 ($SD = 0.17$) among wild-caught pop-

ulations and 0.33 ($SD = 0.21$) among common-garden-reared populations (Table 3). This suggests that wing shape does not vary with size in the same way in all of our populations; however, given the small magnitudes of the allometry vectors, their direction will be poorly estimated.

Call structure covaries more with wing size than with shape

To understand the degree to which call structure covaried with morphological features of the forewing, we used two-block partial least squares (PLS). The PLS analyses of the covariance between wing size and call structure revealed differing patterns of covariance among populations and between rearing environments (Table 4). In all six populations and both rearing environments, the PLS vector was supported by the results of a permutation test (1000 iterations, all $P < 0.001$). Interestingly, while the vector correlations between these PLS vectors spanned the range from very small (wild-caught WA vs. TAS = 0.01) to very large (common-garden ACT vs. SL = 0.93), the average was an absolute vector correlation of 0.45 with a standard deviation of 0.27 (Table 5). This suggests that populations differ to varying degrees in the relationship between size and particular aspects of call structure.

The PLS analyses of the covariance between wing shape and call structure returned five vectors of covariance for each population and rearing environment. We found that only the first PLS vector for the wild-caught WA population was supported by permutation test (1000 iterations, $P = 0.01$, see Fig. 7). This single supported vector had small negative coefficients on three call traits (TN = -0.07 , CIPD = -0.25 and DF = -0.33) and moderate negative coefficients on the other two (CPN = -0.63 and ICD = -0.63) (see supplementary material for tables of all coefficients from these PLS analyses).

Call–shape covariance correlates poorly with divergence

The magnitudes of the two inter-population major (1st) PLS axes – one each for the field-caught genera-

Table 2 MANOVA table for the analysis of population/generation differences in wing shape.

	Wilk's λ	Approx. F	d.f.	P-value	Partial R^2	Marginal R^2	Adjusted R^2
Intercept	0.50	7.62	34,255	<0.001	–	–	0.23
Population	0.20	2.84	170,1269.2	<0.001	<0.01	0.07	
Generation	0.60	4.91	34,255	<0.001	<0.01	0.05	
Wing size	0.84	1.40	34,255	0.08	<0.01	0.04	
Pop. \times Gen.	0.25	2.39	170,1269.2	<0.001	0.04	0.16	
Pop. \times size	0.48	1.20	170,1269.2	0.05	0.02	0.06	
Gen. \times size	0.83	1.49	34,255	0.05	0.01	0.05	

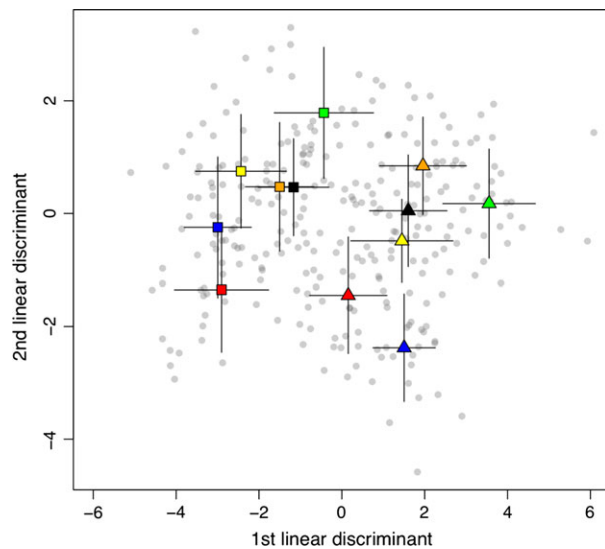


Fig. 6 Wing shape variation between populations and between generations: population mean linear discriminant scores for the first and second linear discriminant functions of call phenotype calculated with population and rearing environment as grouping variables. Populations are colour-coded as per the legend of Fig. 2. The square points are for wild-collected males, and the triangular points represent common-garden-reared males. The bars extend 1 standard deviation either side of the mean.

tion and common-garden-reared generations – were comparatively small at $6.2e-3$ and $2.3e-3$, respectively. Because we have data from only six populations, the number of permutations that are available in the data is only 720 (6!) and so permutation tests are under-powered, but the P -values from permutation tests were large for both between-group PLS analyses (720 iterations, $P = 0.17$, and 720 iterations, $P = 0.49$, respectively). The vector correlations between the between-

group and within-group major PLS axes were spread over quite a wide range, from 0.04 to 0.45 for the wild-caught generation and from 0.02 to 0.39 for the common-garden-reared generation (see Table 6). Notably, none of the within-population major PLS axes were very tightly correlated with the between-population major PLS axis, suggesting that any covariance between calls and forewing shape is not implicated in population divergence.

