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Interrelationships Between Bones, Muscles, and Performance: Biting in the Lizard *Tupinambis merianae*

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Abstract The origins of and potential constraints on the evolution of phenotypic diversity remain one of the central questions in evolutionary biology. The vertebrate skeleton is governed by historical, developmental, architectural, and functional constraints that all play a role in establishing its final form. Whereas the factors underlying shape variation in single bones are fairly well understood, this is less so the case for complex assemblages of bones as observed in the cranium or mandible. It is often suggested that the final phenotype must reflect the mechanical constraints imposed

by the loading of the skeleton as bones remodel to withstand loading. Yet, in the cranium, in contrast to the mandible, the final phenotype is likely constrained by demands other than loading including the protection of sensory systems and the brain. Architectural design constraints may further limit the final form of complex units like the vertebrate cranium. Here we use geometric morphometric approaches to quantify the shape of the cranium and mandible in a lizard and test whether the observed shape co-varies with both the muscles attaching to these structures as well as functional traits such as bite force. Our results show that co-variation between the cranium and mandible is significant and likely driven by the muscles that link the two systems. Moreover, our results show that the patterns of co-variation are stronger between the mandible and ventral side of the cranium. Muscular cross sectional areas, bite force, and the ventral side of the cranium also co-vary more than the dorsal side of the cranium does with muscle properties and function. Finally, our results show sex-specific patterns of co-variation with males showing a stronger degree of integration between the cranium, mandible, muscles and bite force suggesting that constraints on bite force drive the evolution of cranial shape to a greater extent in males compared to females.

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Introduction

Despite enormous advances in our understanding of evolutionary processes, questions concerning the drivers and origins of phenotypic diversity remain central in evolutionary biology (e.g. Chen et al. 2013; Cox and Rabosky

2013; Fulton et al. 2013; Losos et al. 2013). Whereas it is commonly assumed that historical, developmental, architectural, and functional constraints all play a role in establishing the phenotype of an organism (Seilacher 1970; Gould 2002), the relative roles and importance of these factors in constraining or driving phenotypic diversity remain poorly known. The factors underlying shape variation in single bones are fairly well understood (Currey 2002, 2003). Indeed, it is often suggested that the final phenotype reflects the mechanical constraints imposed by the loading of the skeleton as bones remodel continuously in response to mechanical loading induced by movement and muscle forces (e.g. Murray and Drachman 1969; Pontzer et al. 2006). Consequently, mice raised on different diets (hard versus soft) differ in the shape of the mandible reflecting the different loading regimes (Renaud et al. 2010). Although this has been demonstrated for vertebrate long bones and mandibles subjected to repeated loading (e.g. Sharir et al. 2011; Slizewski et al. 2013), in more complex bones or bone assemblages such as the cranium, the final phenotype is likely constrained by additional demands besides mechanical loading (Hallgrímsson et al. 2009). For example, some bones in the vertebrate cranium have been shown to function solely to protect sensory systems and the brain (Ross 2001). This is often reflected in the complex growth patterns of such structures (Clabaut et al. 2009; Monteiro and Abe 1997). Moreover, architectural design constraints may further limit the final form of complex units like the vertebrate cranium (Barel et al. 1989, but see Hulsey et al. 2007).

Even in complex structures composed of multiple bones and governed by multiple functional and spatial demands, however, we may expect co-variation between shape and function (Klingenberg and Marugan-Lobon 2013). Indeed, if the demands on functional performance are strong then the integration between the different structural elements of a single functional unit should be greater. An ideal system to test this hypothesis in is the vertebrate feeding system. Not only is the feeding system subjected to constraints on function imposed by natural selection on, for example, feeding efficiency or bite force (Herrel et al. 1999; Verwaijen et al. 2002), it must also function to protect the brain and sensory organs and plays an important role during respiration and drinking (Schwenk 2000). Moreover, differences in the selective pressures acting on the cranial system between the sexes in sexually dimorphic lizards (Huyghe et al. 2005, 2009, 2010; Husak et al. 2006) can result in sexual dimorphism in head shape (e.g. Herrel et al. 2007; Kaliontzopoulou et al. 2008), and allow a test of the hypothesis that these stronger demands on bite force in males should lead to greater co-variation without the confounding effects of phylogeny. Essentially, males and females are predicted to differ in the degree of co-variation

between the cranium, the mandible, the associated musculature and functional traits, as selection on bite force is greater in males due to the combined action of sexual and natural selection.

