

Bite performance surfaces of three ecologically divergent Iguanidae lizards: relationships with lower jaw bones

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Received 21 January 2019; revised 18 April 2019; accepted for publication 19 April 2019

Traits that interact to perform an ecologically relevant function are expected to be under multivariate non-linear selection. Using the lower jaw morphology as a biomechanical model, we test the hypothesis that lower jaw bones of lizards are subjected to stabilizing and correlational selection, associated with mechanical advantage and maximum bite force. We used three closely related tropidurine species that differ in size, head shape and microhabitat: *Eurolophosaurus nanuzae*, *Tropidurus hispidus* and *Tropidurus semitaeniatus*. We predicted a common pattern of correlational selection on bones that are part of in-levers or part of the out-lever of the lower jaw. The predicted pattern was found in *E. nanuzae* and *T. hispidus*, but this could not be shown to be statistically significant. For *T. semitaeniatus*, we found significant disruptive selection on a contrast involving the surangular, and also significant directional selection on linear combinations of traits in all species. The results indicate that the non-linear selection on lower jaw bones does not reflect an optimum to enhance mechanical advantage in all species. Divergent functional demands and specific ecological contexts of species seem relevant in shaping patterns of selection on morphology.

ADDITIONAL KEYWORDS: comparing performance surfaces – diet – ecomorphology – morphological integration – multivariate stabilizing selection – performance gradients – Tropidurinae.

INTRODUCTION

According to the morphology–performance–fitness paradigm, variation in morphology predicts variation in functional performance, and variation in performance, in turn, predicts fitness differences among individuals (Arnold, 1983). This perspective has been used to advance our understanding of the coevolution of morphology and performance in several study systems (e.g. Huey & Bennet, 1987; Arnold & Bennet, 1988; Losos, 1990; Kohlsdorf *et al.*, 2004; Herrel *et al.*, 2005; Gomes *et al.*, 2009; Kohlsdorf & Navas, 2012; Zagar *et al.*, 2017). Using this ecomorphological theory, one can estimate performance gradients (the direct effect of each morphological trait on some performance measure) and selection gradients (the direct effect

of performance on fitness) using multiple linear regressions (Lande & Arnold, 1983; Arnold, 1983). This theory has been extended to incorporate the role of behaviour and ecological context (e.g. Arnold, 1988; Garland & Losos, 1994; Husak, 2006; Calsbeek *et al.*, 2007; McCormick *et al.*, 2018), as well as of performance/life-history trade-offs, when the same traits affect more than one functional performance or life-history feature (e.g. Ghalambor *et al.*, 2003; Toro *et al.*, 2004; Hughye *et al.*, 2005; Kohlsdorf *et al.*, 2008; Herrel *et al.*, 2009; Barros *et al.*, 2011; Lailvaux & Husak, 2014). However, an underappreciated extension of the ecomorphological theory goes beyond estimating just linear performance gradients to also estimate non-linear performance gradients and performance surfaces (Arnold, 2003). While the linear terms are related to the effects of directional selection on trait means, the non-linear terms are associated with the effects of stabilizing, disruptive and correlational

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selection on trait variances and covariances (Phillips & Arnold, 1989; Arnold, 2003).

The importance of estimating non-linear performance gradients is highlighted by studies of functional complexes comprising multiple traits that perform a particular function. When traits interact to perform a specific function, the theory of morphological integration predicts that average fitness associated with one trait will depend on the phenotypic values of the other traits that perform the same function (i.e. traits will have fitness interactions; Olson & Miller, 1958; Riedl, 1978; Cheverud, 1984). Such fitness interactions among traits define a correlated selection function in which higher fitness is achieved when trait values change together. Correlated selection surfaces imply that stabilizing and correlational selection mould trait variances and covariances, and may ultimately determine the pattern of trait correlations within a population (Lande, 1980; Cheverud, 1984; Schwenk & Wagner, 2001; Hansen & Houle, 2004). The well-studied beak traits in Darwin finches provide a good empirical system to exemplify the connection between morphological integration and the ecomorphological theory. Beak width, depth and length coevolve in predictable ways as diets diverge in the finch radiation (Lack, 1947; Grant & Grant, 2011). Specifically, in *Geospiza fortis* the effect of beak width on bite force depends on depth but not on beak length (Herrel *et al.*, 2005). In other words, variation in performance is related to covariation in morphology. In particular, the high integration between beak width and depth has apparently been promoted by multivariate selection induced by seed feeding, which has shaped the underlying genetic system as well as the more accessible phenotypic covariation (Abzhanov *et al.*, 2006; Mallarino *et al.*, 2011).

Despite extensive empirical support for the morphological integration and the ecomorphology framework, predictions from the two perspectives are rarely combined (but see Brodie, 1992; Brodie *et al.*, 1995; Calsbeek & Irshick, 2007). We argue that such a combination allows researchers to make specific predictions about how the pattern of multivariate non-linear selection connects with functional ecology. Studies of multivariate selection in general tend to ignore correlational selection. It has been notoriously difficult to gather compelling empirical evidence for this particular kind of stabilizing selection that is thought to produce morphological integration (Blows, 2007; Kingsolver *et al.* 2012; Haller & Hendry, 2014). This difficulty may be partially related to a weaker direct association between non-linear variation in morphology and fitness, compared to a stronger relationship between morphology and functional performance. This may be true especially

if performance mediates the effects of morphology on fitness (Arnold, 1983). Therefore, our approach has the potential to reveal multivariate stabilizing selection on traits that could otherwise be difficult to demonstrate using morphology and fitness.

In this study, we use a simple system to test the hypothesis of multivariate stabilizing selection on functional traits related to morphological integration. We conduct our test by measuring traits of the lower jaw and assessing bite performance in the same sample of lizards. Bite force in lizards plays major roles in food acquisition/manipulation and fighting in territorial species (Herrel *et al.*, 2001a; Lailvaux *et al.*, 2004; Huyghe *et al.*, 2005; Husak *et al.*, 2006; Lailvaux & Irshick, 2007). Bite force also affects reproductive output in species such as the collared lizards (Lappin & Husak, 2005), indicating that differences in bite force scale up to differences in fitness. Although the statistical association of head dimensions and bite force is well established in lizards (e.g. Herrel *et al.*, 1999, 2001b; Huyghe *et al.*, 2005; Stayton, 2006; Anderson *et al.*, 2008), those associations have rarely been approached from the perspective of morphological integration (but see Fabre *et al.*, 2014). Studying individual lower jaw bones rather than head dimensions has the advantage of offering a direct link between mechanical design and performance capacity (Wainwright, 2007). This linkage is possible because the lower jaw can be interpreted as a lever system that transmits input force from muscles to the out-lever arm to produce an output force (McGowan, 1999; Stayton, 2006). The ratio between input and output force is known as the mechanical advantage (MA) of a lever. The higher MA, the higher bite force. Consequently, we predict that the lower jaw bones of lizards are under stabilizing and correlational selection that define the optimum associated with MA, and therefore also with bite force. We expect to find that non-linear as well as linear variation in lower jaw lengths explain variation in bite force. We test this prediction in three species of tropidurine lizards that are closely related but ecologically divergent. These species, *Eurolophosaurus nanuzae*, *Tropidurus hispidus* and *Tropidurus semitaeniatus*, vary in overall body size, head shape and habitat usage (Fig. 1A), features that are potentially relevant in producing differences in bite performance. Nevertheless, given that we expect the functioning of the lower jaw to be similar across species, we predict the same pattern of multivariate non-linear selection for the three species, specifically, correlational selection on bones that are part of in-levers or part of the out-lever of the lower jaw. Therefore, we expect a similar shape of species performance surfaces that relate linear and non-linear variation in morphology with variation in bite force.