Discussion

As animal behaviour may be more plastic than morphology, it is perhaps unsurprising that studies have found that an animal's behaviour may evolve more rapidly than its morphology (Moore, 1997; Moore *et al.*, 1998; Puniamorthy *et al.*, 2009). Moreover, a single suite of morphological characteristics must produce an entire behavioural repertoire (e.g. Bennet-Clark & Ewing, 1969), and that repertoire is often complex and plastic (West-Eberhard, 1989; Stirling & Roff, 2000; Bailey & Zuk, 2008). Selection on behaviour is assumed to apply indirect selection on related morphology (Wcislo, 1989; West-Eberhard, 2003), but this link is most often studied where behaviour and morphology are tightly linked and variation is more or less qualitative (e.g. Pener & Yerushalmi, 1998; Moczek & Emlen, 2000; Nijhout, 1999). The morphology of the forewings of male field crickets is interesting in this respect because forewings function primarily as musical instruments with which males produce an advertisement call (Alexander, 1962; Zuk, 1987; Andersson, 1994; Gerhardt & Huber, 2002), which is subject to sexual selection (Nocke, 1971; Bennet-Clark & Bailey, 2002; Bennet-Clark, 2003; Montealegre, 2009). Moreover, in this system, we are able to bring together quantitative morphometric data with data on behaviour that displays

	Common-Garden-Reared					
	ACT	KL	SA	SL	TAS	WA
Wild-caught						
ACT	0.19 <0.01–0.47	0.39 <0.01–0.48	0.06 <0.01–0.6	0.25 <0.01–0.48	0.12 <0.01–0.5	0.07 <0.01–0.5
KL	0.46 0.01–0.62	0.52 <0.01–0.52	0.41 <0.01–0.55	0.53 0.01–0.56	0.43 <0.01–0.45	0.63 <0.01–0.56
SA	0.33 <0.01–0.52	0.43 <0.01–0.44	0.52 0.01–0.5	0.41 <0.01–0.51	0.13 0.01–0.45	0.35 0.02–0.49
SL	0.58 <0.01–0.53	0.60 0.01–0.58	0.58 <0.01–0.42	0.56 0.01–0.48	0.04 0.01–0.5	0.57 0.02–0.51
TAS	0.41 0.01–0.56	0.39 0.01–0.53	0.60 0.01–0.61	0.71 <0.01–0.49	0.25 <0.01–0.51	0.58 <0.01–0.5
WA	0.20 <0.01–0.46	0.21 0.01–0.41	0.43 <0.01–0.52	0.28 <0.01–0.47	0.14 <0.01–0.49	0.28 0.01–0.43

ACT, Australian Capital Territory; KL, Kioloa; SA, South Australia; SL, Smith's Lakes; TAS, Tasmania; WA, Western Australia.

Table 3 Vector correlation matrix for vectors of allometry modelled by population and rearing environment. Correlations between wild-caught populations are below the diagonal, correlations between common-garden-reared populations are above the diagonal, and correlations within populations across generations are on the diagonal in bold. Below each correlation are 95% bootstrapped confidence intervals. (All correlations are expressed in absolute terms.)

Table 4 Results of PLS analyses of the covariance between wing centroid size and call structure within populations and generations. As wing size is a univariate measure, each analysis returns an RV coefficient of 1, and single PLS vector with coefficients for each of the five call traits. *P*-values for each vector were calculated from a permutation test (1000 iterations).

	Population					
	ACT	KL	SA	SL	TAS	WA
Wild-caught						
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CPN	0.53	-0.39	-0.06	0.46	0.07	0.37
TN	0.71	-0.56	0.94	0.62	-0.64	-0.65
ICD	0.41	0.03	0.11	0.60	0.41	0.65
CIPD	0.19	-0.72	0.21	0.05	-0.55	-0.04
DF	-0.13	-0.14	0.23	-0.22	-0.33	0.13
Common-garden-reared						
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CPN	0.19	0.52	0.68	0.51	-0.59	-0.04
TN	-0.05	0.14	0.35	0.77	-0.56	-0.87
ICD	-0.08	-0.28	0.15	0.23	-0.11	-0.24
CIPD	-0.33	0.79	-0.41	-0.10	-0.09	-0.14
DF	-0.92	-0.08	-0.48	-0.28	-0.57	0.41

CPN, chirp pulse number; TN, trill number; ICD, inter-call duration; CIPD, chirp inter-pulse interval; DF, dominant frequency.

quantitative genetic variation that is under selection (Hunt *et al.*, 2007; Pitchers *et al.*, 2013b), allowing us to test for more subtle associations with wing form than have been investigated previously.

