Nonlinear selection and the evolution of variances and covariances for continuous characters in an anole

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Abstract

The pattern of genetic variances and covariances among characters, summarized in the additive genetic variance-covariance matrix, **G**, determines how a population will respond to linear natural selection. However, **G** itself also evolves in response to selection. In particular, we expect that, over time, **G** will evolve correspondence with the pattern of multivariate nonlinear natural selection. In this study, we substitute the phenotypic variance-covariance matrix (**P**) for **G** to determine if the pattern of multivariate nonlinear selection in a natural population of *Anolis cristatellus*, an arboreal lizard from Puerto Rico, has influenced the evolution of genetic variances and covariances in this species. Although results varied among our estimates of **P** and fitness, and among our analytic techniques, we find significant evidence for congruence between nonlinear selection and **P**, suggesting that natural selection may have influenced the evolution of genetic constraint in this species.

Introduction

The classic breeder's equation, $R = h^2 S$, predicts that the evolutionary response (R) to selection (S) will vary in direct proportion to the heritability of the trait (h^2; Falconer & MacKay, 1996; Roff, 1997). However, this picture is incomplete because selection usually acts simultaneously on multiple traits that are genetically correlated. Under these circumstances, it is the matrix of additive genetic variances and covariances (the **G** matrix) that determines the single generation response to linear selection (Lande, 1979; Lande & Arnold, 1983; Arnold *et al.*, 2001).

The single generation response to selection on a given trait is a function of the additive genetic variance for the trait, the linear selection gradient acting on it, **β**, the selection gradients on other traits, and the genetic covariance between them (Lande & Arnold, 1983). In fact, the predicted response to selection is simply the sum

of these factors, i.e. $R_i = V_A(i)\beta_i + \sum_{j=1}^{n, i \neq j} Cov_A(i,j)\beta_j$ for n traits, in which $V_A(i)$ is the additive genetic variance for trait i, and $Cov_A(i, j)$ is the additive genetic covariance between traits i and j (Lande & Arnold, 1983). β_i, the selection gradient, is just the partial regression coefficient of the ith phenotypic trait on fitness (Lande & Arnold, 1983). This equation can also expressed in matrix form as $\Delta \bar{z} = \mathbf{G}\boldsymbol{\beta}$, in which **β** is a vector containing the linear selection gradients for all the traits in the analysis, and $\Delta \bar{z}$ is a vector containing the changes in the mean phenotype for all traits (Lande & Arnold, 1983). When **β** does not align with an eigenvector of **G**, the effect of **G** is that it will tend to bias the course of evolution away from that which maximizes the increase in mean fitness in the population (Via & Lande, 1985; Schluter, 1996, 2000; Arnold *et al.*, 2001). By contrast, when the direction of selection, **β**, and a major eigenvector of **G** are aligned, evolution by natural selection can be greatly facilitated by **G** (Schluter, 1996).

What is obvious from this theory of quantitative trait evolution by natural selection is that **G** plays a central role, either in determining the amount of evolution in response to a particular level of selection (primarily via

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the additive genetic variances), or in determining the course of evolution in response to a particular direction of selection (via the additive genetic covariances between characters). Unfortunately and despite the importance of **G**, the factors that affect its evolutionary dynamics are poorly understood (Turelli, 1988; Jones *et al.*, 2003; Revell, 2007; Arnold *et al.*, 2008).

Theoretical and empirical studies both suggest that selection, particularly multivariate nonlinear selection, can play a very important role in the evolution of **G** (Lande, 1980; Cheverud, 1984; Turelli, 1988; Arnold *et al.*, 2001; Jones *et al.*, 2003). In particular, under natural selection, uncorrelated mutation, and genetic drift, **G** is expected to evolve in response to γ, the matrix of quadratic and correlational terms in the multivariate second order polynomial regression of phenotype on fitness (i.e. $w = \alpha + \beta\mathbf{z} + \frac{1}{2}\mathbf{z}'\gamma\mathbf{z} + \varepsilon$). Here, w is individual fitness, α is the regression intercept, $\mathbf{z}$ is a vector of phenotypic trait values, and ε is random error. Specifically, we expect alignment between the eigenvectors of **G** and $-\gamma^{-1}$ to evolve as the linear component selection flattens. At this point **G** should respond only to the pressure of mutational input, the loss of genetic variability due to drift, and the stabilizing or disruptive and correlational influence of quadratic selection (Cheverud, 1984).

The prediction that the **G** matrix should evolve to align with the curvature of the individual selection surface, given by γ, arises as follows. Lande (1980) provided the following equation for change in the **G** matrix across a generation under linear and quadratic selection: $\Delta\mathbf{G} = \mathbf{G}(\gamma - \beta\beta')\mathbf{G} + \mathbf{U}$. Here all terms have been defined except for **U** which contains the mutational inputs of genetic variation and covariation each generation. At equilibrium (when **G** is no longer changing, i.e. $\Delta\mathbf{G} = \mathbf{0}$) we can assume that $\beta = \mathbf{0}$, because the population will be at the optimum where linear selection is absent. Therefore, rearrangement of the previous expression gives: $-\mathbf{G}\gamma\mathbf{G} = \mathbf{U}$. If we assume that mutational variance is equal and uncorrelated among traits (i.e. $\mathbf{U} = k\mathbf{I}$, where **I** is the identity matrix), then we can solve for **G** at equilibrium as $\mathbf{G}_{eq} = -k\gamma^{-1/2}$ (Lande, 1980; Cheverud, 1984). In this study, we usually compare **G** to $-\gamma^{-1}$ (as is common in similar studies, e.g. Blows *et al.*, 2004), rather than to $-\gamma^{-1/2}$, because if the latter can be evaluated it will have the identical eigenvectors in the same rank order as $-\gamma^{-1}$ (which can nearly always be evaluated; note that $-\gamma^{-1}$ also has the same eigenvectors as γ, although their order is rearranged). Alignment of the individual selection surface and **G** has been predicted in theory (e.g. Lande, 1980; Cheverud, 1984; Arnold *et al.*, 2001), found in simulation studies (e.g. Jones *et al.*, 2003; Revell, 2007), but tested for explicitly in few prior instance that we know of (Brodie, 1992, 1993; Conner & Via, 1993; Blows *et al.*, 2004; McGlothlin *et al.*, 2005; Hunt *et al.*, 2007).

The actual value of **G** found in a natural population will be a function not only of the strength and pattern of selection, but also of the mutation rates and mutational variances, particularly as these vary among traits, as well as of the mutational covariances and the effective population size (Lande, 1980; Arnold *et al.*, 2001; Jones *et al.*, 2003). These factors are generally quite difficult to measure empirically. The mutation rate has been calculated in some studies (e.g. Kimura, 1968), and the mutational variance-covariance matrix has been estimated in very few (e.g. Camara & Pigliucci, 1999; Camara *et al.*, 2000). Selection studies are more common (Endler, 1986; Kingsolver *et al.*, 2001), but few have looked for a correspondence between **G** and multivariate selection (e.g. Brodie, 1992; Blows *et al.*, 2004; McGlothlin *et al.*, 2005; Hunt *et al.*, 2007).

Iguanian lizards in the genus *Anolis*, commonly referred to as anoles, are one of the most diverse and interesting groups of taxa in the new world tropical region (Losos, 2009). With approximately 350 species in its ranks, *Anolis* is the second most species rich genus of vertebrates (behind the neotropical frog genus *Pristimantis*, formerly *Eleutherodactylus*; Hedges *et al.*, 2008), and the richest genus of amniotes. Even aside from its species richness, the genus *Anolis* has become a textbook example of ecological and morphological convergence in an adaptive radiation (Futuyma, 1998; Schluter, 2000; Losos, 2009). On the separate Greater Antillean islands of the Caribbean, anoles have independently diversified into similar ecological specialists with concomitant morphological adaptations (Williams, 1972; Losos, 1990a,b, 2009; Losos *et al.*, 1998). As a consequence, anoles have become among the best studied adaptive radiations of animals (Schluter, 2000). Nonetheless, little is known about the evolutionary quantitative genetics of anoles (but see Revell *et al.*, 2007).