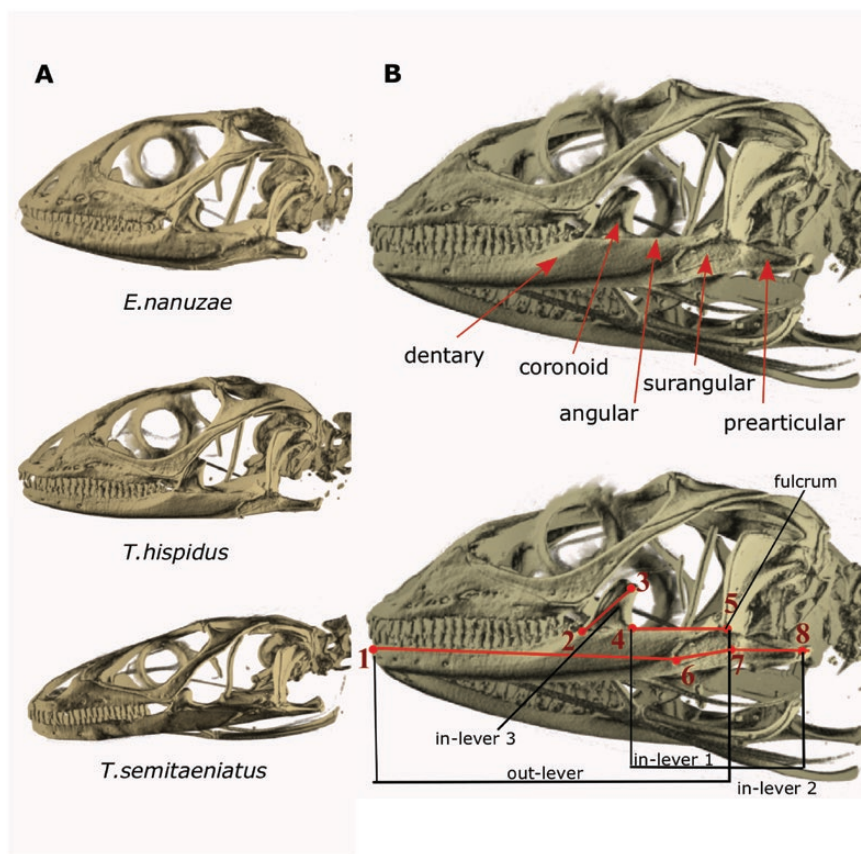


Figure 1. Representative skulls of the three Tropicurinae species (A) and linear distances in the lizard lower jaw (B). A, the skulls are not in scale, *E. nanuzae* being smaller than *T. semitaeniatus*, and much smaller than *T. hispidus* (see Table 1). Notice the flatter skull of *T. semitaeniatus*. B, the out-lever arm is composed of dentary and surangular lengths. In-lever 1 is the angular length and the insertion site of adductor muscles. In-lever 2 is the prearticular length and insertion site of the m. depressor. Finally, in-lever 3 is the height of the coronoid bone and the insertion site for adductor and m. pseudotemporalis muscles (attached to the tendinous bodenaponeurosis).

MATERIAL AND METHODS

LIZARD SPECIES AND ECOLOGICAL DIVERGENCE

Active individuals of *Eurolophosaurus nanuzae* ($N = 29$), *Tropidurus hispidus* ($N = 31$) and *Tropidurus semitaeniatus* ($N = 30$) were noose-captured at three Brazilian localities, Serra do Cipó (MG), Vale do Jequitinhonha (MG) and Morro do Chapéu (BA), respectively. *E. nanuzae* (Rodrigues, 1981) is an open-habitat species, endemic to rocky meadows in the Cerrado Brazilian biome, and is characterized by a smaller body size compared to the other tropidurines (Rodrigues, 1987; Galdino *et al.*, 2006). *T. hispidus* (Spix, 1825) is a generalist species that uses different types of microhabitats in the Cerrado and the Caatinga biomes (e.g. leaf litter, logs; Pelegrin *et al.*, 2017), a semi-arid environment in north-east Brazil. *T. hispidus* is the largest species of the genus (Rodrigues, 1987), is very territorial and uses aggressive behaviour in male–male combats (Diaz-Urriarte, 1999). *T. semitaeniatus* (Spix,

1825), a rock-dweller that lives in rock crevices mostly in the Caatinga biome, is characterized by noticeable dorsoventral flattening of the body (Rodrigues, 1981; Fig. 1A). Despite their differing morphologies, the three species appear to have similar diets, eating mostly ants, termites and plant matter (Kiefer, 1998; Kolodiuk *et al.*, 2010; Ribeiro & Freire, 2011; Pelegrin *et al.*, 2017). However, dietary differences between species are most likely to shape differences in phenotypic means rather than in trait covariance/correlation patterns.

MEASUREMENT OF MAXIMUM BITE FORCE

Bite force was measured at a field laboratory at each site using a portable force transducer device (Kistler Inc., Wintherthur, Switzerland). Lizards were transported to a field lab and placed in plastic boxes supplied with heat lamps so that they reached field-active body temperatures (around 37 °C), measured

with a cloacal thermometer before the biting trials. Field-active body temperatures reflect ecologically relevant temperatures at which lizards normally bite, and temperatures used during the trials were congruent with preferred temperatures for tropidurine lizards (Kohlsdorf & Navas, 2006). Individuals were stimulated to bite the device, positioned in front of the jaws, by gentle taps on the snout. Five repetitions of bite force were recorded, each separated by an interval of 30–40 s (e.g. Irschick & Meyers, 2007). For analysis, we used the maximum bite force of each individual. After quantifying bite force, all individuals were killed via an intraperitoneal overdose of tyopental solution, fixed with 10% formalin, preserved in 70% ethanol and deposited at the Herpetological Collection of Ribeirão Preto (CHRP-USP) for future use in other projects. All procedures were in accordance with Brazilian laws and were approved both by CEUA (Ethics Committee for Animal Research from University of São Paulo, protocol number 12.1.156.53.5) and by IBAMA (permit number: 35624-1). We did not expect any major impact on the natural populations of removing around 30 individuals because these species are very abundant in their natural habitats, and our sampling corresponded on average to less than 10% of the total number of adult individuals estimated for the population. All individuals were sexed and snout–vent length (SVL) was measured before preservation.