Wing shape and divergence

Our analyses show differences in forewing morphology among populations, and to a lesser extent between wild-caught and laboratory-reared cohorts of crickets.

This adds to the differences in call structure we have previously reported for these populations (Pitchers *et al.*, 2013b). The broad variation in both call structure and morphometric data allowed us to quantify the covariance between wing size and shape with aspects of call structure. When animals are reared in common-garden conditions, any remaining inter-population variation is indicative of inter-population genetic differences (Mousseau & Roff, 1995; Mousseau & Howard, 1998; Simmons, 2004). Given the large geographical distance between our populations (Fig. 1), they will certainly have experienced a myriad of different abiotic environmental conditions and thus might be predicted to have diverged through local adaptation. Previous work (Pitchers *et al.*, 2013b) has demonstrated inter-population variation in call structure, but our current study suggests that this divergence is accompanied by a similar pattern of differences in both the size and geometric shape of the forewings. Moreover, in the case of wing shape, we find an interaction between population and generational effects, indicating inter-population differences in the importance of environmental conditions. These different responses to common-garden rearing conditions expressed in genetically divergent populations is suggestive of a genotype-by-environment interaction for wing shape (Ingleby *et al.*, 2010). Interestingly, the traits that differed most among populations were related to the temporal patterning of calls (TN and ICD; Pitchers *et al.*, 2013b); though, the directions of strongest multivariate selection identified in Hunt *et al.* (2007; \mathbf{g}_3 and \mathbf{g}_4) had loading of similar magnitude for both these traits (TN and ICD) and for dominant frequency (DF). It appears that wing shape does not neatly explain this pattern, given our finding that where the shape-calls covariance had statistical support, the moderately strong coefficients were for ICD and CPN (both negative), with smaller coefficients for

Table 5 Vector correlation matrix for vectors of covariance between wing size and call structure (PLS vectors) calculated by population and generation. Correlations between wild-caught populations are below the diagonal, correlations between common-garden-reared populations are above the diagonal, and correlations within populations between generations are on the diagonal in bold. (All correlations are expressed in absolute terms.)

	Common-Garden-Reared					
	ACT	KL	SA	SL	TAS	WA
Wild-caught						
ACT	0.09	0.42	0.65	0.93	0.69	0.81
KL	0.12–0.64	0.84	0.09	0.51	0.68	0.53
SA	0.32	0.04–0.85	0.11	0.63	0.65	0.77
SL	0.2–0.85	0.01–0.9	0.02–0.8	<0.01–0.78	0.06–0.73	0.01–0.77
TAS	0.20	0.21	0.70	0.91	0.56	0.79
WA	0.14–0.61	0.1–0.89	0.07–0.81	0.03–0.8	0.06–0.91	0.11–0.87
ACT	0.50	0.58	0.27	0.21	0.51	0.39
KL	0.1–0.97	0.11–0.52	0.02–0.89	0.03–0.87	0.17–0.62	0.06–0.51
SA	0.05	0.12	0.08	0.19	0.01	0.45
SL	0.02–0.87	0.03–0.76	0.01–0.8	0.09–0.62	0.03–0.57	0.1–0.78

PLS, partial least-squares; ACT, Australian Capital Territory; KL, Kioloa; SA, South Australia; SL, Smith's Lakes; TAS, Tasmania; WA, Western Australia.

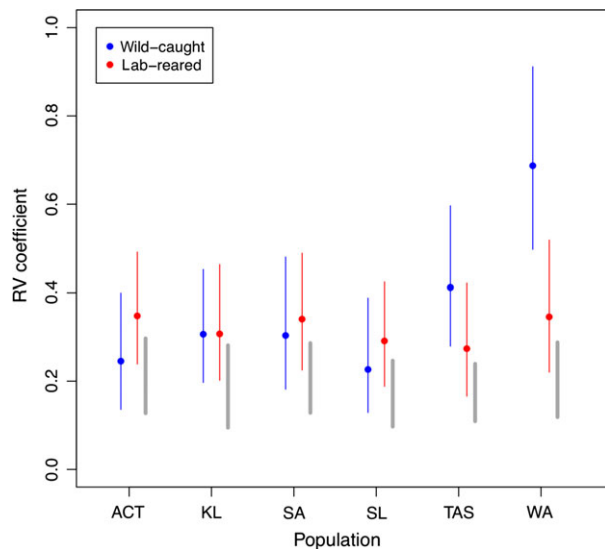


Fig. 7 RV coefficients and bootstrapped 95% confidence intervals from PLS analyses of the covariation between call structure and forewing shape within populations and generations. The grey bars indicate the extent of the 95% of the distribution of RV coefficients under permutation.