The Puerto Rican crested anole, *Anolis cristatellus*, is among the most common and ubiquitous members of the vertebrate fauna of the Puerto Rican bank islands, which include the main island of Puerto Rico, and its close geographic neighbours. *Anolis cristatellus* is found at very high natural densities (e.g. Genet, 2002), particularly in the tropical lowland areas in which it is most common, and has been quite well studied (e.g. Huey & Webster, 1976; Fleishman *et al.*, 1993; Leal & Rodríguez-Robles, 1995; Perry *et al.*, 2003; Leal & Fleishman, 2004; Glor *et al.*, 2007; Revell *et al.*, 2007). Unfortunately, the natural habitat for *A. cristatellus*, lowland dry and mesic tropical forest, has been largely decimated by human activities on the main island which are concentrated in coastal lowland areas (Birdsey & Weaver, 1987; Thomlinson *et al.*, 1996).

To our knowledge, this habitat destruction has thus far produced no serious impact on population densities of *A. cristatellus*, as there is no evidence of population declines in this species in anthropogenically modified and disturbed environments. However, habitat destruction and alteration has probably substantially altered the selective regime for populations of this species.
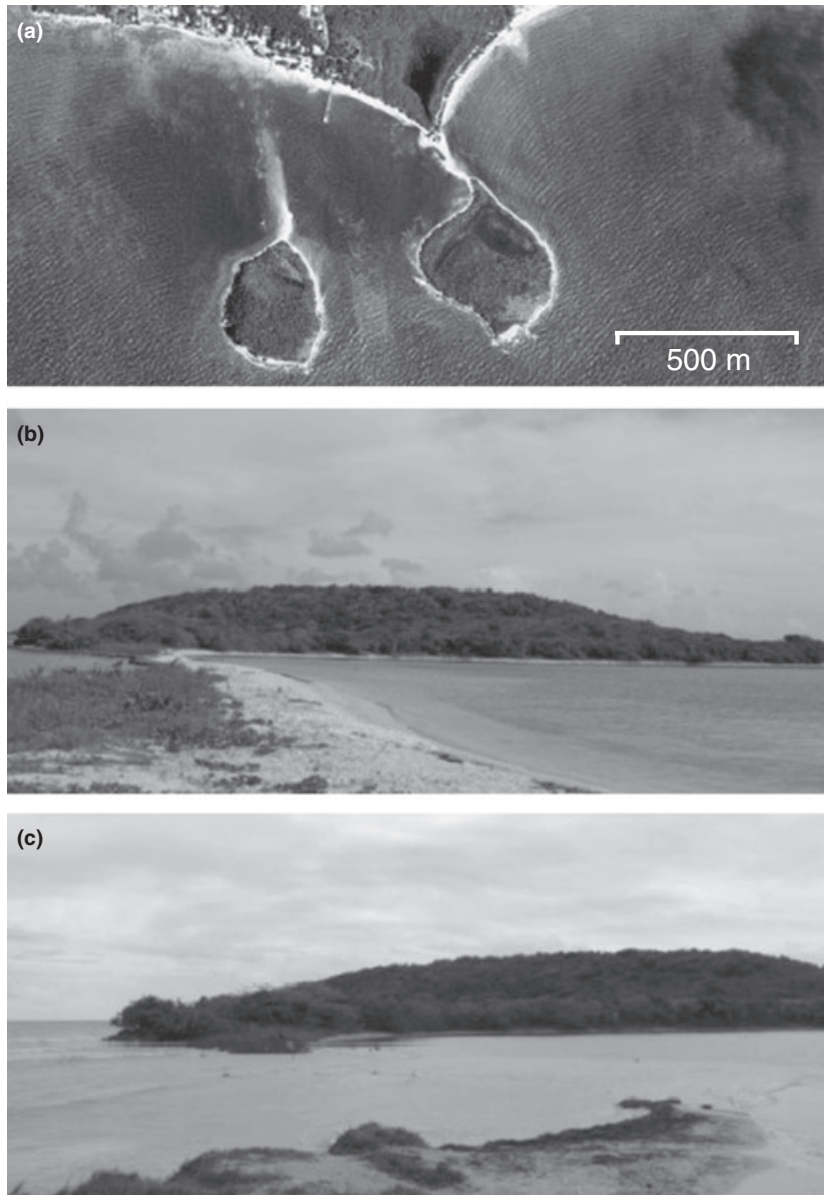
Fig. 1 Three views of the study site, Cayo de Tierra, Vieques. (a) Aerial view. Cayo de Tierra is the more easterly (rightmost) of the two cays. (b) View of cay when connected by sand bar. (c) View when unconnected. (Aerial view from Google Maps.)

Consequently, in our efforts to measure linear and nonlinear selection potentially reflective of that experienced during the majority of the evolutionary history of this species, we sought out an environment relatively free from recent human modification. This led us to the off-shore island of Vieques. Vieques is a moderate sized Caribbean island – approximately 32 km long at its longest, and approximately 7 km wide at its widest. Although much of the island was once cleared for sugar cane farming, the 62 year U.S. Navy presence on the island (finally ending in 2003) prevented extensive anthropogenic development and thus allowed natural vegetation to re-grow in many areas. Whether or not this secondary forest well approximates the historic condi-

tions on the isle is unknown. However, we feel that it probably represents a selective milieu more consistent with historical conditions than that which is often found in the highly developed lowland areas of the main island. We chose the intermittently insular/peninsular cay, Cayo de Tierra, off the southern coast of Vieques as our study area for several reasons including its complete lack of human habitation, its isolation from adjacent populations of *Anolis cristatellus*, and its relatively manageable dimensions. Figure 1 shows aerial and terrestrial views of the cay.

In this study, we detail efforts to (i) elucidate multivariate linear and nonlinear natural selection in a population of *A. cristatellus* and (ii) compare the structure

of nonlinear selection to that of genetic variances and covariances. In so doing, we will examine the hypothesis that multivariate nonlinear selection has influenced the evolution of quantitative genetic parameters in this species.

Materials and methods

Data collection

In June of 2006 we captured, measured, marked, and released 675 adult male *A. cristatellus* from an approximately 2.0 ha area of Cayo de Tierra, Vieques (Fig. 1). Our study plot was bordered on the northwest and northeast by a lagoon, on the southwest by the Caribbean Sea, but had an open (and thus 'leaky') southeastern forest verge (Fig. 1a). Very high lizard densities prohibited a completely exhaustive sampling of the cay. When an individual was captured, we flagged the tree upon which it was first observed and collected GPS latitude and longitude information for its collection locality. The individual was then temporarily removed from the field, measured for five morphological characters, and weighed. The five morphological measurements collected externally from each animal with a ruler or digital calipers included: snout-to-vent-length (a measure of body length commonly collected from reptiles and amphibians), jaw length, head width, forelimb length and hindlimb length. We generally collected all measurements of bilaterally symmetrical characters from the right side of the animal; however, in the event that the right side showed injury or damage, we collected the measurement from the left side instead. We then permanently marked each animal using nontoxic injectable coloured elastomers (Losos *et al.*, 2004). We used a marking scheme in which marks were placed on three of eight possible limb segments, uniquely identifying each marked lizard.

After each lizard was marked and measured, it was returned to the field. We used the flagging and GPS coordinates to return lizards as close as possible to their locations of initial capture to ensure minimal disruption in the animals' individual habits.