Here we use geometric morphometric approaches to quantify the shape of the cranium and mandible in a lizard and test whether the shape of these two structures co-varies. Furthermore, we test whether the shape of the cranium and mandible co-varies with 1) the force generating capacity (i.e. muscle cross sectional area) of the muscles attaching to these structures and 2) bite force. Specifically, we predict that shape co-variation will be greater between the mandible and the associated musculature and bite force than between the cranium and the associated muscles given that the cranium is involved in additional functions including the protection of the brain and sensory structures. Moreover, given the different selective pressures driving the evolution of bite force in both sexes we predict that the degree of co-variation will be greater in males compared to females given the combined natural and sexual selection pressures exerted on bite force in males. We here use a large omnivorous species of lizard, *Tupinambis merianae*, as a model system. This species is of interest as it is known to be dimorphic in head size, head shape, and bite force (Herrel et al. 2009; Fabre et al. in press). Moreover, it has been shown that the cranial structure changes shape during growth involving an enlargement of the adductor area, suggesting selection on biting in adults which may drive patterns of co-variation (Monteiro and Abe 1997).

Materials and Methods

Animals and Husbandry

The animals used in this study were obtained from the Jacarezário at the Universidade Estadual Paulista (Rio Claro, São Paulo, in southeastern Brazil), which runs a conservation breeding program for tegu lizards and other reptiles (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis reg. 1-35-94-1088-8). At this laboratory, the lizards are kept in small groups (5–10 individuals) in outdoor pens (5 m × 10 m or 2 m × 2 m) and are provided with water and ground shelters, as well as shade and sunny areas for thermoregulation. In spring and summer, the animals are fed three times a week with ground beef, fruits, and/or 1-d-old whole chickens.

Bite Forces

In vivo bite forces were measured using an isometric Kistler force transducer (9311B; range ± 5.000 N; Kistler, Switzerland) mounted on a purpose-built holder and

Table 1 Muscle physiological cross sectional areas for the individuals used in this study

ind	Sex	svl	hl	bf	Ptlat	Ptmed	MAME	PsTS	PsTP	MAMP
19	m	270	61.20	240.29	2.3	2.6	4.5	0.6	0.8	0.4
15	m	357	70.97	278.29	5.3	2.5	7.2	1.5	1.7	1.0
27	m	370	80.09	423.59	11.1	4.7	6.6	1.4	1.3	1.9
55	m	372	76.05	357.65	9.0	5.6	9.9	1.4	1.2	0.7
1	m	385	86.30	429.18	15.2	3.6	5.7	1.4	1.3	1.4
17	m	385	80.63	411.29	12.1	2.1	6.5	1.2	0.9	1.0
50	m	393	81.54	502.94	12.7	5.4	9.3	2.3	1.2	1.9
34	m	405	99.65	464.94	23.1	9.1	12.1	5.5	1.4	1.8
39	m	405	87.86	534.24	20.8	8.1	12.1	4.0	2.7	2.8
8	m	425	92.42	451.53	21.8	5.3	8.3	2.5	2.4	1.8
69	f	297	62.53	184.41	2.6	1.4	3.5	1.4	1.4	1.5
22	f	300	66.38	233.59	2.9	1.7	3.0	0.8	1.0	1.2
14	f	310	62.15	312.94	3.3	2.2	3.4	0.9	1.0	1.6
10	f	322	61.46	185.53	3.2	3.2	3.5	0.7	0.8	1.0
40	f	333	66.79	260.41	4.3	0.9	2.5	1.4	0.8	0.8
7	f	335	63.95	235.82	3.7	1.9	5.7	1.0	1.1	1.2
72	f	335	65.05	301.76	3.3	1.8	2.0	0.8	0.8	0.3
4	f	345	70.67	228.00	4.5	2.7	5.9	1.5	1.4	1.5
42	f	348	69.30	245.88	3.3	1.5	2.7	1.0	0.3	0.7