Table 6 Vector correlations between the major PLS axis calculated between populations and the major PLS axis calculated within populations. Estimates were made separately for the wild-caught and common-garden-reared samples.

	Wild-caught	Common-garden-reared
ACT	0.23	0.3
KL	0.41	0.26
SA	0.39	0.29
SL	0.23	0.39
TAS	0.04	0.02
WA	0.45	0.02

PLS, partial least-squares; ACT, Australian Capital Territory; KL, Kioloa; SA, South Australia; SL, Smith's Lakes; TAS, Tasmania; WA, Western Australia.

CIPD, DF and TN. The degree to which these differences between populations reflect natural selection, drift or their interaction therefore remains unclear and is of potential importance for future study.

In addition to the biomechanical interest of the wing shape–call structure system, we sought to measure the potential influence that the covariance between shape and call structure has on the evolution of call structure. Having shown that both call structure and the wing shape vary among our six populations, we quantified the between-population shape–call covariance. Our between-population PLS analyses returned RV coefficients that were orders of magnitude smaller than those

calculated *within* populations. Although the power of these analyses is not great – due to a small sample size of populations – this difference suggests that the covariance between call structure and wing shape is unlikely to be a strong influence on the direction of the divergence among populations. Moreover, the directions of the major between-population PLS axes show a broad range or vector correlations with the major within-population PLS axes, indicating that the form of shape–call covariance between populations is a poor predictor of the shape–call covariance *within* a population. We would not expect this pattern of the shape–call structure covariance where driving or directing the divergence of these populations – although with the caveat that the small magnitude of these vectors makes estimates of their directions less accurate.

Call structure and morphology

The harp on a cricket wing acts as part of a mechanical resonating system, and therefore, changes in the size and shape of the harp are expected to influence the frequency of sound produced (Prestwich *et al.*, 2000; Bennet-Clark, 2003). Specifically, all else being equal, larger harps should produce lower-frequency chirps. Indeed, frequency has typically been found to be negatively correlated with harp area in a range of field cricket species (Simmons, 1995; Simmons & Ritchie, 1996; Scheuber *et al.*, 2003a; Jacot *et al.*, 2005). This has led to the hypothesis that a male's body size may be signalled to females via the frequency of his call (Bennet-Clark, 1999). However, despite the widespread assumption of a negative association between body size and frequency, none of the above-mentioned studies report such a relationship (although they do report a correlation between harp area and body size), nor do a number of other studies where the relationship between frequency and body size has been measured (Simmons, 1988; Webb & Roff, 1992; Simmons & Zuk, 1992; Sakaluk *et al.*, 1992; Gray, 1997; Ryder & Siva-Jothy, 2000; Ferreira & Ferguson, 2002; Bateman *et al.*, 2004). In fact, a literature review by Verburgt & Ferguson (2009) revealed that this straightforward putative association between male size and call frequency is neither simple nor ubiquitous. In our study, we found that forewing size and harp size are very tightly associated ($r > 0.94$) and so a larger wing will bear a larger resonant area and could therefore be expected to emit a lower frequency.

A number of studies have viewed this association in the context of a three-way relationship between body size, frequency and harp size (e.g. Simmons & Ritchie, 1996; Jacot *et al.*, 2004), but our work shows that this relationship is also highly variable among populations and even between rearing environments (Table 4). As there was a negative correlation between wing size and dominant frequency overall ($r = -0.37$), we might

expect the PLS axes from our analyses of the covariance between wing size and call structure to load negatively for dominant frequency. However, this clearly was not the case for all populations (Table 4). Our findings suggest that, far from being a straightforward signal of body size, the structure of calls in *T. commodus* may vary in information content among populations and even in response to environmental plasticity. This possibility is particularly interesting given that female choice in this species is known to be condition dependent (Hunt *et al.*, 2005).