In February of 2007, we returned to our study site on Cayo de Tierra and captured, humanely euthanized, measured, and preserved 600 adult males from the original study site and its immediate vicinity. Prior to preservation, we collected the same external measurements as those listed above. We recorded and definitively identified any individuals that had been marked in the prior season. The rate of mark loss was very low (<1% of marks). In all but three of 142 recaptures, marked animals could be unambiguously identified. Of these, two animals had been marked incorrectly, and one had lost two of three tags (thus eliminating any hope of successful identification).

Upon returning from the field we used Varian Image View and Acquisition, and Kevex X-ray Source Interface 4.1.3 software to obtain digital radiographs of each specimen. We collected the following 13 linear measurements from each digital X-ray: head length, jaw length, metacarpal length on the third digit, radius length, ulna length, humerus length, pectoral girdle width, pelvic girdle width, femur length, tibia length, fibula length, metatarsal length on the third toe, and, finally, first phalangeal bone length on the third toe. For each bilaterally symmetrical linear measurement, we obtained measures from both the right and left side. We checked and re-measured left-right differences in excess of 10% (15% for metatarsal, metacarpal, and phalangeal measurements). As with external measurements, if either side of the animal was injured or damaged, we used only the intact side for measurement; however, for all individuals from which accurate measurements could be obtained from both sides, we averaged bilaterally symmetrical traits. This yielded a total of 13 morphological measurements per animal. Because we deemed 13 to be too large a number of characters for meaningful analysis and interpretation (the number of estimated parameters for both variance-covariance matrix and quadratic selection model calculation proliferate with the square of the number of characters), we agglomerated the linear measurements from X-rays as follows: hindlimb length = phalanx + metatarsal + tibia + femur lengths; forelimb length = metacarpal + radius + humerus lengths; and we dropped ulna, fibula, and pectoral girdle widths from all the main analyses. This left us with a total of five measurements (or agglomerated measurements) from each X-rayed individual (hindlimb length, forelimb length, jaw length, head width and pelvic width).

Substitution of P for G

Cheverud (1988) conjectured that under many circumstances the matrix of phenotypic variances and covariances (**P**) might be a suitable substitute for **G** in evolutionary analyses. As **G** is estimated by way of breeding experiment whereas **P** is estimated from individuals sampled by whatever means, error in **G** decreases as a function of the number of families in the study rather than as a function of the number of individuals. This led Cheverud (1988) to suspect that empirical estimates of **P** based on large samples might provide a better proxy for **G** than do direct estimates of **G** from breeding experiments (due largely to high error in the empirical estimation of **G**). Cheverud (1988) was able to support this conjecture with a meta-analysis of published data showing that the phenotypic correlation structure approached the genetic correlation structure as the effective sample sized used to estimate the latter increased. The most logical explanation for a pattern in which the discordance between **G** and **P** is greatest when the sample size is small but decreases for larger *n*, is that

such discordance is likely attributable to error in the estimation of **G** rather than to real differences between **G** and **P**. Several subsequent authors have also found considerable resemblance between genetic and phenotypic correlation structures for a wide variety of traits and species (e.g. Roff, 1995; Koots & Gibson, 1996; Reusch & Blanckenhorn, 1998; Waitt & Levin, 1998).

Although Cheverud's conjecture has been the subject of considerable contention and remains controversial (e.g. Willis *et al.*, 1991), under many circumstances obtaining a reasonable estimate of **G** is prohibitively challenging or impractical. This is particularly true for members of nonmodel species, many of which are of greatest interest to evolutionary investigators (e.g. Gracey & Cossins, 2003; Lee & Mitchell-Olds, 2006). As direct estimates of the genetic variance-covariance matrix for *A. cristatellus* are not yet available, in this study we apply Cheverud's (1988) conjecture and calculate the phenotypic variance-covariance matrix, **P**, then substitute it for the **G** matrix in all subsequent evolutionary analyses. Obviously, we would have preferred to study **G** instead of **P** in this study. We hope that our estimates of selection can be used for future analysis of **G** if that matrix becomes available for *A. cristatellus* or other closely related species. Furthermore, we note that discordance between **G** and **P** is likely to increase type II rather than type I error when we substitute **P** for **G** in evolutionary analyses (Revell *et al.*, 2007). Finally, by comparing the curvature of the natural selection surface to the phenotypic variance covariance matrix, we are indirectly testing the hypothesis that the **G** matrix is influenced by natural selection. However, we are directly testing the hypothesis that the phenotypic integration of the traits in our study (measured by **P**) is shaped in part by nonlinear selection (Olson & Miller, 1958; Cheverud, 1982; Brodie, 1992; Arnold, 2005). Although we do not focus on morphological integration in the present article, this is a very active area of research (e.g. Schlichting & Pigliucci, 1998; Pigliucci & Preston, 2004; discussed in Arnold, 2005).

P-matrix estimation

Because all our linear measurements covaried strongly with size (as is common in *Anolis* lizards and vertebrates generally), we first removed the effect of size from both linear external and digital X-ray measurements by calculating: $\mathbf{R} = \mathbf{Y} - \mathbf{X}(\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{Y}$, in which **R** contains the size corrected residuals, **X** is a matrix containing a column of 1.0s and a column with the measurements for size (SVL in this study), and **Y** is a matrix containing all non size traits (4 external traits or 5 skeletal measurements from X-rays). This is the standard linear regression procedure for size correction.

Once we had removed size as described above, we were able to calculate phenotypic variance-covariance matrices (**P** matrices) for the size free residuals. We

estimated **P** using the standard unbiased estimator for the covariance matrix, given as $\mathbf{P} = \mathbf{R}'\mathbf{R}/(n - 1)$. Here, **R** is a matrix containing the size free residuals for each trait in columns, as above. There is no need to subtract the mean of **R** when calculating the variances and covariances because size-correction via linear regression has given each column of residuals a mean value of 0.0. We calculated two versions of **P**: the 4×4 matrix $\mathbf{P}_{ex}$ (in which 'ex' denotes 'external' measurements), containing the size-free variances and covariances for each of the four externally measured characters (hindlimb and forelimb lengths, jaw length and head width); and the 5×5 matrix $\mathbf{P}_{in}$ ('in' denotes 'internal'), containing the size-corrected variances and covariances for each of the five characters or agglomerated traits measured from digital radiographs (hindlimb and forelimb summed lengths, jaw length, head width and pelvis width). For the latter, we used only the specimens collected during the recapture component of this study (as we had no access to X-ray equipment in the field), thus $n_{in} = 600$. However, for the former matrix we used all individuals for which we had obtained external measurements. This included 675 individuals from the capture period, and then 600 in the recaptured series. 139 of these ($142 - 3$, from above) overlapped between series and were thus excluded (we excluded the measurements from the capture series arbitrarily). Thus, $n_{ex} = 600 + 675 - 142 = 1136$ (we also had to leave out a small number of individuals due to missing data, thus the realized sample sizes for both matrices were actually $n_{ex} = 1134$ and $n_{in} = 598$).

We obtained standard errors for the matrix elements using a delete-one jackknife procedure (Manly, 1997). These were obtained by first estimating the sample variance for each element of **P** as follows: $\text{var}(P_{ij}) = \frac{1}{n-1}\sum_{k=1}^{n}\left(nP_{ij} - (n-1)P_{ij,-k}\right)^2$, in which $P_{ij,-k}$ is the estimate of the i,jth element of **P** obtained with the kth sample excluded. The term in parentheses (i.e. $nP_{ij} - (n-1)P_{ij,-k}$) is a pseudovalue for P_{ij} (Manly, 1997). We can then compute the standard error of P_{ij} as $\sqrt{\text{var}(P_{ij})/n}$ (Manly, 1997).