Individuals (ind) are ranked by sex and by snout-vent length. Snout-vent length (svl) and head length (hl) are in mm, bite force (bf) in newton (N) and physiological cross-sectional areas in cm². Ptlat, m. pterygoideus pars lateralis; Ptmed, m. pterygoideus pars medialis; MAME, m. adductor mandibulae externus; PsTS, m. pseudotemporalis pars superficialis; PsTP, m. pseudotemporalis pars profundus; MAMP, m. adductor mandibulae posterior

connected to a Kistler charge amplifier (5995A; Kistler, Switzerland; see Herrel et al. 1999; Anderson et al. 2008 for a more detailed description of the setup). When the free end of the holder was placed between the jaws of an animal, prolonged and repeated biting resulted. The place of application of bite forces on the transducer was standardized for all animals by mounting a metal bridge on the bite plates (see Herrel et al. 1999). Consequently all animals were biting at the level of the premaxillary teeth. Gape angle was standardized by moving the bite plates away from each other for larger animals resulting in gape angles in between 20° and 30° for all animals. Measurements were repeated five times for each animal, and the maximum value obtained during such a recording session was considered to be the maximal bite force for that individual (see Herrel et al. 2009; Table 1).

Dissections

Nineteen animals (ten males and nine females) were sacrificed for anatomical studies using an intramuscular injection of an overdose of pentobarbital after having their bite forces measured. Heads were dissected and all jaw muscle bundles were removed one by one and weighed on an Ohaus Scout Pro electronic balance. Muscle length was

measured from origin to insertion using digital calipers (Mitutoyo). Muscle fiber lengths were determined by cutting muscles lengthwise and measuring the length of the fibers from origin to insertion using digital calipers (Mitutoyo). Physiological cross sectional areas were determined by multiplying the mass of each muscle by muscle density (1.06 g/cm³; Mendez and Keys 1960) and dividing by muscle fiber length (Table 1). We did not correct for pennation angles as complex muscles were dissociated into individual muscle bundles composed of fibers running in the same direction. Total muscle cross sectional areas for each muscle were calculated as the sum of the cross sectional area of each muscle bundle.

Geometric morphometrics

After dissection, skulls were cleaned by hand and photographs were taken in lateral view of the mandible and in dorsal and ventral views of the cranium with a grid as background for scaling purposes. On the mandible 11 homologous type I landmarks and ten type II landmarks were taken (Fabre et al. in press). On the dorsal view, 27 type I and 16 type II landmarks were taken on one half of the cranium (Fabre et al. in press). On the ventral view, 23 type I and 17 type II landmarks were taken. All landmarks

were taken in TPSDig (V. 2.17; available at: <http://life.bio.sunysb.edu/morph/>). Landmarks were always taken by the same person (A-CF). Two repetitions were done for each individual in order to assess measurement error which was found to be low.

Analyses

Shape variation of the cranium and mandible was assessed using geometric morphometric approaches allowing an analysis of size and shape components independently (Zelditch 2004). Generalised procrustes superimposition (Rohlf and Slice 1990) was performed on the point coordinates using the package Rmorph (Baylac 2012) in the program R (R Development Core Team 2010). Two-block partial least squares analyses (PLS) implemented in the Rmorph library were used to quantify and visualize shape co-variation between the cranium and mandible as well as between muscle and bone shape using pairs of orthogonal vectors of each element (Rohlf and Corti 2000). Vectors were calculated on the co-variance matrices of the Procrustes coordinates of the cranium and the left hemi-mandible of the same individual. To test and statistically assess the significance and the magnitude of the shape covariation between the cranium and the mandible, we performed an RV test in R (Escoufier 1973; Klingenberg 2009) using the function RV.rtest in the ade4 library (Dray and Dufour 2007). The RV-coefficient provides a metric of the strength of co-variation between two structures and returns a value ranging between 0 and 1 (Goswami and Polly 2010). This coefficient is more analogous to a correlation coefficient. Its calculation mainly involves the division of the covariance between the two sets of variables by the variance of each data set or block. When the RV-coefficient is 0, no co-variation between structures is present; when the RV-coefficient is 1 the two structures are perfectly integrated. We compared the RV-coefficient estimated from 100 permutations. A *p* value is associated and allows one to assess the significance level of the observed signal (Escoufier 1973; Klingenberg 2009). The *P*₉₅-value is calculated by comparison of the observed RV coefficient to those obtained after re-sampling. If the RV coefficient was higher than those obtained from permuted blocks, then its associated *P*₉₅-value is considered as significant.