We cannot be certain that the call structure traits measured here are the only important ones; though, we do know that they are under sexual selection (Hunt *et al.*, 2007). However, we believe that it is important for researchers interested in the biomechanics of cricket stridulation to broaden their efforts to include potential relationships with temporal components of call structure. As both frequency (Libersat *et al.*, 1994; Hennig & Weber, 1997) and temporal structure (Stabel *et al.*, 1989; Balakrishnan & Pollack, 1996; Hennig & Weber, 1997) are important in both species recognition and female choice, such an effort might allow for more synthesis between biophysical and evolutionary approaches to cricket sexual signaling. In addition to the temporal patterning and dominant frequency of calls that we did measure, there are other potentially important call traits that we did not, for example frequency modulation (Simmons & Ritchie, 1996; Desutter-Grandcolas, 1998; Hirtenlehner *et al.*, 2013). This is a particularly interesting avenue for future research in the light of evidence that females may be able to infer information about the fluctuating asymmetry of males' wings from the frequency modulation of their calls (Simmons & Ritchie, 1996; Hirtenlehner *et al.*, 2013).

The forewings of male crickets are principally used in signalling, rather than for flight, and so presumably the principal agent of selection acting on them is the acoustic preference of potential mates. Female *T. commodus* express preferences for multiple temporal and spectral call parameters (Brooks *et al.*, 2005; Hunt *et al.*, 2007), which suggests that multiple aspects of the call are likely to be informative about male 'quality', whether that be in terms of 'good genes' or as a signal of condition. There was some evidence in this study for intra-population patterns of covariation between shape and call structure, although less might be naively predicted based on the function of the forewings. Despite this, there is considerable inter-population variation for both call structure and shape. This covariation between populations may be shaped by selection, drift or both. Future studies should examine how multivariate selection on call structure and wing shape might be shaped by differences in female preference across these populations. Understanding how wing morphology serves to 'translate' information about male phenotype/condition

into call properties may therefore offer researchers the opportunity to link multivariate sexual selection to complex adaptation.

Acknowledgments

This work was supported by NERC, through a studentship granted to WP. JH and TT were funded by NERC and JH by a Royal Society Fellowship and Equipment Grant. WP and ID were funded by NIH grant 1R01GM094424-01. The paper was greatly improved by the comments from two anonymous reviewers.

References

- Adams, D.C. & Otárola-Castillo, E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**: 393–399.
- Adams, D.C. & Rohlf, F.J. 2000. Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proc. Natl. Acad. Sci. USA* **97**: 4106–4111.
- Alexander, R.D. 1962. Evolutionary change in cricket acoustic communication. *Evolution* **16**: 443–467.
- Andersson, M.B. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ, USA.
- Arnold, S.J. 1992. Constraints on phenotypic evolution. *Am. Nat.* **140**: S85–S107.
- Bailey, N.W. & Zuk, M. 2008. Acoustic experience shapes female mate choice in field crickets. *Proc. Biol. Sci.* **275**: 2645–2650.
- Balakrishnan, R. & Pollack, G.S. 1996. Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Anim. Behav.* **51**: 353–366.
- Bateman, P.W., Ferguson, J. & Ferreira, M. 2004. The influence of physical and acoustic experience on sequential mate preference in the cricket *Gryllus bimaculatus*. Is song important? *J. Insect Behav.* **17**: 843–855.
- Bennet-Clark, H.C. 1999. Resonators in insect sound production: How insects produce loud pure-tone songs. *J. Exp. Biol.* **202**: 3347–3357.
- Bennet-Clark, H.C. 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *J. Exp. Biol.* **206**: 1479–1496.
- Bennet-Clark, H.C. & Bailey, W.J. 2002. Ticking of the clockwork cricket: the role of the escapement mechanism. *J. Exp. Biol.* **205**: 613–625.
- Bennet-Clark, H.C. & Ewing, A.W. 1969. Pulse interval as a critical parameter in the courtship song of *Drosophila melanogaster*. *Anim. Behav.* **17**: 755–759.
- Bentley, D.R. & Hoy, R.R. 1972. Genetic control of the neuronal network generating cricket (*Teleogryllus gryllus*) song patterns. *Anim. Behav.* **20**: 478–492.
- Bentsen, C.L., Hunt, J., Jennions, M.D. & Brooks, R. 2006. Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*. *Am. Nat.* **167**: E102–E116.
- Breuker, C.J., Patterson, J.S. & Klingenberg, C.P. 2006. A single basis for developmental buffering of drosophila wing shape. *PLoS ONE* **1**: e7.
- Brooks, R., Hunt, J., Blows, M.W., Smith, M.J., Bussière, L.F. & Jennions, M.D. 2005. Experimental evidence for