In addition to the matrices calculated above, we also generated a second estimate of $\mathbf{P}_{in}$ from a series of $n = 140$ individuals collected in 2005 from the same locality as that used in this study (see Revell *et al.*, 2007). Although we do not present the results from these analyses, this matrix was extremely highly correlated with the version estimated herein (element-by-element correlation, $r = 0.996$), and as such we use only $\mathbf{P}_{ex}$ and $\mathbf{P}_{in}$ from the current study in all subsequent analyses.

Selection analysis

The selection analysis component of this study involved several different types of data and analysis. Firstly, the phenotypic data came in two forms: externally from 1136

wild caught lizards, 536 of which were never recaptured; and internally from digital X-rays of 600 captured, humanely euthanized and preserved specimens.

Secondly, fitness was measured in two different ways. We estimated fitness in the set of 675 initially marked lizards to be 1.0 if the lizard was recaptured, and to be 0.0 if the lizard failed to be recaptured. Two assumptions are implicit in this estimate: that the vast majority of lizards originally caught were recaptured if alive at the time of re-sampling; and that survivorship over the approximately 9 month period of the study was (or would normally be) strongly correlated with reproductive output (we excluded a small number of lizards from this analysis for various reasons such as injury during capture or death prior to marking, making our realized sample size $n = 661$).

In addition, we estimated fitness in the set of 600 captured and preserved specimens from body condition, calculated as size-corrected body mass. The implicit assumption of estimating fitness in this way is that lizards with good body condition (i.e. fatter lizards) will on average sire more progeny. As body condition probably correlates with territory size or quality in this highly territorial species, we thought this to be a reasonable supposition *a priori*. Some field and experimental evidence from anoles and other lizards supports this assumption. For example, in a supplemental feeding experiment, Licht (1974) observed that *A. cristatellus* that were fed supplemental food became more robust and in some cases expanded their territories to the exclusion of other previously dominant males. Stamps & Eason (1989) found that juvenile *A. aeneus* with larger territories exhibited higher growth rates, reversing the direction of causality but nonetheless resulting in an expected relationship between growth and territory size (although in this case the authors measured only overall size, not size-corrected mass). Jenssen *et al.* (2005) found that body mass controlling for overall size significantly predicted the outcome of aggressive encounters in a laboratory experiment with *A. carolinensis*. In non-anoline iguanian lizards, body mass controlling for size has also been found to correlate with mating success (in the marine iguana, *Amblyrhynchus cristatus*; Wikelski *et al.*, 1996). However, in at least one instance it has been suggested that poor body condition was reflective of higher metabolic costs associated with reproductive behaviours (in the painted dragon lizard, *Ctenophorus pictus*; Olsson *et al.*, 2007). In this case one might expect a negative correlation between body condition and reproductive fitness; however, this relationship has not been reported for anoles. The success of our models, of course, depends on the degree to which the performances measures estimated herein (survival across an arbitrary time interval, and adult body condition) map to reproductive fitness in this species (Arnold, 1983, 2003).

Once each measure of fitness was calculated, we fit second-order multivariate polynomial selection models to the fitnesses and our size-corrected phenotypic data, following Lande & Arnold (1983); also see Stinchcombe *et al.*, 2008) and as discussed above. We evaluated the type I error probability (i.e. significance) of the second-order polynomial regression models using both parametric tests and random permutation of fitness values among individuals.

Finally, we estimated nonlinear and correlational selection in a wholly different way by computing the vector of linear selection differentials, **s**, and the matrix, **C**, containing the nonlinear within generation selection differentials. This latter matrix is computed as follows: $\mathbf{C} = \mathbf{P}^* - \mathbf{P} + \mathbf{ss}'$ (Lande & Arnold, 1983; Arnold *et al.*, 2001). Here, $\mathbf{P}^*$ is the phenotypic variance-covariance matrix after selection, **P** is as previously defined, and **s** is the linear selection differential. The term $\mathbf{ss}'$ is added to account for the change in **P** due to linear selection (Lande & Arnold, 1983). The expected relationship between **C** and γ is $\mathbf{C} = \mathbf{P}\gamma\mathbf{P}$ (Arnold *et al.*, 2001), so to solve for the curvature matrix, γ, we just computed $\gamma = \mathbf{P}^{-1}\mathbf{C}\mathbf{P}^{-1}$.

We can estimate $\mathbf{P}^*$ by calculating the phenotypic variance-covariance matrix for only those individuals who have a fitness value of 1.0 (having been sampled both in 2006 and 2007). As this procedure can be performed using both external and internal measurements, we can compare the quadratic selection matrices obtained to both versions of **P** ($\mathbf{P}_{ex}$ and $\mathbf{P}_{in}$), as previously described.

In addition to these analyses, we also performed secondary canonical analyses of the nonlinear selection (γ) matrices (Blows & Brooks, 2003). Using this procedure we can identify the independent multivariate trait dimensions with the greatest curvature on the fitness surface. Our analysis follows Blows & Brooks (2003) and is presented in detail in Appendix S1, along with the results. For contrasting opinions on the advantages of diagonalization in evolutionary quantitative genetic analyses and selection analyses see Blows (2007a,b) and Brodie & McGlothlin (2007).

Comparison of $-\gamma^{-1}$ and P

To test the hypothesis that **G** will evolve to significantly align with the selection surface under nonlinear and correlational selection, we compared the two matrices (applying Cheverud's conjecture, as noted above) using four different techniques. First we computed the element-by-element Pearson product moment correlation between $-\gamma^{-1}$ and **P**, and compared this correlation to a null distribution for $-\gamma^{-1}$ obtained by randomly permuting fitness values among individuals. Next, we used vector correlation to compare the leading eigenvector of **P** ($\mathbf{p}_{max}$) to the leading eigenvector of $-\gamma^{-1}$ (which we denote ω_{max}, due to approximate alignment of $-\gamma^{-1}$ and the Gaussian selection surface matrix ω under weak stabilizing selection; Lande, 1979). This analysis follows

Schluter (1996, 2000) and Arnold *et al.* (2001). We used the subspace method of Blows *et al.* (2004); following Krzanowski, 1979), to compare the overall alignment of the partial subspaces defined by the first two eigenvectors of $-\gamma^{-1}$ and **P**. We also compared the observed differences between the major eigenvectors and the subspaces of $-\gamma^{-1}$ and **P** to those obtained by random permutation to estimate a type I error probability (*P*-value) for each test. Finally, we used the method of Hunt *et al.* (2007) to estimate and compare the nonlinear selection in the dimensions of γ defined by major eigenvectors of **P**, and *vice versa*. The correlation and major eigenvector analyses are relatively straightforward. The methods of Krzanowski (1979; also Blows *et al.*, 2004) and Hunt *et al.* (2007) deserve some additional details. For definitive descriptions of the application of both methods to problems highly similar to those treated herein, the reader should refer to Blows *et al.* (2004) and Hunt *et al.* (2007).

For the analysis based on Krzanowski (1979) we first computed two matrices, **A** and **B**. **A** is an $m \times 2$ matrix containing the first two eigenvectors (standardized to unit length) of $-\gamma^{-1}$ (in columns), whereas **B** is similarly dimensioned but containing the first two eigenvectors of **P**. We used only the first two eigenvectors because one cannot use more than half of the eigenvectors of each matrix and obtain a sensible result, and our analyses contained four or five traits (Krzanowski, 1979; Blows *et al.*, 2004). Next, we calculated the matrix, **S**, containing the minimum angles between the vectors of **A** and **B**, as $\mathbf{S} = \mathbf{A'BB'A}$. The arccosine of the square root of the leading eigenvalue of **S** is the smallest angle between any pair of orthogonal axes in each subspace, and subsequent eigenvalues are the squared cosines of subsequent, larger angles (Krzanowski, 1979). Thus, the similarity of the subspaces can be assessed by summing the (in this case) two eigenvalues of **S**, $\sum_i \lambda_i(\mathbf{S})$, which can be interpreted as the sum of squared cosines of the minimum angles between the two subspaces. This sum of squares will range from 0.0 if the matrices are wholly dissimilar, to 2.0 (in this case; or the number of eigenvectors used, in general) if the matrices share their major eigenvectors in common (Blows *et al.*, 2004). We also computed standard errors on the summed squared cosines of the minimum angles using a delete-one jackknife approach, as previously described (see above; Manly, 1997).