Next, we ran principal component analyses on the muscle cross sectional area data as these directly reflect the force generation capacity of a muscle. We ran analyses for all individuals as well as for males and females separately. We tested whether these principal components summarizing variation in muscle cross sectional area were correlated with *in vivo* bite force data for the same individuals using multiple regression analyses and did so for all individuals as well as for each sex separately. Finally, we explored

relationships between muscle cross sectional areas with the cranium and mandible shape using PLS analyses and RV-tests as described higher. This was done using data for all individuals as well as data for males and females separately.

Results

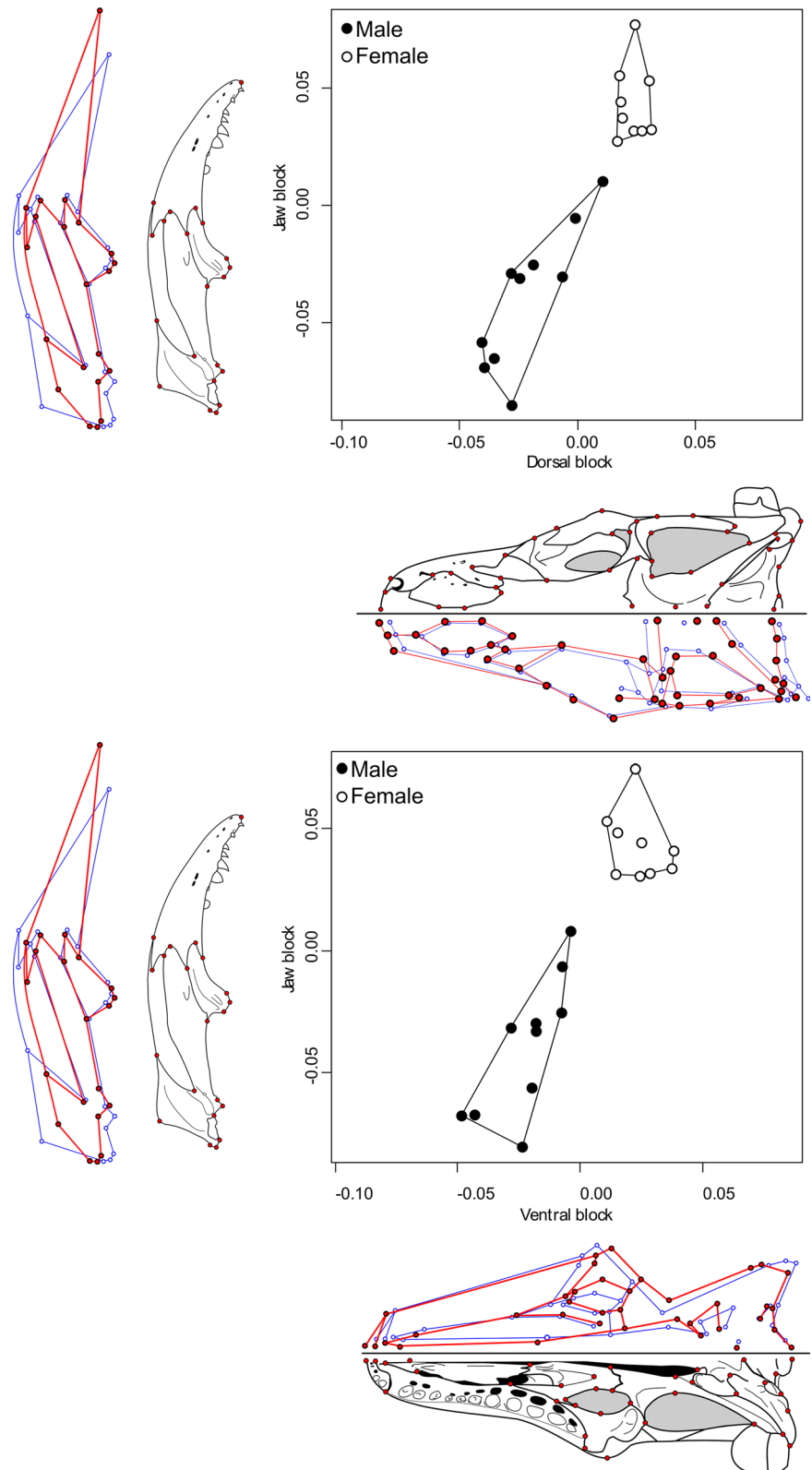
Co-variation Between Cranium and Mandible