- multivariate stabilizing sexual selection. *Evolution* **59**: 871–880.
- Chen, S., Lee, A.Y., Bowens, N.M., Huber, R. & Kravitz, E.A. 2002. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl. Acad. Sci. USA* **99**: 5664–5668.
- Cheverud, J.M. 1984. Quantitative genetics and developmental constraints on evolution by selection. *J. Theor. Biol.* **110**: 155–171.
- Desutter-Grandcolas, L. 1998. Broad-frequency modulation in cricket (Orthoptera, Grylloidea) calling songs: two convergent cases and a functional hypothesis. *Can. J. Zool.* **76**: 2148–2163.
- Dryden, I.L. & Mardia, K.V. 1998. *Statistical Shape Analysis*. Wiley, Hoboken, NJ, USA.
- Elliott, C. & Koch, U.T. 1985. The clockwork cricket. *Naturwissenschaften* **72**: 150–153.
- Ferreira, M. & Ferguson, J.W.H. 2002. Geographic variation in the calling song of the field cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) and its relevance to mate recognition and mate choice. *J. Zool.* **257**: 163–170.
- Fox, J. & Weisberg, S. 2009. *An R Companion to Applied Regression*, 2nd edn. R package version. Sage, Thousand Oaks, CA.
- Gerhardt, H.C. & Huber, F. 2002. *Acoustic Communication in Insects and Anurans*. University of Chicago Press, London, UK.
- Gómez, J.M., Perfectti, F. & Camacho, J.P.M. 2006. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *Am. Nat.* **168**: 531–545.
- Gray, D. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Anim. Behav.* **54**: 1553–1562.
- Hennig, R.M. & Weber, T. 1997. Filtering of temporal parameters of the calling song by cricket females of two closely related species: a behavioral analysis. *J. Comp. Physiol. A.* **180**: 621–630.
- Hill, K., Loftus-Hills, J. & Gartside, D. 1972. Pre-mating isolation between the Australian field crickets *Teleogryllus commodus* and *T. oceanicus* (Orthoptera : Gryllidae). *Aust. J. Zool.* **20**: 153–163.
- Hirtenlehner, S., Kung, S., Kainz, F. & Römer, H. 2013. Asymmetry in cricket song: female preference and proximate mechanism of discrimination. *J. Exp. Biol.* **216**: 2046–2054.
- Hunt, J., Blows, M.W., Zajitschek, F., Jennions, M.D. & Brooks, R. 2007. Reconciling strong stabilizing selection with the maintenance of genetic variation in a natural population of black field crickets (*Teleogryllus commodus*). *Genetics* **177**: 875–880.
- Hunt, J., Brooks, R. & Jennions, M.D. 2005. Female mate choice as a condition-dependent life-history trait. *Am. Nat.* **166**: 79–92.
- Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussière, L.F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024–1027.
- Ingleby, F.C., Hunt, J. & Hosken, D.J. 2010. The role of genotype-by-environment interactions in sexual selection. *J. Evol. Biol.* **23**: 2031–2045.
- Jacot, A., Scheuber, H. & Brinkhof, M.W.G. 2004. Costs of an induced immune response on sexual display and longevity in field crickets. *Evolution* **58**: 2280–2286.
- Jacot, A., Scheuber, H. & Brinkhof, M.W.G. 2007. The effect of age on a sexually selected acoustic display. *Ethology* **113**: 615–620.