MICRO-CT SCANNING AND LINEAR DISTANCES ON LOWER JAW BONES

We assessed the skulls of all lizards for which we recorded maximum bite force using an X-ray micro-computed tomography system (SkyScan 1176, Konitch, Belgium) available at the Biosciences Institute at the University of São Paulo, Brazil. The scans were obtained at 18- μ m resolution (70 kV), using aluminium filters of 0.5 mm or 0.2 mm. Cross-sections of the skulls were reconstructed using the NRecon software (SkyScan) with parameter values as follows: attenuation coefficient thresholds of 0.0 and 0.1, ring artefact reduction of 2.0–3.0, beam-hardening correction of 30% and smoothing of 2.0. We used the TINA Manual Landmarking Tool software (Schunke *et al.*, 2012) to place eight 3D landmarks on bone sutures or bone tips of the five lower jaw bones (Supporting Information, Table S1). From these landmarks, we extracted five linear distances, each corresponding to a single lower jaw bone (Fig. 1B). We placed the 3D landmarks twice on each specimen to calculate repeatability, which quantifies the proportion of variation in linear distances due to biological differences among individuals and not due to measurement error (measured within individuals). Repeatability was calculated following Lessells &

Boag (1987): $r = s_A^2 / (s^2 + s_A^2)$; where s_A^2 is the among-individual variance (each individual represented by two measurements) and s^2 is the within-individual variance.

Given that different muscles participate in the production of bite force (Groning *et al.*, 2013), we characterized three in-levers of the lizard jaw (in-levers 1, 2 and 3) using the lengths of the angular, coronoid and prearticular bones and their corresponding muscular insertions (Fig. 1B). In-lever 1 is the angular length and the insertion site of adductor muscles, strongly related to the force produced when closing the lizard mouth. In-lever 2 is the height of the coronoid bone and the insertion site for adductor and m. pseudotemporalis muscles (attached to the tendinous bodenaponeurosis), also associated with closing the mouth. Finally, in-lever 3 is the length of the prearticular bone and insertion site of the m. depressor, which is involved in opening the mouth (Groning *et al.*, 2013). The lengths of the dentary and surangular bones were used to represent the closing out-lever arm. Increased MA that enhances bite force can be achieved by increasing the length of bones related to closing in-levers or reducing the out-lever length.

NON-LINEAR PERFORMANCE GRADIENTS

We estimated non-linear performance gradients using ordinary least-square multiple regression, following Lande & Arnold (1983). We first tested for effects of sex, SVL and their interaction on maximum bite force and on jaw linear distances using univariate and multivariate analysis of variance (type III sum of squares MANOVA), respectively (Supporting Information, Table S2). Although maximum bite force may differ between sexes in lizards (e.g. Lailvaux *et al.*, 2004; Herrel *et al.*, 2006; Fabre *et al.*, 2014), we did not expect differences between males and females in the pattern of multivariate non-linear selection on the jaw bones because the lower jaw has the same biomechanical design in both sexes. Hence, we removed sexual differences in the means of maximum bite force and in the linear distances. Because absolute performance measures should be converted to relative performance (mean of 1.0) to interpret performance gradients (Lande & Arnold, 1983), we divided each individual value of maximum bite force of males and females by its within-sex mean, for each species. Consequently, we simultaneously removed sex differences in maximum bite force and obtained relative performance measures (with mean = 1.0) within each species (Table S3). For *E. nanuzae* and *T. semitaeniatus*, this procedure also removed SVL effects on maximum bite force, but for *T. hispidus*, SVL still had a significant effect (because of differences within males). We mean-centred the within-sex linear

distances, that is, we calculated individual differences from males to the mean of males, and the differences of females from the mean of females. As a consequence, we removed the effect of SVL on the linear distances in the three species, which was mainly due to differences between sexes (Table S3).

We used the following multiple regression to estimate the non-linear performance gradients:

$$f = \alpha + \sum_{i=1}^n \beta_i z_i + \sum_{i=1}^n \frac{1}{2} \gamma_{ii} z_i^2 + \sum_{i \neq j} \gamma_{ij} z_i z_j + \varepsilon, \quad (1)$$

where f is relative performance, α is the intercept, β_i is the directional performance gradient for trait z_i , γ_{ii} is the quadratic performance gradient for trait z_i , γ_{ij} is the quadratic performance gradient for traits z_i and z_j , ε is the error term, and N is sample size within species (Lande & Arnold, 1983; Phillips & Arnold, 1989). We included the linear β_i terms in the regression to account for potential effects of directional selection on the shape of the performance surface (especially when the traits have a skewed distribution). A multiple regression with just the linear terms was used to estimate the β_i coefficients, which we interpret as the slope of the performance surface. The γ_{ii} and γ_{ij} terms indicate the curvature (convex or concave) and tilt (positive or negative) of the performance surface, interpreted as the strength of stabilizing/disruptive and correlational selection, respectively. After computing the non-linear performance gradients, we constructed species performance γ -matrices in which the γ_{ii} coefficients for each lower jaw bone comprise the diagonal elements and the γ_{ij} coefficients are off-diagonal elements. The performance γ -matrix reflects the total non-linear multivariate selection on the traits (stabilizing/disruptive on diagonal and correlational selection off-diagonal). We also performed the non-linear regression using variance standardized traits, to check whether differences in variance among traits would affect the γ -matrices (see Supporting Information, Tables S5–7). All calculations and analyses were performed using the R programming environment (R Core Team, 2018).

CANONICAL ANALYSIS OF THE PERFORMANCE γ -MATRIX

To simplify interpretation of the pattern of multivariate selection, the γ -matrix can be diagonalized into a matrix of eigenvectors and corresponding eigenvalues (Phillips & Arnold, 1989). The eigenvectors of γ reflect the linear combination of the original traits that are under non-linear selection. The magnitude and sign of the associated eigenvalue indicate the strength of non-linear selection and whether the surface is convex (negative eigenvalue) or concave (positive eigenvalue) in the direction corresponding to a

particular eigenvector. We predicted that the pattern of non-linear effects of lower jaw bones on maximum bite force would be different for in-levers and the out-lever. In particular, we expected that at least one eigenvector of the γ -matrix would correspond to a contrast between in-lever and out-lever, indicating correlational selection within in-lever or within out-lever, but not between them. We also expected an associated negative eigenvalue, reflecting stabilizing selection on a morphological dimension that is relevant for MA.

Eigenvector–eigenvalue analysis is equivalent to rotating the original trait axes to a new set with a new matrix of multivariate curvature (Λ) that has the eigenvalues of γ on its main diagonal (λ_i) and zeros elsewhere. This rotation can be written as:

$$\Lambda = M^T \gamma M, \quad (2)$$

where M is an orthogonal matrix whose columns are the eigenvectors of γ normalized to unit length (Phillips & Arnold, 1989). The expected contrast should emerge as opposing signs for in-lever bones and out-lever bones in the same eigenvector, indicating that the pattern of correlational selection favours correlations within the in-lever or the out-lever, but not between the different sets of bones. We tested the statistical significance of the eigenvalues associated with each eigenvector using a permutation test adapted from Reynolds *et al.* (2009), following the procedure described by Lewis *et al.* (2011) and Chenoweth *et al.* (2012). We provide further details and a R script in the Supporting Information.