We used the method of Hunt *et al.* (2007) to compare the nonlinear selection in the dimensions of γ defined by major eigenvectors of **P**, and *vice versa*. The expectation in

this case is that stabilizing selection should be relatively weak (or positive, i.e. disruptive) in dimensions of high variance in **P**; whereas stabilizing selection should be strong in dimensions in which there is little variance in **P**. We computed quadratic selection both in the orthogonal major axes of **P**, and along the major axes of the selection matrix, γ. As we computed selection in two ways for each of our two phenotypic datasets, and we analyzed the relationship between selection and trait variability in two ways for each analysis of selection (along the eigenvectors of **P** and γ), we obtained results from eight separate analyses. This is a different approach to the analysis of the relationship between selection and genetic variability than the prior three methods because it focuses less on the correlation between $-\gamma^{-1}$ and **P**, and more on the general prediction that trait combinations with high genetic and phenotypic variability should be under less severe stabilizing selection (and *vice versa*). Because it focuses on the measurement of selection on trait combinations, rather than on the original traits directly, it is in some ways analogous to the canonical analysis of the selection surface described in Appendix S1 (also Blows & Brooks, 2003).

Results

P-matrix estimation

As previously noted, we calculated two versions of **P** in this study. The first, $\mathbf{P}_{ex}$, was based wholly on external measurements for the following characters: hindlimb length, forelimb length, jaw length and head width. Controlling for size, the residual variance-covariance and correlation matrices are given in Table 1. The largest residual correlation is between forelimb and hindlimb lengths; however, we found a smaller, but substantial, correlation between jaw length and head width (Table 1). All variances and covariances, and correlations, were significantly nonzero. We also provide the eigenvalues and eigenvectors, and percentage of variance explained by each eigenvector in the Appendix (Table S2.1).

We also calculated a second standard **P** matrix based on analogous measurements collected internally (from digital X-rays), with the addition of one further character that was not measured externally (pelvis width). We denote this matrix $\mathbf{P}_{in}$, which was based on linear measurements collected from digital X-rays for the following characters (or agglomerated characters, see

Table 1 Phenotypic correlations above the diagonal and covariances below. Traits were measured externally in mm on $n_{ex} = 1134$ individuals, and then size-corrected by calculating the residuals from ordinary least-squares linear regressions.

Trait	Hindlimb	Forelimb	Jaw length	Head width	Variance
Hindlimb	–	0.653 (0.02)	0.082 (0.03)	0.162 (0.03)	2.678 (0.12)
Forelimb	1.178 (0.07)	–	−0.075 (0.03)	0.178 (0.03)	1.217 (0.06)
Jaw length	0.093 (0.04)	−0.057 (0.02)	–	0.302 (0.03)	0.473 (0.02)
Head width	0.113 (0.02)	0.084 (0.01)	0.089 (0.01)	–	0.182 (0.01)

Standard errors in parentheses were obtained by jackknifing.

Trait	Hindlimb	Forelimb	Jaw length	Head width	Pelvis width	Variance
Hindlimb	–	0.769 (0.02)	0.248 (0.04)	0.219 (0.04)	0.237 (0.04)	1.444 (0.09)
Forelimb	0.645 (0.04)	–	0.338 (0.04)	0.251 (0.04)	0.289 (0.04)	0.488 (0.03)
Jaw length	0.146 (0.02)	0.116 (0.02)	–	0.427 (0.03)	0.265 (0.04)	0.240 (0.01)
Head width	0.101 (0.02)	0.067 (0.01)	0.080 (0.01)	–	0.319 (0.04)	0.147 (0.01)
Pelvis width	0.080 (0.01)	0.057 (0.01)	0.037 (0.01)	0.035 (0.00)	–	0.079 (0.01)

Table 2 Phenotypic correlations above the diagonal and covariances below. Trait measures in mm were obtained from X-rays or by summing long bone measurements on X-rays, and were size corrected, as in Table 1.

Jackknife standard errors in parentheses.
More details are available in the text; sample size was n_{in} = 598 individuals.

Methods): hindlimb length, forelimb length, jaw length, head width and pelvis width. Controlling for size, the residual variance-covariance and correlation matrices are given in Table 2. We also give the eigenvalues, eigenvectors and percentage of variance explained by each eigenvector in the Appendix (Table S2.2).

The correlation between analogous correlations or covariances from $\mathbf{P}_{ex}$ and $\mathbf{P}_{in}$ is quite high (0.85 and 0.99 respectively); however, there are some substantive differences. Residual variances and covariances from internal measurements are usually substantially lower then their externally measured counterparts and correlations higher (Tables 1, 2). This suggests, as one might expect, that analogous measurements are collected with lower measurement error from digital X-ray images than they are from external measurement using calipers or a ruler.

Selection analysis

We conducted three distinct selection analyses in this study. Each used a different proxy for fitness, a different phenotypic dataset, or a different model estimation procedure. The results are as follows.

The first regression selection model, in which we used only external measurements and in which we scored fitness as 0.0 (if an animal was not recaptured) or 1.0 (otherwise), was almost entirely nonsignificant, suggesting little if any relationship between phenotype and this measure of fitness. The full model was nonsignificant according to parametric tests ($F_{14,646}$ = 1.106, P = 0.349), and all but one coefficient (the linear selection coefficient on jaw length) was also individually nonsignificant. We

nonetheless report the full set of coefficients for the model in the Appendix (Table S2.3). The significances of individual model coefficients obtained by 9999 random permutations of fitness values among individuals and those obtained by comparison to the *t*-distribution were almost identical, but the former were slightly more conservative and thus we use these here and throughout. We also used 9999 random permutations for all randomization tests described herein.

The second regression selection model, in which we used internal X-ray measurements and scored fitness as residual log-transformed body mass (body condition), was highly significant. The full model was significant ($F_{20,577}$ = 7.361, P < 0.001); however, only two of 21 estimated coefficients (20 selection coefficients and the model intercept) were statistically significant, with two additional marginally significant coefficients (Table 3; intercept not shown). Three of these four coefficients were quadratic terms, indicating significant positive correlational selection between jaw length and head width, and marginally significant negative correlational selection between pelvis width and jaw length (Table 3). All model coefficients and significant type I error probabilities are reported or indicated in Table 3. All but one of the diagonal elements in the matrix were negative, suggesting a trend towards stabilizing selection on most traits (albeit a nonsignificant one).

The third selection model involved calculating the selection differentials for both the means (**s**) and the phenotypic variances and covariances (**C**), as described above. The linear and quadratic terms in the model were then estimated by computing $\boldsymbol{\beta} = \mathbf{P}^{-1}\mathbf{s}$ and $\boldsymbol{\gamma} = \mathbf{P}^{-1}\mathbf{C}\mathbf{P}^{-1}$,

γ-matrix	Hindlimb	Forelimb	Jaw length	Head width	Pelvis width	β-vector
Hindlimb	−0.0024	0.0074	−0.0206	0.0099	−0.0005	0.0026
Forelimb	–	−0.0319	0.0405	−0.0773	0.0435	−0.0091
Jaw length	–	–	−0.0187	0.1361**	−0.1158[†]	0.0220
Head width	–	–	–	0.0191	−0.1047	0.1912*
Pelvis width	–	–	–	–	−0.1336	0.0612[†]

Table 3 Linear and nonlinear selection gradients from the quadratic regression based selection analysis of adult body condition and internal morphological data.