- Jacot, A., Scheuber, H., Kurtz, J. & Brinkhof, M.W.G. 2005. Juvenile immune status affects the expression of a sexually selected trait in field crickets. *J. Evol. Biol.* **18**: 1060–1068.
- Klingenberg, C.P. 2010. Evolution and development of shape: Integrating quantitative approaches. *Nat. Rev. Genet.* **11**: 623–635.
- Klingenberg, C.P. & Ekau, W. 1996. A combined morphometric and phylogenetic analysis of an ecomorphological trend: pelagization in Antarctic fishes (Perciformes: Nototheniidae). *Biol. J. Linn. Soc.* **59**: 143–177.
- Klingenberg, C.P. & McIntyre, G.S. 1998. Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with procrustes methods. *Evolution* **52**: 1363–1375.
- Klingenberg, C.P., Debat, V. & Roff, D.A. 2010. Quantitative genetics of shape in cricket wings: developmental integration in a functional structure. *Evolution* **64**: 2935–2951.
- Klingenberg, C.P., McIntyre, G.S. & Zaklan, S.D. 1998. Left-right asymmetry of fly wings and the evolution of body axes. *Proc. Biol. Sci.* **265**: 1255–1259.
- Koch, U.T., Elliott, C.J., Schäffner, K.-H. & Kleindienst, H.-U. 1988. The mechanics of stridulation of the cricket *Gryllus campestris*. *J. Comp. Physiol. A* **162**: 213–223. Springer.
- Kutner, M.H., Nachtsheim, C.J. & Neter, J. 2003. *Applied Linear Regression Models*. McGraw-Hill, Irwin.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**: 292–305.
- Lasbleiz, C., Ferveur, J.-F. & Everaerts, C. 2006. Courtship behaviour of *Drosophila melanogaster* revisited. *Anim. Behav.* **72**: 1001–1012.
- Libersat, F., Murray, J.A. & Hoy, R.R. 1994. Frequency as a releaser in the courtship song of two crickets, *Gryllus bimaculatus* (de Geer) and *Teleogryllus oceanicus*: a neuroethological analysis. *J. Comp. Physiol. A* **174**: 485–494.
- Losos, J.B. 1990a. Concordant evolution of locomotor behaviour, display rate and morphology in Anolis lizards. *Anim. Behav.* **39**: 879–890.
- Losos, J.B. 1990b. The evolution of form and function: morphology and locomotor performance in West Indian Anolis lizards. *Evolution* **44**: 1189–1203.
- McGlothlin, J.W., Parker, P.G., Nolan, V. & Ketterson, E.D. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. *Evolution* **59**: 658–671.
- Mhatre, N., Montealegre-Z, F., Balakrishnan, R. & Robert, D. 2012. Changing resonator geometry to boost sound power decouples size and song frequency in a small insect. *Proc. Natl. Acad. Sci. USA* **109**: E1444–E1452.
- Moczek, A.P. & Emlen, D.J. 2000. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**: 459–466.
- Montealegre-Z, F. 2009. Scale effects and constraints for sound production in katydid (Orthoptera: Tettigoniidae): correlated evolution between morphology and signal parameters. *J. Evol. Biol.* **22**: 355–366.
- Moore, A.J. 1997. The evolution of social signal: Morphological, functional, and genetic integration of the sex pheromone in *Nauphoeta cinerea*. *Evolution* **51**: 1920–1928.