A two-block PLS analysis performed on the shape data for the mandible and the cranium in dorsal view was significant across all individuals with the first PLS explaining 77 % of the total co-variation in the data set. However, only the first PLS axis was significant as indicated by the RV coefficient and associated test ($RV = 0.77$; $P = 0.01$). This analysis shows that the shorter and more robust mandibles (i.e. with a taller mandibular body) on the negative side of the first PLS axis and characterizing males are associated with crania with shorter snouts and larger adductor chambers (Fig. 1). A two-block PLS analysis performed on the shape data for the mandible and the cranium in ventral view was also significant ($RV = 0.64$; $P = 0.01$) with the first PLS axis explaining 84.6 % of the overall variation (Fig. 1). Here again, the robust mandibles characterizing males are associated with crania with shorter snouts yet larger adductor areas (Fig. 1). For males these results remained significant (mandible–dorsal shape: $RV = 0.68$, $P = 0.01$; mandible–ventral shape: $RV = 0.72$, $P = 0.01$) with the first PLS explaining 49 and 60.8 % of the total co-variation in the two analyses respectively (Fig. S1). Shapes associated with the extremes of the first PLS axes also indicated that shorter, more robust mandibles are associated with crania with shorter snouts and larger adductor areas. For females only the PLS between the jaw and the cranial shape in ventral view was significant ($RV = 0.65$; $P = 0.05$) and explained 69.3 % of the total co-variation (Fig. S2). The associated shapes link a slightly more curved and deeper mandible with a cranium that has a slightly longer snout and more anteriorly positioned quadrate (Fig. S2).

Muscle, Bite Force, and Shape Covariation

A principal component analysis performed on the muscle cross sectional areas extracted two axes together explaining over 90 % of the variation in the data set (Table 2). In the analysis including all individuals, the first axis was most strongly and negatively determined by the m. pterygoideus pars lateralis even though all muscles loaded negatively on this axis suggesting that this axis represents overall muscle cross sectional area (Table 2). This indicates that males

Fig. 1 Results of a two-block partial least squares analyses performed on **a** the mandible and the shape of the cranium in dorsal view; **b** the mandible and the shape of the cranium in ventral view. Illustrated are the landmarks taken on the cranium and mandible as well as the shapes representing the extremes along the first PLS axis (*red* positive; *blue* = negative). *Closed symbols* represent males, *open symbols* females (Color figure online)



and females differ predominantly in the cross sectional area the muscles with males having stronger muscles, and especially a bigger m. pterygoideus lateralis cross sectional

area (Fig. 2a). The analysis performed for males only showed similar results with the m. pterygoideus lateralis explaining most of the variation in the data set and

Table 2 factor loadings of the principal component analysis performed on the muscle physiological cross-sectional areas

	PC1	PC2
<i>All individuals</i>		
% variation explained	81.31	6.80
Ptlat	−0.62	−0.52
Ptmed	−0.45	0.58
MAME	−0.41	0.37
PsTS	−0.41	−0.43
PsTP	−0.27	0.28
<i>Males</i>		
% variation explained	80.90	9.00
Ptlat	−0.61	0.74
Ptmed	−0.40	−0.54
MAME	−0.27	−0.29
PsTS	−0.57	−0.25
PsTP	−0.26	−0.08
<i>Females</i>		
% variation explained	50.92	29.44
Ptlat	−0.08	0.11
Ptmed	−0.38	−0.76
MAME	−0.58	−0.04
PsTS	−0.14	0.56
PsTP	−0.70	0.32

Bold variables are those contributing most to each axis. Ptlat, m. pterygoideus pars lateralis; Ptmed, m. pterygoideus pars medialis; MAME, m. adductor mandibulae externus; PsTS, m. pseudotemporalis pars superficialis; PsTP, m. pseudotemporalis pars profundus

differentiating smaller from larger males (Fig. 2b). In females, however, the m. pseudotemporalis profundus explained by far most of the variation in the data set (Table 2, Fig. 3c).

The principal components summarizing variation in muscle cross sectional areas were significantly correlated with in vivo bite force ($R^2 = 0.84$, $P < 0.001$) across all individuals. Whereas for males only this result held ($R^2 = 0.89$, $P = 0.045$) for females the relationship between muscles and bite force was not significant ($R^2 = 0.40$, $P = 0.81$).