BITE PERFORMANCE SURFACES

We plotted performance surfaces for each species in the new rotated trait space. In particular, we estimated the best quadratic approximation of the surfaces, using only the eigenvectors of the performance γ -matrices associated with statistically significant non-linear or linear selection. We used the R function ‘outer’ to obtain a grid of relative maximum bite force values calculated using the linear and non-linear coefficients of the significant eigenvectors (θ_i and λ_i , respectively). The θ_i coefficients were computed using only the linear terms and the individual scores ($y_i = m_i^t \mathbf{z}$) on the m_i eigenvectors in the multiple regression, and using those scores to run the multiple regression again in canonical form:

$$f = \alpha + \theta^t \mathbf{y} \quad (3)$$

where $\theta = M^t \beta$ (Box & Draper, 1987; Bisgaard & Ankenman, 1996) and β is the column vector of directional performance gradients. We tested the significance of linear selection using the individual scores on the eigenvectors of the γ -matrices using

permutation tests in all species (Table S4). We then used the R function ‘persp’ to plot performance surfaces and ‘contour’ to plot contour lines. We also plotted the performance surfaces using another technique (thin-plate splines), which is more flexible, to accommodate complex surfaces (see Supporting Information).

RESULTS

MAXIMUM BITE FORCE AND LOWER JAW LINEAR DISTANCES

The three tropidurine species differ in mean maximum bite force (Kruskal–Wallis test, $\chi^2 = 12.606$, d.f. = 2, $P = 0.002$; Fig. 2A). Maximum bite force in *T. hispidus* is more variable [coefficient of variation (CV) = 0.67] and has a higher mean than in *E. nanuzae*

(CV = 0.19) and *T. semitaeniatus* (CV = 0.28; Table 1). The distribution of maximum bite force in *T. hispidus* is greatly right-skewed (Fig. 2A), mostly because of the variation within males, although variation in females is also high (CV_{females} = 0.36, CV_{males} = 0.48). The male *T. hispidus* population can be roughly divided into two groups: smaller males having lower bite force (SVL = 101.23 ± 5.9 mm, maximum bite force = 46.6 ± 13.05 N) and larger males that have higher bite force (SVL = 113.34 ± 1.97 mm, maximum bite force = 103.68 ± 21.77 N). Lower jaw linear distances are highly repeatable in all three species, ranging from 0.82 to 0.99, except for the angular bone in *E. nanuzae*, which has a repeatability value of 0.72. However, this lower repeatability is expected for very small distances because the error in placing the landmarks is proportionally higher for smaller

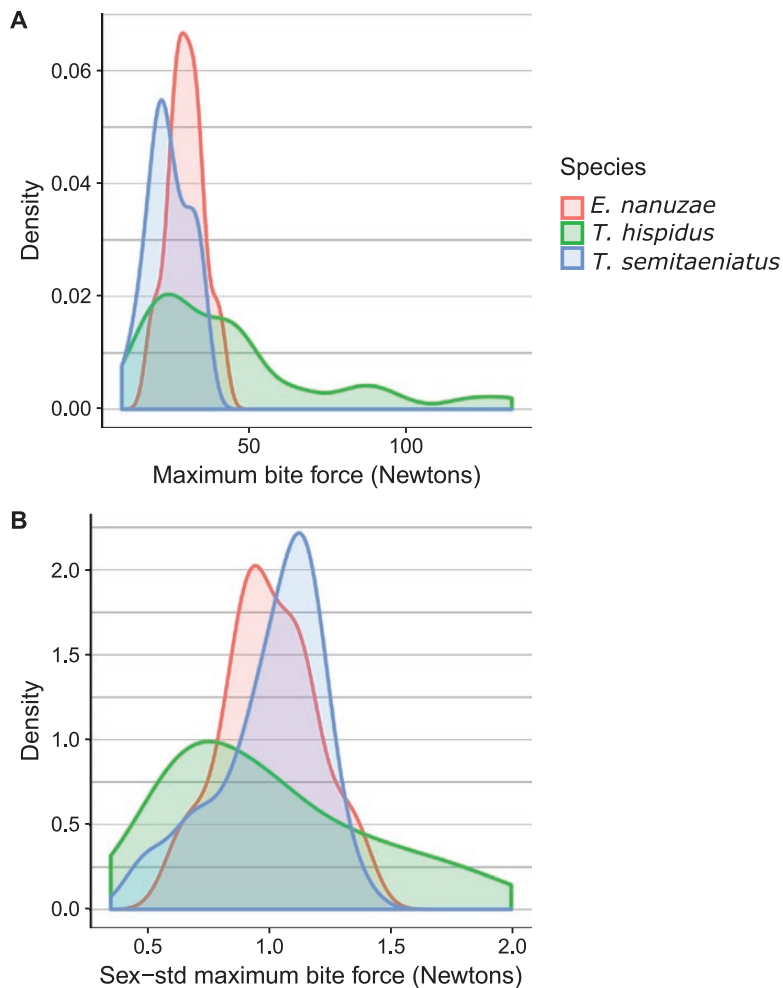


Figure 2. Species differences in maximum bite force for raw (A) and sex-standardized data (B). A, species differ in their average maximum bite force, *T. hispidus* exhibiting the highest values. Maximum bite force was also more variable in *T. hispidus*, being characterized by a marked right-skewed distribution. B, relative maximum bite force was obtained by dividing individual values by the within-sex average value. All species have a mean = 1.0, but *T. hispidus* still presents larger variance in relative maximum bite force.

Table 1. Means \pm SD of original lower jaw traits and maximum bite force and of mean-centred traits and relative maximum bite force

	<i>E. nanuzae</i>	<i>T. hispidus</i>	<i>T. semitaeniatus</i>
Raw variables			
Maximum bite force (N)	29.91 + 5.67	45.02 + 30.32	24.56 + 6.81
SVL (mm)	53.74 + 2.65	94.60 + 12.11	69.48 + 6.03
Coronoid (mm)	1.72 + 0.13	3.47 + 0.68	1.98 + 0.28
Angular (mm)	1.18 + 0.15	2.34 + 0.51	2.00 + 0.33
Dentary (mm)	10.05 + 0.66	17.15 + 2.48	12.76 + 1.14
Surangular (mm)	1.97 + 0.20	2.97 + 0.62	2.19 + 0.30
Prearticular (mm)	1.65 + 0.12	3.06 + 0.59	2.33 + 0.36
Sex-standardized variables			
Relative maximum bite force	1.0 + 0.18	1.0 + 0.41	1.0 + 0.21
Coronoid (mm)	0.0 + 0.13	0.0 + 0.39	0.0 + 0.17
Angular (mm)	0.0 + 0.15	0.0 + 0.35	0.0 + 0.23
Dentary (mm)	0.0 + 0.65	0.0 + 1.27	0.0 + 0.43
Surangular (mm)	0.0 + 0.19	0.0 + 0.31	0.0 + 0.20
Prearticular (mm)	0.0 + 0.12	0.0 + 0.35	0.0 + 0.29

Relative maximum bite force corresponds to the maximum bite force in males and females divided by each within-sex mean. The mean-centred lower jaw distances correspond to the difference between individual male and female values and the corresponding within-sex mean. SVL, snout-vent length.

distances. In sex-centred distances, *T. hispidus* has more variable lower jaw traits than *E. nanuzae* and *T. semitaeniatus*, but the dentary bone has the highest variation in all three species (Table 1).