* < 0.01; ** < 0.05; [†] < 0.10.
The **β**-vector (final column) contains the linear terms in the quadratic selection model, whereas the **γ**-matrix contains the nonlinear terms in the model. As **γ** is symmetrical, only the upper diagonal elements are reported. Significant coefficients (by permutation) are indicated by symbols.

following Lande (1980) and Lande & Arnold (1983); also see appendix to Arnold *et al.*, 2001). We performed these analyses with both the external measurements and the internal measurements and agglomerated measurements from X-ray images. In both cases, any individual that had appeared in 2006 and 2007 samples was included in the post-selection calculations (whereas all samples were included in the preselection mean and variance-covariance matrix estimates). We estimated the type I error probability (*P*-value) of each selection coefficient by random permutations of fitness, each time re-computing the selection differentials and gradients. The results from these analyses on external and internal measurements are presented in Tables 4 and 5. A number of coefficients are significant or marginally so in each case (Tables 4 and 5). In some cases these coefficients are difficult to interpret. For example, the selection differential analysis on the external morphological data indicated significant positive correlational selection between relative hindlimb length and jaw length (Table 4), and the analysis based on internal measurements indicated disruptive selection on both hindlimb and forelimb lengths, and, perhaps most surprisingly, significant negative correlational selection between limb lengths (Table 5).

As previously mentioned, we also performed canonical analysis on each of our estimated selection surfaces following Blows & Brooks (2003). Results from these analyses are presented in Appendix S1 and Tables S1.1–S1.4.

Comparison of $-\gamma^{-1}$ and P

We analyzed the alignment of the selection surface with **P** in several different ways. First, we computed the

element by element correlation between the selection surface, $-\gamma^{-1}$, and **P**. As we computed γ and **P** each in several ways, this analysis yielded a number of different results. Invariably, the observed correlation between $-\gamma^{-1}$ and **P** was moderate to quite strong (Table 6); however, in no case was it significant by permutation tests.

We also compared the major eigenvector of **P** ($\mathbf{p}_{max}$) with the major eigenvector of the individual fitness surface ($\boldsymbol{\omega}_{max}$), using a vector correlation. In this case, we also found strong alignment and in one instance this alignment was also quite significant (Table 7). We have provided all the eigenvectors and eigenvalues for $-\gamma^{-1}$ obtained in each of our selection models in the Appendix (Tables S2.4–S2.7).

We followed Blows *et al.* (2004) and compared the alignment of the multidimensional subspaces of **P** and the selection surface. We calculated the sum of the eigenvalues of the matrix, **S**, defined to find the minimum angles between the first two eigenvectors of **P** and the first two eigenvectors of $-\gamma^{-1}$, as described in the methods. We found significant alignment of **P** with $-\gamma^{-1}$ by the subspace method when the latter matrix was estimated using the selection differential procedure (Table 8; see Methods); however, measures of alignment computed when $-\gamma^{-1}$ was estimated via least-squares regression were invariably nonsignificant. We found some discordance between our significance assessed by permutation and the standard errors estimated using a delete-one jackknife approach (Manly, 1997; Table 8). This may be because our jackknife parameter estimates were upwardly biased for the selection differential based analyses (in particular, many pseudovalues in the jackknife distribution had $\sum_i \lambda_i(\mathbf{S}) > 2$, which makes no sense as $\sum_i \lambda_i(\mathbf{S}) = 2$ indicates perfect alignment of the eigenvectors. We are not sure how to resolve this issue, but in the absence of an obvious resolution, we would guess that our *P*-values obtained by permutation are probably robust.

Finally, we also followed Hunt *et al.* (2007) and computed the curvature of the selection surface (γ) along the eigenvectors of **P**, as well as the variability in **P** along the eigenvectors of γ. We performed both analyses for each of our four selection models, described above. We expected to find that variability in **P** was associated with the largest values for curvature in γ (i.e. weak

Table 4 Selection gradient estimates from the selection differential based analysis of external morphological data.

γ-matrix	Hindlimb	Forelimb	Jaw length	Head width	$\boldsymbol{\beta}$-vector
Hindlimb	–0.0489	0.0370	0.0517*	0.1420	–0.0407
Forelimb	–	–0.0556	–0.0202	–0.0867	0.1012
Jaw length	–	–	0.2378	0.2411	–1.1575*
Head width	–	–	–	0.8564	0.0885

* < 0.01.

$\boldsymbol{\beta}$ and γ are as in Table 3. Significant coefficients are indicated by symbols.

Table 5 Selection gradient estimates from the selection differential based analysis of internal morphological data.

γ-matrix	Hindlimb	Forelimb	Jaw length	Head width	Pelvis width	$\boldsymbol{\beta}$-vector
Hindlimb	0.2786*	–0.9024*	0.2306	0.0693	–0.0075	0.0014
Forelimb	–	2.6681*	–0.9053	–0.0859	–0.8631	–0.0048
Jaw length	–	–	0.8356	0.0475	1.1116[†]	0.0116
Head width	–	–	–	0.8795	0.2649[†]	0.1005**
Pelvis width	–	–	–	–	–0.0556	0.0321[†]

* < 0.05; [†] < 0.10.

$\boldsymbol{\beta}$ and γ are as in Tables 3 and 4. Significant coefficients are indicated by symbols.

Table 6 Pearson product moment correlation coefficients for the correlation between **P** and the individual selection surface.

Method	Data	$r(-\gamma^{-1}, \mathbf{P})$	P (permutation)
Least-squares	External	−0.8859	0.8746
	Internal	0.8871	0.1396
Selection differential	External	0.8514	0.3301
	Internal	0.9407	0.7142

Type I error probabilities (P-values) were obtained by permutation.

Table 7 Vector correlation between the leading eigenvectors of **P** ($\mathbf{p}_{max}$) and the individual selection surface ($-\gamma^{-1}$, given as ω_{max}).

Method	Data	$r(\mathbf{p}_{max}, \omega_{max})$	P (permutation)
Least-squares	External	0.2802	0.7594
	Internal	0.9907	0.0193
Selection differential	External	0.9442	0.1882
	Internal	0.9640	0.5128

Type I error probabilities were obtained by permutation.

Table 8 Alignment of the subspaces of **P** and the individual selection surface.

Method	Data	$\sum_i \lambda_i(\mathbf{S})$	P (permutation)
Least-squares	External	0.9028 (1.2519)	0.6858
	Internal	1.0480 (0.5404)	0.3503
Selection differential	External	1.6198 (0.7407)	0.0003
	Internal	1.0819 (1.7242)	0.0175

A value of $\sum_i \lambda_i(\mathbf{S}) = 2$, would in this case indicate perfect alignment (proportionality) of the two matrices. Standard errors, obtained using a delete-one jackknife approach, are reported in parentheses. Type I error probabilities were obtained by random permutation of the fitness values among individuals.

stabilizing selection or disruptive selection). What we found instead was not a monotonic increase in the variability of **P** with decreased stabilizing selection and increased disruptive selection, but maximum variability in **P** along orthogonal dimensions with relatively little quadratic selection of any kind (stabilizing or disruptive). Figure 2 shows the relative nonlinear selection and relative variance (scaled to the interval −1,1 and 0,1 in each case) from these analyses. The intermediate mode between nonlinear gradients and phenotypic variance is clearly evident. Full details of these results are given in Appendix S2 (Tables S2.8–S2.11).