- Moore, A.J., Wolf, J.B. & Brodie, E.D. III. 1998. The influence of direct and indirect genetic effects on the evolution of behavior: social and sexual selection meet maternal effects. In: *Maternal Effects as Adaptations* (T.A. Mousseau & C.W. Fox, eds), pp. 22–41. Oxford University Press, Oxford.
- Mousseau, T.A. & Howard, D.J. 1998. Genetic variation in cricket calling song across a hybrid zone between two sibling species. *Evolution* **52**: 1104–1110.
- Mousseau, T.A. & Roff, D.A. 1995. Genetic and environmental contributions to geographic variation in the ovipositor length of a cricket. *Ecology* **76**: 1473–1482.
- Nijhout, H.F. 1999. Control Mechanisms of Polyphenic Development in Insects. *BioScience* **49**: 181–192. Oxford University Press.
- Nocke, H. 1971. Biophysics of sound production by the tegmina of crickets. *J. Comp. Physiol. A* **74**: 272–314.
- Pener, M.P. & Yerushalmi, Y. 1998. The physiology of locust phase polymorphism: an update. *J. Insect Physiol.* **44**: 365–377.
- Phillips, P.C. & Arnold, S.J. 1989. Visualizing multivariate selection. *Evolution* **43**: 1209–1222.
- Phillips, P.C. & McGuigan, K. 2006. Evolution of Genetic Variance-Covariance Structure. In: *Evolutionary Genetics: Concepts and Case Studies* (C.W. Fox & J.B. Wolf, eds), pp. 310–325. Oxford University Press, New York.
- Pitchers, W., Pool, J.E. & Dworkin, I. 2013a. Altitudinal clinal variation in wing size and shape in African *Drosophila melanogaster*: One cline or many? *Evolution* **67**: 438–452.
- Pitchers, W.R., Brooks, R., Jennions, M.D., Tregenza, T., Dworkin, I. & Hunt, J. 2013b. Limited plasticity in the phenotypic variance-covariance matrix for male advertisement calls in the black field cricket, *Teleogryllus commodus*. *J. Evol. Biol.* **26**: 1060–1078.
- Pollack, G.S. & Hoy, R. 1979. Temporal pattern as a cue for species-specific calling song recognition in crickets. *Science* **204**: 429–432.
- Prestwich, K.N., Lenihan, K.M. & Martin, D.M. 2000. The control of carrier frequency in cricket calls: A refutation of the subalar-tegmina resonance/auditory feedback model. *J. Exp. Biol.* **203**: 585–596.
- Puniamoorthy, N., Ismail, M.R.B., Tan, D.S.H. & Meier, R. 2009. From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae). *J. Evol. Biol.* **22**: 2146–2156.
- Rasband, W.S. 1997–2014. *ImageJ*, US. National Institutes of Health, Bethesda, MD, USA. <http://imagej.nih.gov/ij/>.
- Ritchie, M.G., Couzin, I.D. & Snedden, W.A. 1995. What's in a song? Female bushcrickets discriminate against the song of older males. *Proc. R. Soc. Lond. B Biol. Sci.* **262**: 21–27.
- Rohlf, F.J. & Corti, M. 2000. Use of two-block partial least-squares to study covariation in shape. *Syst. Biol.* **49**: 740–753.
- Ryder, J.J. & Siva-Jothy, M.T. 2000. Male calling song provides a reliable signal of immune function in a cricket. *Proc. Biol. Sci.* **267**: 1171–1175.
- Sakaluk, S.K., Burpee, D.M. & Smith, R.L. 1992. Phenotypic and genetic variation in the stridulatory organs of male decorated crickets, *Gryllodes sigillatus* (Orthoptera: Gryllidae). *Can. J. Zool.* **70**: 453–457.
- Scheuber, H., Jacot, A. & Brinkhof, M.W.G. 2003a. Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. *Anim. Behav.* **65**: 721–727.
- Scheuber, H., Jacot, A. & Brinkhof, M.W.G. 2003b. The effect of past condition on a multicomponent sexual signal. *Proc. Biol. Sci.* **270**: 1779–1784.
- 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. 2004. Genotypic variation in calling song and female preferences of the field cricket *Teleogryllus oceanicus*. *Anim. Behav.* **68**: 313–322.
- Simmons, L.W. 1988. Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus* (Degeer). *Anim. Behav.* **36**: 372–379.
- Simmons, L.W. & Ritchie, M.G. 1996. Symmetry in the songs of crickets. *Proc. Biol. Sci.* **263**: 1305–1311.
- Sinervo, B. & Svensson, E. 2002. Correlational selection and the evolution of genomic architecture. *Heredity* **89**: 329–338.
- Stabel, J., Wendler, G. & Scharstein, H. 1989. Cricket phonotaxis: localization depends on recognition of the calling song pattern. *J. Comp. Physiol. A* **165**: 165–177.
- Stirling, G. & Roff, D. 2000. Behaviour plasticity without learning: phenotypic and genetic variation of naive *Daphnia* in an ecological trade-off. *Anim. Behav.* **59**: 929–941.
- R Core Development Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Simmons, L.W. & Zuk, M. 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.
- Verburgt, L. & Ferguson, J.W.H. 2009. Mate choice in field crickets: can females acoustically detect male body size? *J. Ethol.* **28**: 141–151.
- Wagner, W.E. & Sullivan, B.K. 1995. Sexual selection in the Gulf Coast toad, *Bufo valliceps*: female choice based on variable characters. *Anim. Behav.* **49**: 305–319.
- Wcislo, W.T. 1989. Behavioral environments and evolutionary change. *Annu. Rev. Ecol. Syst.* **20**: 137–169.
- Webb, K.L. & Roff, D.A. 1992. The quantitative genetics of sound production in *Gryllus firmus*. *Anim. Behav.* **44**: 823–832.
- West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press, New York, NY, USA.
- West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**: 249–278.
- Zuk, M. 1987. Variability in attractiveness of male field crickets (Orthoptera: Gryllidae) to females. *Anim. Behav.* **35**: 1240–1248.

Supporting information

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

Table S1 P-values of PLS analyses of the covariance between wing shape and call structure within populations and generations.

Table S2 Vector correlation matrix for the 1st PLS vector of covariance between wing shape and call structure calculated by population and generation.

Table S3 A summary table for the literature cited on the predicted link between body size and measures of the advertisement call in a number of cricket species.

Received 30 April 2014; revised 20 July 2014; accepted 23 July 2014

Data deposited at Dryad: doi:10.5061/dryad.1kp0s