SPECIES BITE PERFORMANCE Γ -MATRICES

Comparisons in the original trait space

Although the γ -matrices vary among species, the pattern of non-linear performance gradients for the coronoid and prearticular bones is similar in the three species (Table 2). Although these patterns suggest non-linear selection, caution need to be exercised because most non-linear gradients are not significant (significant only in some *T. hispidus*; see Table 2).

Comparisons in the rotated trait space

We extracted the eigenvectors of these matrices to investigate the pattern of correlational selection, expressed as the signs of the loadings on each eigenvector. For both *E. nanuzae* and *T. hispidus*, we found evidence for correlational selection favouring the coordinated action of bones within the in-lever and within the out-lever, as predicted. In *E. nanuzae*, we found a contrast between [dentary + surangular] and the remaining bones, whereas in *T. hispidus*, we found a contrast between dentary and [coronoid + prearticular], expressed in the m_5 eigenvector in both species (Table 3). This in-lever/out-lever contrast seems to be under stabilizing selection, as indicated by the negative eigenvalue associated with this multivariate

direction (Table 3). In contrast, the lower jaw bones in *T. semitaeniatus* appeared to experience two different selective regimes, neither of which reflects correlational and stabilizing selection on an in-lever/out-lever contrast. A contrast between the angular and all other bones seems to be under stabilizing selection (m_3), whereas disruptive selection appears on a dimension that contrasts the surangular and all other bones (m_2 , Table 3). The strengths of non-linear selection associated with the highlighted contrasts among bones (i.e. the eigenvalues associated with the eigenvectors) are significant only at $\alpha = 0.1$ (Fig. 3).

LINEAR PERFORMANCE GRADIENTS

Although only the β value for the dentary in *T. hispidus* is statistically significant in the original trait space (Table 4), we found significant linear selection on a contrast between [coronoid + dentary] and the other bones (eigenvector m_2) and on a contrast of the surangular and all other bones (m_4) for *E. nanuzae*; by contrast in *T. semitaeniatus*, linear selection is on a contrast between the angular and all other bones (Table 4). For *T. hispidus*, allometric size is the major multivariate dimension under directional selection, with the dentary having the highest contribution to this multivariate direction. The linear models using only the significant eigenvectors explain a low amount of variation in relative maximum bite force in both *E. nanuzae* (multiple $R^2 = 0.26$) and *T. semitaeniatus* ($R^2 = 0.14$), but a high amount of variation in *T. hispidus* ($R^2 = 0.58$). Adding the quadratic coefficients to the

Table 2. Performance γ -matrices based on maximum bite force in the three Tropidurinae species

	Coronoid	Angular	Dentary	Surangular	Prearticular
<i>E. nanuzae</i>					
Coronoid	-3.600				
Angular	-0.344	3.760			
Dentary	0.252	-1.422	0.724		
Surangular	2.886	2.561	-1.227	-1.386	
Prearticular	-8.378	-5.556	2.229	1.630	-5.368
<i>T. hispidus</i>					
Coronoid	4.609				
Angular	-0.537	-2.217			
Dentary	0.331	0.944*	-0.522*		
Surangular	-1.923	-1.952	-0.057	2.544	
Prearticular	-1.883	0.119	1.126	-0.230	-5.448**
<i>T. semitaeniatus</i>					
Coronoid	-5.275				
Angular	2.220	-4.421			
Dentary	1.130	0.716	0.362		
Surangular	-0.988	3.246	-1.239	0.106	
Prearticular	-0.186	2.650	-0.312	-0.070	-0.183

The γ coefficients correspond to non-linear direct effects of each jaw distance on relative maximum bite force. * $P < 0.05$, ** $P < 0.01$.

Table 3. Eigenvectors of performance γ -matrices and their associated strengths of non-linear (λ) selection associated with maximum bite force

	Eigenvectors of γ				
	m_1	m_2	m_3	m_4	m_5
<i>E. nanuzae</i>					
Coronoid	0.361	0.701	0.078	-0.151	-0.591
Angular	0.699	-0.499	-0.436	0.102	-0.247
Dentary	-0.279	0.292	-0.888	-0.193	0.107
Surangular	0.228	-0.108	0.125	-0.923	0.263
Prearticular	-0.500	-0.404	-0.001	-0.280	-0.713
Eigenvalue (λ)	8.957	1.133	0.179	-0.958	-15.180
<i>T. hispidus</i>					
Coronoid	0.851	-0.463	0.113	-0.103	-0.194
Angular	0.066	0.403	0.179	-0.890	-0.095
Dentary	0.035	0.149	0.935	0.235	0.219
Surangular	-0.504	-0.759	0.242	-0.323	-0.088
Prearticular	-0.126	0.159	0.152	0.195	-0.947
Eigenvalue (λ)	5.997	2.468	-0.162	-3.263	-6.104
<i>T. semitaeniatus</i>					
Coronoid	-0.016	-0.261	-0.069	0.778	0.567
Angular	0.453	-0.332	-0.109	0.407	-0.712
Dentary	-0.308	-0.764	-0.457	-0.332	0.040
Surangular	0.714	0.157	-0.550	-0.226	0.336
Prearticular	0.436	-0.462	0.687	-0.259	0.240
Eigenvalue (λ)	2.680	1.124	-0.319	-4.248	-8.648

m_1 to m_5 correspond to eigenvectors of the γ -matrices, and the signs of their loadings indicate the pattern of correlational selection. Negative λ values indicate stabilizing selection, whereas positive λ values indicate disruptive selection on the combination of traits.