Discussion

In this study we first estimated natural selection in a population of Caribbean *Anolis* lizards and then compared the curvature of the individual selection surface to the pattern of variances and covariances for phenotypic characters. The motivation in so doing was to determine
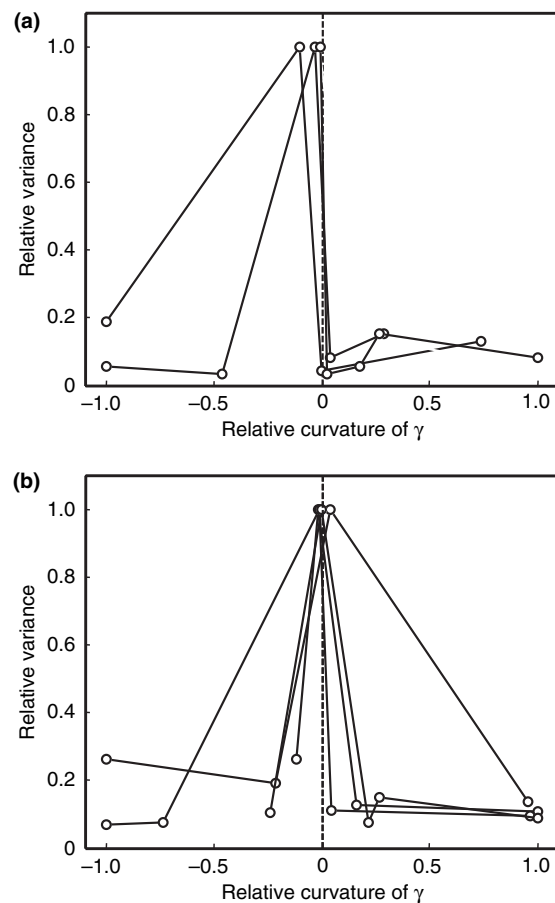

Fig. 2 (a) Relative variance on each eigenvector of **P**, against the relative magnitude of quadratic selection on that eigenvector. Each set of connected points represents of set of values from a different selection analysis. Eigenvalues and selection coefficients have been rescaled to be on the interval (0,1) and (−1,1), respectively. Unscaled measures are in Appendix S2. (b) Relative variance in **P** on each eigenvector of the matrix of nonlinear selection, γ, against the nonlinear selection coefficients (eigenvalues) on the eigenvectors of γ. Variances and coefficients have been rescaled, as in (a).

the role played by natural selection in the evolution of genetic variances and covariances in this species (e.g. Blows *et al.*, 2004; Hunt *et al.*, 2007), using phenotypic measures as a proxy (Cheverud, 1988; Roff, 1995).

Although our nonlinear natural selection analyses provided a mixture of nonsignificant and significant results, we did find, in several cases, significant alignment of the individual selection surface with the phenotypic variance-covariance matrix, **P**, as predicted by theory. This finding implies that selection in the form of curvature in the individual selection surface may have played a role in the evolution of **G** for *Anolis cristatellus*. By contrast, in multivariate analyses of the strength of selection on major eigenvectors of **P**, or (conversely) the variability in **P** in the orthogonal directions of curvature

on the selection surface, γ, we actually found a result contrary to expectation. That is, we found the maximum level of phenotypic variability in directions under weakest stabilizing or disruptive selection, whereas we would have expected more variability in dimensions under stronger disruptive selection (i.e. towards the right side of the abscissa in Fig. 2a,b).

The phenotypic variances and covariances

We estimated two different phenotypic variance-covariance matrices (**P** matrices) in this study. One was obtained from external (i.e. ruler and caliper) measures collected from 1134 different individuals, and the second was obtained from internal (i.e. digital X-ray) measures collected from 598 different individuals. After size correction, four of the five characters (hindlimb and forelimb lengths, jaw length and head width) measured internally have approximate analogues measured externally. In fact, with pelvis width removed from the internal matrix, both versions of **P** were highly correlated (Tables 1 and 2; $r = 0.986$). However, in general the variances estimated from external measures were larger, and the correlations between traits smaller, than those computed for the analogous measures collected internally (Tables 1 and 2). This suggests that measurements collected from digital X-rays have decreased measurement error relative to external measurements (also see Revell *et al.*, 2007).

Even after correcting for size, forelimb and hindlimb lengths are highly phenotypically correlated in this species. The next largest correlation between any pair of characters is between relative jaw length and head width. These findings are consistent with an earlier study on the same species (Revell *et al.*, 2007). Future studies are needed to determine if limb lengths and head dimensions evolve as developmental modules (Cheverud, 1996), and if this pattern of covariance between characters is genetic and general to other anoles as well.

The pattern of natural selection

In this study we found some limited evidence for significant linear, nonlinear and correlational selection (Tables 3–5). To obtain measures of natural selection we first calculated fitness from mark-recapture data collected for adult males as well as from body condition. We analyzed these data in two ways. First, we fit a least-squares regression model including all linear and second-order (including correlational) terms, and second, we calculated selection differentials for the mean and variance-covariance matrix (Lande & Arnold, 1983).

Nonsignificant findings may indicate that the species is not experiencing directional or nonlinear selection at present; however, there are a number of other biologically plausible explanations for the shortage of significant selection coefficients in this study. Aside from an insufficiency of power, these arise from practical and experimental limitations in our study.

Firstly, we sampled only adult males. Prior studies of natural selection in anoles have usually sampled a wider size range of individuals in the population (e.g. Losos *et al.*, 2004, 2006; Calsbeek & Irschick, 2007; Calsbeek & Smith, 2007; Calsbeek, 2009). We chose to sample adult males mainly for several reasons in this study. Firstly, from a practical perspective, the very high absolute population size of our study site would have been overwhelming had we not concentrated our efforts on only a subset of all individuals in it. Prior studies on anoles have concentrated on populations much smaller in size (e.g. Losos *et al.*, 2004). Male anoles are conspicuous and relatively easy to catch, whereas females and juveniles in this species are evasive (females) or secretive, increasing the difficulty of including these individuals in the study. Secondly, females in particular may be subject to a conflicting pattern of natural selection, which might have made a concordant pattern between **G** and γ even more difficult to identify. Finally, and most importantly, almost all of the significant comparative studies of the adaptive radiation of anoles have been conducted analyzing adult male morphology (e.g. Losos, 1990a,b; Losos *et al.*, 1998; but see Butler *et al.*, 2007), and as such we decided to retain male morphology as the target of analysis in the present article. However, if selection is strong but takes place only prior to adulthood or primarily on females (with only a correlated effect on male morphology), then it would almost certainly fail to be detected by our study.

Secondly, in most published selection studies proxies for fitness (usually survivorship, although sometimes growth) are used in lieu of direct measures, although there are a growing number of studies in which genetic parentage analysis has been used to estimate fitness directly (DeWoody, 2005; e.g. Lewis *et al.*, 2000; Blouin-Demers *et al.*, 2005; Reichard *et al.*, 2009; also see Morgan & Conner, 2001). In this study we substitute adult survivorship and body condition as two separate and independent measures of fitness; however, their relationship to reproductive success is unverified for our species (although some prior evidence suggests that robust lizards might be fit; e.g. Licht, 1974; Wikelski *et al.*, 1996; Jenssen *et al.*, 2005). In as much as we failed to find strong evidence for natural selection, it may be because we used performance variables as proxies for fitness that were only weakly correlated with lifetime reproductive success in this population (Arnold, 2003).