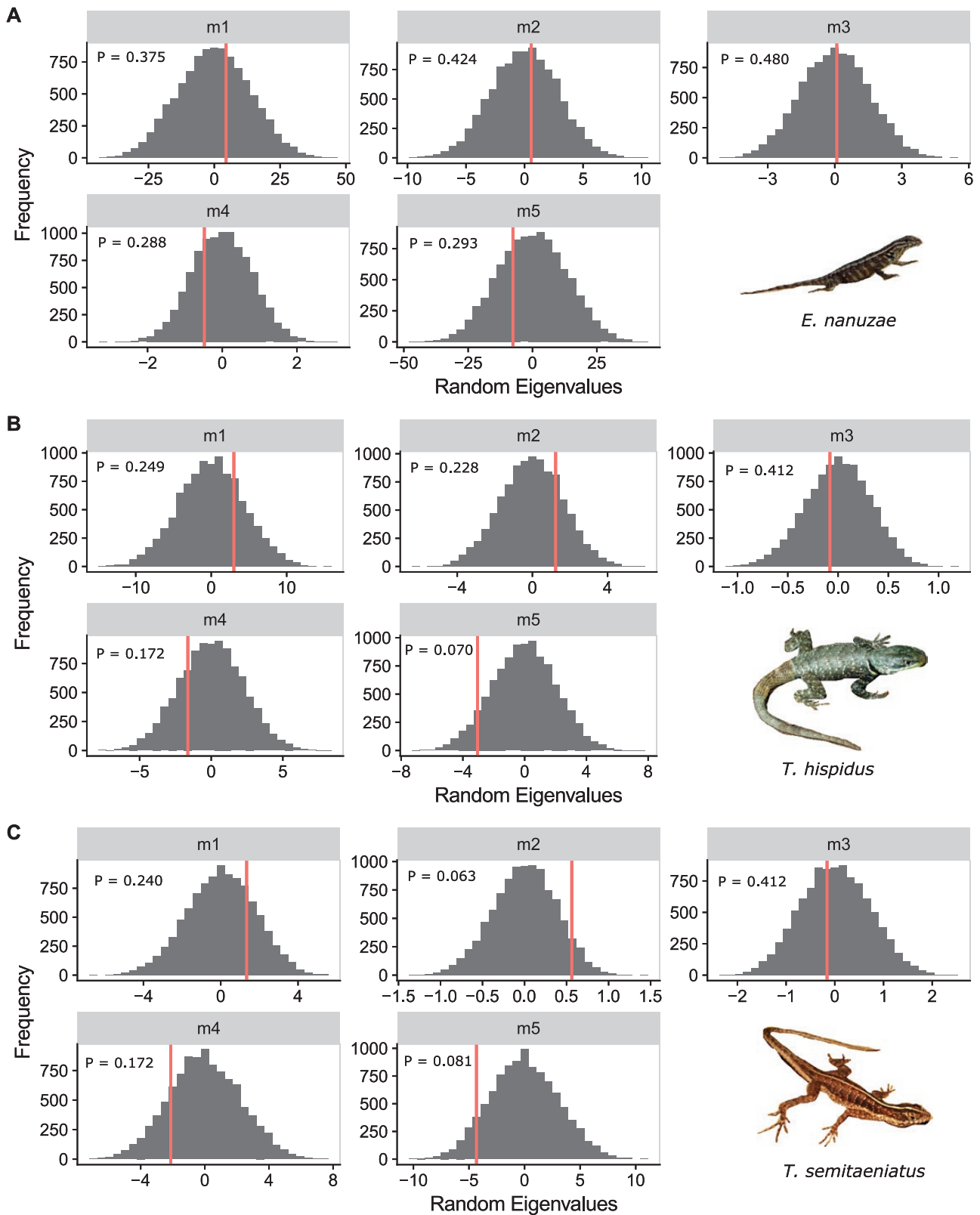


Figure 3. Permutation tests for eigenvalue significance of each eigenvector of the species γ -matrices. We reshuffled relative maximum bite force across individuals 1000 times, and re-ran the double regression, but keeping the individual scores on each eigenvector constant. We then constructed a null distribution for the eigenvalues and calculated how many times we could obtain values that were higher than the empirical one (red lines).

Table 4. Linear performance gradients on individual jaw traits (β) and on eigenvectors (θ) of species γ -matrices

	<i>E. nanuzae</i>		<i>T. hispidus</i>		<i>T. semitaeniatus</i>	
Traits	β	Std Err	β	Std Err	β	Std Err
Coronoid	0.297	0.349	0.153	0.234	0.346	0.244
Angular	-0.357	0.299	-0.076	0.203	-0.055	0.204
Dentary	0.065	0.093	0.201*	0.091	0.178	0.109
Surangular	-0.338	0.211	-0.040	0.196	-0.094	0.229
Preatticular	-0.503	0.361	0.123	0.224	-0.052	0.156
Eigenvectors	θ	Std Err	θ	Std Err	θ	Std Err
m_1	0.014	0.338	0.137	0.241	-0.175	0.209
m_2	0.645*	0.298	-0.021	0.175	0.199*	0.096
m_3	0.080	0.126	0.200**	0.060	-0.083	0.132
m_4	0.359*	0.168	0.136	0.215	0.223	0.254
m_5	0.189	0.386	-0.091	0.233	0.198	0.235

The β coefficients and associated standard errors (SE) derive from linear regressions of relative maximum bite force on mean-centred lower jaw traits. The θ coefficients and associated standard errors derive from the linear regression using individual scores on eigenvectors m_1 to m_5 of each species γ -matrix. *Permutation $P < 0.05$, **permutation $P < 0.01$.

model in *T. hispidus* does not improve the amount of variance explained in relative maximum bite force ($R^2 = 0.59$), but practically doubles the variance explained in *T. semitaeniatus* ($R^2 = 0.26$). The results are similar when using variance-standardized data, except for less directional selection in *T. semitaeniatus* (see [Supporting Information, Tables S5–S7](#)). In summary, although the three species have combinations of traits under directional selection, these multivariate directions vary among species.

SHAPE OF THE BITE PERFORMANCE SURFACES

Contrary to our prediction, the three species have different shapes of their bite performance surfaces when analyses were performed using the most relevant morphological dimensions (i.e. eigenvectors that have significant linear selection and marginally significant non-linear selection, [Fig. 4](#)). For *E. nanuzae*, the surface has no curvature because jaw bone morphology has only linear effects on maximum bite force ([Fig. 4A](#)). In particular, a linear effect in direction m_2 increases maximum bite force, and this effect arises from increasing the lengths of the coronoid and dentary. For *T. hispidus*, the performance surface has a convex curvature in one of the multivariate dimensions, suggesting stabilizing selection on the predicted in-lever/out-lever contrast (m_5). However, the strongest effect on maximum bite force is associated with linear selection on allometric size (m_3 , [Fig. 4B](#)). In *T. semitaeniatus*, the surface is a saddle because one dimension has a convex curvature (contrast of angular and all other bones), while the other dimension has a concave curvature, suggesting disruptive selection ([Fig. 4C](#)). Higher maximum bite force is achieved by the two extreme bone morphologies along m_2 , a

longer surangular and the other the bones shorter, or a shorter surangular and the other bones longer. The strongest difference when looking at the surfaces using thin-plate splines is the much lower curvature found for m_5 in both *T. hispidus* and *T. semitaeniatus*, but a signal of disruptive selection in *T. semitaeniatus* is still seen ([Supporting Information, Fig. S1](#)).

DISCUSSION

RELATIONSHIP BETWEEN LOWER JAW BONES AND BITE FORCE IS COMPLEX

The study of phenotypic traits associated with measures of fitness and performance has an important bearing on the evolution of complex morphologies. Although we did not measure fitness in the lizard species, we argue that a good biomechanical/functional understanding of trait complexes can provide specific predictions about how non-linear selection mediates functional performance. Thus, in this study, we focused on the performance aspect of the total selection that acts on traits. Of course, differences in functional performance do not necessarily translate into a demographic contribution to the next generation, as differences in fitness do ([Franklin & Morrissey, 2017](#)). Nevertheless, non-linear variation in morphology may have stronger effects on functional performance than on relative fitness. In the case of traits that form a functional complex, a focus on non-linear effects on performance may be more illuminating than a focus on total fitness effects ([Arnold, 1983, 2003](#)). For example, by interpreting the five bones of the lower jaw of lizards as a simple lever system, we predicted that tropidurine species would exhibit a favoured intermediate phenotype reflecting the different functional roles of

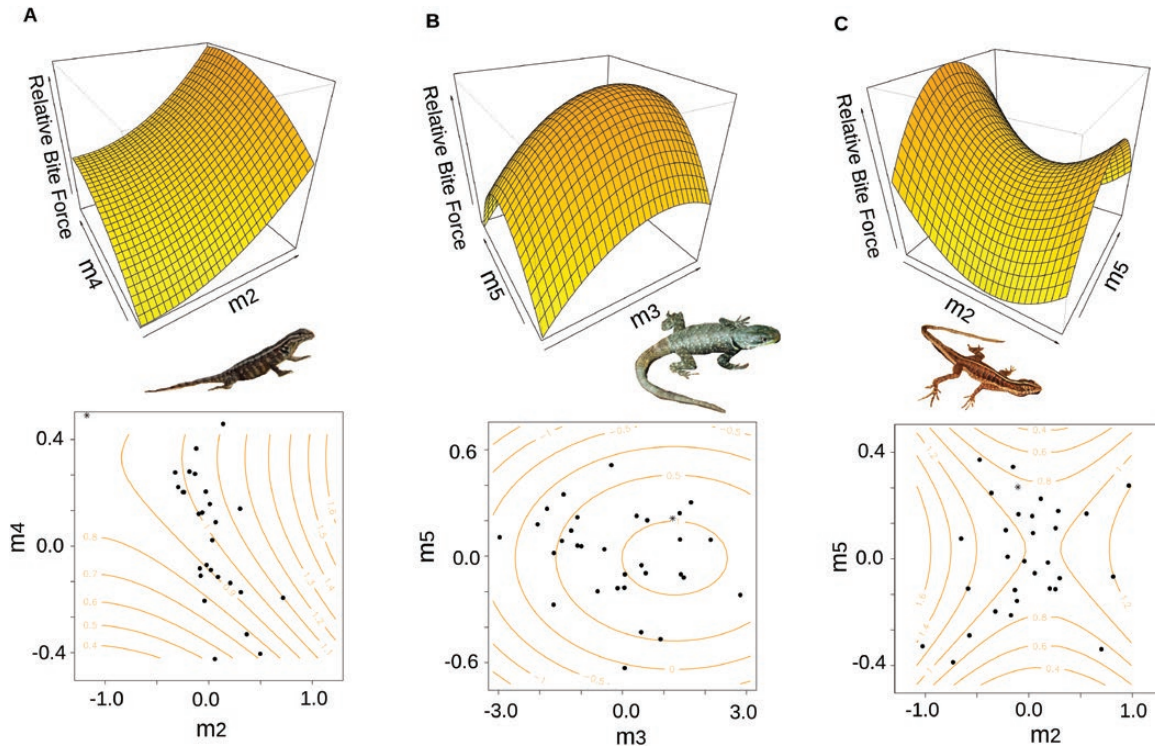


Figure 4. Performance surfaces and contour plots for the significant eigenvectors of the γ -matrices in the three lizard species. The surfaces correspond to the best quadratic approximation of the relationship between individual scores on the eigenvectors of γ and relative maximum bite force. Asterisks indicate the stationary point. Whereas the surface for *E. nanuzae* (A) suggests directional selection on contrasts between traits, the surface for *T. hispidus* (B) suggests an intermediate optimum, but also directional selection on allometric size (m_3), while in *T. semitaeniatus* (C) the surface is a saddle with disruptive and directional selection on m_2 , a contrast involving the surangular.

in-levers and the out-lever on maximum bite force. Our results partially confirmed that expectation, although not all aspects of the pattern of correlational selection in bite performance reflect expectations for enhancing mechanical advantage.

The lower jaw may be involved in other functions that lead to a different pattern of selection than expected for enhancing mechanical advantage. Functional trade-offs may reflect biomechanical constraints, when the same traits are important for more than one function, and/or conflicting selection pressures (Aerts *et al.*, 2000; Vanhooydonck & Van Damme, 2001; Kohlsdorf *et al.*, 2008; Barros *et al.*, 2011). Alternatively, other non-measured traits may be relevant for bite force, such as muscle architecture (e.g. fibre type, pennation angle) and muscle orientation (i.e. how much a muscle wraps around the bones; Groning *et al.*, 2013; Wittorski *et al.* 2016).

Analysing selection in a rotated trait space enabled us to test and verify our predictions based on mechanical advantage, but we were unable to show that those new dimensions experienced stabilizing selection. This type of failure to demonstrate significant non-linear selection is a common issue in empirical studies

of multiple traits, even when sample sizes are much higher than in our study. The problem of detecting non-linear selection on multiple traits has been extensively discussed (Kingsolver *et al.*, 2001, 2012; Blows & Brooks, 2003; Blows, 2007; Haller & Hendry, 2014), especially because evolutionary thinking relies heavily on models of adaptation that postulate multivariate stabilizing selection (Lande, 1976, 1986; Hansen, 1997; Butler & King, 2004). Blows & Brooks (2003) have proposed that stabilizing selection may be stronger on combinations of traits than on single traits and argued that canonical analysis provides a way to identify those strongly selected combinations. In other words, the eigenvectors of the gamma matrix may be the key to finding trait combinations that are the targets of non-linear selection (Phillips & Arnold, 1989; Blows & Brooks, 2003; Blows, 2007; Chenoweth *et al.*, 2012).

For instance, we found evidence of non-linear selection on a combination of traits in *T. semitaeniatus* that represents a contrast between the length of the surangular and the lengths of the other lower jaw bones, but the evidence is for disruptive selection instead of stabilizing. The saddle-shaped bite

performance surface of *T. semitaeniatus* suggests that more than one type of interaction between bones can provide proper biting within the same population, associated with the length of the out-lever in relation to the in-lever. Disruptive selection on phenotypic traits has been also identified by other studies of how limb length affects sprint speed in lizards (Calsbeek & Irschick, 2007), how vertebral numbers affect crawling speed in snakes (Arnold & Bennet, 1988), and how anti-predator behaviour and colour pattern affects survival in snakes (Brodie, 1992). Although we do not expect saddle-shaped fitness surfaces to be evolutionary stable, the existence of morphological dimensions under disruptive selection suggests recurrent production of intermediate phenotypes with reduced fitness. This kind of persistent disruptive selection may reflect competition among individuals such that intermediate phenotypes experience stronger competition (Hendry, 2017). Therefore, our study contributes to the perception that disruptive selection may be as common as stabilizing selection on multiple traits, if not more common when associated with functional performance.