Finally, we were neither able to mark nor recapture all of the adult male lizards that inhabited our study plot on either trip, and furthermore the plot was leaky, i.e. it lacked hard boundaries on all sides (Fig. 1a). This means that it is quite likely that some marked lizards that were not recaptured were either simply missed or migrated out of the plot, causing us to incorrectly score them as deceased. As stated earlier, this failure was due in large

part to an embarrassment of riches: our study site (Cayo de Tierra, Vieques; Fig. 1) had more lizards than we could catch. In future studies, we might do better to anticipate the extraordinarily high (but largely undocumented, although see Genet, 2002) population densities of *A. cristatellus* throughout its range. We note that the selection differential analysis is less susceptible to this problem than is the regression analysis. This is because the method does not assume that all individuals not recaptured are unfit – rather it assumes only that **P*** is computed from a random sample of fit individuals, and **P** from a random sample of individuals fit and unfit.

Although in some cases measures of nonlinear selection were significant, these measures were independent or even negatively correlated among different datasets, measures of fitness, and estimation procedures (Tables 3–5; Table S2.3). This suggests that, even inasmuch as differential adult survivorship and body condition have significant morphological bases (Tables 3–5), body condition and adult survivorship are not likely to both be good proxies for fitness, if either is. Corroborating this interpretation, body condition when initially captured is only a weak and nonsignificant indicator of whether an animal will be recaptured ($t_{254.8} = 0.789$, one-tailed $P = 0.215$). Field studies examining the relationship between body condition, adult survivorship, and lifetime reproductive success in this species would be helpful in clarifying this matter.

Congruence of phenotypic covariances and individual selection

With the caveats already considered above; however, we did identify significant evidence of selection in some analyses. Moreover, we also found significant congruence between the matrix of phenotypic variances and covariances (**P**), which we use as a substitute for **G** herein, and the matrix of nonlinear natural selection (Tables 7 and 8). Congruence between **G** and the quadratic selection surface is expected in theory so long as mutational inputs are equal and uncorrelated among traits (Cheverud, 1984; Arnold, 1992). This assumption is nearly certain to be violated by any correlated pleiotropic mutation among characters (e.g. Jones *et al.*, 2003). However, we feel that testing for a relationship between **G** and quadratic selection is nonetheless a viable means of assessing the importance of selection in the evolution of genetic variances and covariances between characters.

We estimated congruence using four approaches. First we evaluated whole matrix alignment using the very simple Pearson product moment correlation. We then assessed the significance of said correlation by randomly permuting fitness values among individuals and each time recalculating the individual selection surface. Although we found very strong positive congruence in three of four comparisons (Table 6), in no instance was
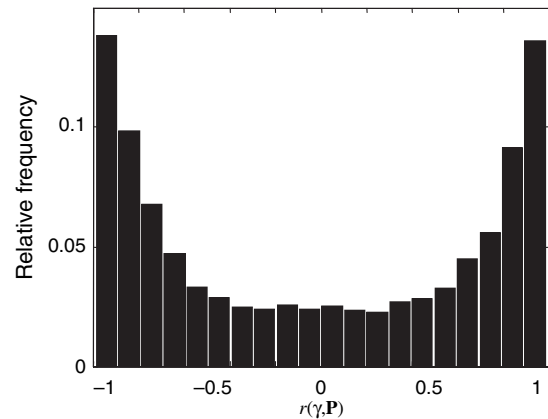

Fig. 3 Null distribution of element-by-element matrix correlations between the individual selection surface and **P** obtained by randomly permuting fitness among individuals. 9999 random permutations were used.

this alignment stronger than one would expect by chance. This is because the permutation distribution for the matrix correlation was usually U-shaped, with very high right and left shoulders (Fig. 3). This makes it nearly impossible to identify a significant excess of matrix congruence by this method. It is not clear to us at the present time whether this is a general property of the test or a unique peculiarity of the data in this study.

We compared the major eigenvectors of **P** and the individual selection surface. As before, strong congruence between the vectors was expected by chance. However, in one analysis significant correspondence was indicated by our permutation tests (Table 7).

Next, we used the method of Blows *et al.* (2004); also Krzanowski, 1979) to compare subspaces from the individual selection surface and the phenotypic matrix. We prefer this method to other methods such as Common Principal Components (Phillips & Arnold, 1999) because it is not as susceptible to changes in the rank-order of eigenvectors in different matrices (Blows *et al.*, 2004). We found significant congruence only from the selection differential analyses (Table 8); however, the alignment in this case was quite highly significant for both the external and internal data.

Finally, we evaluated the curvature of the selection surface in the multivariate dimensions given by the major eigenvectors in **P**; as well as the variability in **P** along the major orthogonal axes of curvature in the selection surface. This analysis, based on Hunt *et al.* (2007), is designed to test the hypothesis that there should be more genetic variability in dimensions of the selection surface with little negative curvature (weak stabilizing selection) or positive curvature (disruptive selection). As such, we expected a monotonic relationship between variability in **P** and curvature in corresponding multivariate dimensions of γ. We did not find

evidence consistent with our expectation (Tables S2.8–S2.11). However, we did uncover the somewhat paradoxical finding of an intermediate mode in the relationship between nonlinear selection and variability in **P** (Fig. 2). We cannot explain this pattern in theory; however, the reader should keep in mind that few multivariate dimensions of curvature in γ were significant in our analysis (Tables S1.1–S1.4).

Despite a shortage of significant evidence of nonlinear selection, we still detect significant alignment of the individual selection surface with **P** by some measures. Although not definitively established by this study, this finding suggests that individual nonlinear selection may have influenced the evolution of the genetic variances and covariances for characters in *A. cristatellus*, as predicted by theory. Future studies might consider estimating the **G** matrix using breeding experiments, and tracking lifetime fitness through juvenile and adult phases of both males and females to definitively establish what we preliminarily identify herein: that there is a significant role for selection in the evolution of genetic constraint in an anole.

Acknowledgments

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Supporting information

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

Appendix S1 Methods and results for canonical analyses of multivariate selection.

Table S1.1 Canonical analysis of γ from the quadratic regression selection analysis on external data is the ith column of **M**, the matrix of eigenvectors in the canonical analysis of the curvature of the selection surface, γ (Table S2.3).

Table S1.2 Canonical analysis of γ from the quadratic regression selection analysis on internal (X-ray) data.

Table S1.3 Canonical analysis of γ from the selection differential analysis on external data.

Table S1.4 Canonical analysis of γ from the selection differential analysis on internal data.

Table S2.1 Eigenvectors ($\mathbf{p}_i$) and eigenvalues (λ_i) of $\mathbf{P}_{ex}$, the phenotypic variance covariance matrix calculated from external measurements.

Table S2.2 Eigenvectors ($\mathbf{p}_i$) and eigenvalues (λ_i) of $\mathbf{P}_{in}$, the phenotypic variance covariance matrix calculated from internal X-ray measurements.

Table S2.3 Quadratic regression selection analysis; external data.

Table S2.4 Eigenvectors and eigenvalues of $-\gamma^{-1}$ and γ. Left-right rank order of the eigenvectors is based on $-\gamma^{-1}$.

Table S2.5 Eigenvectors and eigenvalues of $-\gamma^{-1}$ and γ. Rank order of the eigenvectors is based on $-\gamma^{-1}$. Internal data; least-squares regression selection analysis.

Table S2.6 Eigenvectors and eigenvalues of $-\gamma^{-1}$ and γ. Rank order of the eigenvectors is based on $-\gamma^{-1}$. External data; selection differential analysis.

Table S2.7 Eigenvectors and eigenvalues of $-\gamma^{-1}$ and γ. Rank order of the eigenvectors is based on $-\gamma^{-1}$. Internal data; selection differential analysis.

Table S2.8 Analysis using the method of Hunt *et al.* (2007); external data.

Table S2.9 Analysis using the method of Hunt *et al.* (2007); internal (X-ray) data.

Table S2.10 Variability of **P** evaluated over the eigenvectors of γ; external data.

Table S2.11 Variability of **P** evaluated over the eigenvectors of γ; internal (X-ray) data.

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