DIRECTIONAL SELECTION AS THE MAIN EVOLUTIONARY FORCE ON LOWER JAW MORPHOLOGY

Although some multivariate dimensions appear to be under non-linear selection in the lizards studied, we found much stronger evidence for directional selection acting on some of the combinations of traits. When traits make a strong contribution to performance/fitness (i.e. the amount of variance in performance explained by the traits is high), power to detect selection is increased (Hersch & Phillips, 2004). The possibility of higher power to detect linear effects is highlighted by results in *T. hispidus*, in which the model with only linear effects explained more than half the variance in relative maximum bite force. Directional selection on performance is commonly found in a variety of organisms (Irschick *et al.*, 2008), suggesting that the means of the traits underlying performance might change across generations, if part of the trait variation is heritable, as a response for increased performance. Nevertheless, trait means may evolve under directional selection, while trait covariation remains the same, as long as directional selection does not have strong indirect effects on trait covariances (by eliminating pleiotropic effects for example; Melo & Marroig, 2015) and the pattern of stabilizing and correlational selection remains similar enough across generations.

Interestingly, we found evidence for significant linear selection on eigenvectors of γ that do not experience detectable non-linear selection. Morphological dimensions with very weak curvature on the

performance or fitness surface can be interpreted as ‘performance/selective lines of least resistance’ (Arnold *et al.*, 2001; Arnold, 2003). These lines of least resistance form ridges on the performance surface, allowing more individual variation in the morphology because there is practically no performance penalty in deviating from the mean. Thus, adaptive evolutionary change may be easier on the selective lines of least resistance, and adaptive peaks may preferentially move along these lines (Arnold *et al.*, 2001), because the changes in phenotypic means would not conflict with the pattern of correlational selection on the traits. However, a deeper understanding of peak shifts must connect differences among species to variation in ecological factors.

POTENTIAL ECOLOGICAL DRIVERS OF SPECIES DIFFERENCES IN BITE PERFORMANCE SURFACES

The three species differ in habitat, feeding ecology and mating tactics in ways that may be related to differences that we found in the bite performance surfaces. *T. hispidus* shows large variation in body size and more variable jaw morphology compared to the other two species. The difference in size classes of males translates into large differences in maximum bite force. *T. hispidus* dominant males have larger territories and access to more females than smaller satellite males (Diaz-Uriarte, 1999; R.B. and T.K., pers. obs.). The two classes of males differ in their mating strategy: the larger males fight other males to protect their territories and females, while the smaller males sneak into the larger males’ harems in an attempt to copulate with a female. Thus, our observation of a multivariate dimension that corresponds to allometric size in *T. hispidus*, which is potentially under directional selection, fits the social scenario of this species, and is probably related to intrasexual selection. Studies on other lizard species have shown that larger males with larger bite forces have greater success in male–male combats (e.g. Huyghe *et al.*, 2005; Lailvaux & Irschick, 2007). As a future step, it would be interesting to investigate whether larger males indeed have greater fitness than smaller males. Alternatively, smaller males could reach fitness levels similar to the dominant males, by using different strategies than larger males (sneaking instead of fighting).

The differences that we identified in average maximum bite force between species indicate that the species may respond to different functional demands as they adapt to different resources. Specifically, in *T. hispidus* and *T. semitaeniatus*, the much lower maximum bite force in *T. semitaeniatus* could contribute to divergence in diet, suggested to exist between these two species in syntopy, mostly in terms of prey size/volume rather than diet diversity

(Kolodiuk *et al.*, 2010; Pelegrin *et al.*, 2017). Therefore, our results support the idea that the flattening of the head in *T. semitaeniatus* may have imposed a constraint on prey size (Kohlsdorf *et al.*, 2008; Pelegrin *et al.*, 2017), as also seen in other flattened species (e.g. Broeckhoven & Mouton, 2014), even though the constraint may not be a general phenomenon for all flattened lizards (Herrel *et al.* 2001a).

Differences among species in the degree of variability of the lower jaw bones may be related to the range of microhabitats used by the species. Whereas *E. nanuzae* and *T. semitaeniatus* are found only in rocky habitats and in undisturbed areas, *T. hispidus* is found in urban environments, and therefore it is a generalist in terms of microhabitats, being found on palm trees, shrubs, walls of the houses, rocks and even on building rubble (T.K., pers. obs.). Consequently, *T. hispidus* may have a more diversified diet, when compared to the other two species, that could promote more variable lower jaw morphology. However, given that we found scratches in both males and females presumably derived from biting (R.B., pers. obs.), divergence in biting forces may be related to different strengths of sexual selection associated with agonistic interactions between males and with male–female interactions, and not just restricted to feeding mechanics. A larger study with more *Tropidurus* species is being conducted to explore sexual selection in head morphology and its relationship to bite force.

In conclusion, we have demonstrated linear effects of jaw morphology on bite force, as well as disruptive, non-linear effects. However, the non-linear effects that we found do not support an optimum associated with mechanical advantage. Most trait complexes are probably subjected to multiple selective pressures, arising from selection on different functional properties and in different ecological contexts. Nevertheless, full characterization of the performance surface, especially using combinations of traits instead of single traits, provided important evidence that interactions between traits affect performance and so are likely to shape the evolution of the functional complex.

ACKNOWLEDGMENTS

This work was funded by a national and an overseas post-doctoral fellowship from the Fundação de Amparo a Pesquisa de São Paulo to M.N.S. (FAPESP, projects 2015/19556-4 and BEPE 2016/22159-0), a post-doctoral fellowship from FAPESP to R.B. (project 2013/14125-0) and a thematic project FAPESP 2015/07650-6 to T.K. The licence to collect the specimens was given by IBAMA (SISBIO 33335-2). We wish to thank the reviewer and the editor for comments that helped to improve the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Three-dimensional landmarks used in the lower jaw of the lizards. Landmarks were placed on 3D volumes of skulls using the TINA manual landmarking tool, on both sides of the mandible and twice for each individual. From these landmarks, five linear distances within each lower jaw bone were extracted (see Fig. 1).

Table S2. Effects of sex and SVL on bite force and lower jaw linear distances. We ran univariate and multivariate analysis of variance on each species to assess whether differences in bite force or in the linear distances could be explained by sex, SVL and their interaction.

Table S3. Effects of sex, SVL and their interaction on relative maximum bite force and within-sex mean centred lower jaw linear distances. We re-ran the univariate and multivariate analysis of variance after transforming mean maximum bite force to one and the means of the lower jaw distances to zero. All effects seen before on the raw measurements disappeared, except for an effect of SVL on relative maximum bite force in *T. hispidus*.

Table S4. Normality tests for the lower jaw traits and maximum bite force.

Table S5. Bite performance γ -matrices for variance-standardized lower jaw distances.

Table S6. Eigenvectors of variance-standardized performance γ -matrices and their associated strengths of non-linear (λ) selection associated with maximum bite force. Lower jaw distances were mean-centred and divided by their corresponding standard deviations. m_1 to m_5 are the eigenvectors of the γ -matrices.

Table S7. Linear performance gradients of variance-standardized lower jaw traits. Linear performance gradients on single traits (β) and on eigenvectors of performance γ -matrices.

Figure S1. Performance surfaces and contour plots using thin-plate splines. A, *E. nauzae*; B, *T. hispidus*; C, *T. semitaenistus*. Although there is a much less curvature on m_5 in *T. hispidus* and *T. semitaenistus*, the surfaces are quite similar to the quadratic approximation.