Dorsal cranial shape across all individuals co-varied with the muscle cross sectional areas and the first PLS axis explained 97.43 % of the total covariance (Fig. 3). The associated RV-test suggested that this result is significant ($RV = 0.77$, $P = 0.01$). Ventral cranial shape across all individuals co-varied with the muscle data as well with 97.5 % of the co-variation explained by the first PLS axis. The associated RV was significant ($RV = 0.66$, $P = 0.01$). Finally, the mandible also co-varied with the muscle cross

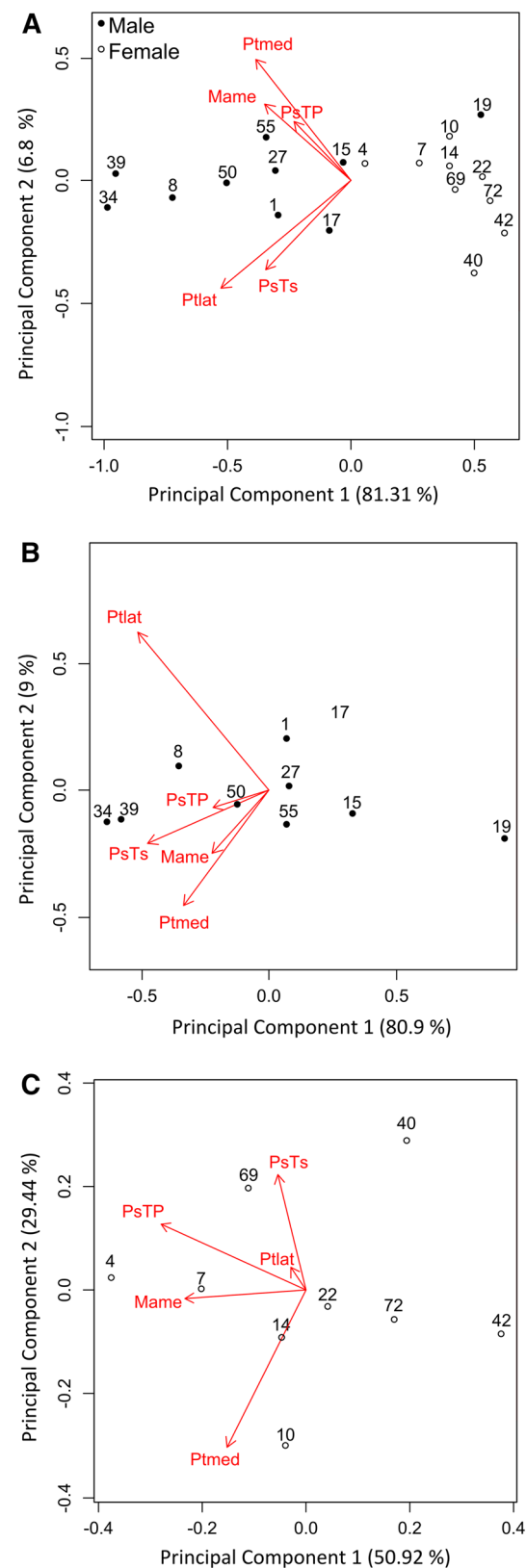


Fig. 2 Results of the principal component analysis performed on the muscle physiological cross sectional data. Illustrated is the distribution of the individuals in the space defined by the first two principal components for all individuals (a), for males only (b), and for females only (c). Loadings of the original variables on the axes are indicated by vectors. Together the first two axes explain over 90 % of the total variability in the data set when considering all individuals and males. For females the first two axes explained just over 80 % of the overall variability in the data set. Closed symbols represent males, open symbols females

sectional areas with the first PLS explaining 98.97 % of the overall covariance. The RV was again significant ($RV = 0.77$; $P = 0.01$). The results when analyzing data for males only were similar (dorsal shape–muscle: PLS = 91.26 %, $RV = 0.61$, $P = 0.01$; ventral shape–muscle PLS = 92.7 %, $RV = 0.51$, $P = 0.04$; mandible shape–muscle: PLS = 96.46 %, $RV = 0.56$, $P = 0.03$; Fig. S3). However, when analyzing data for females only none of the analyses of covariance were significant (RV tests; all $P > 0.05$) and PLS 1 explained at most 63.1 % of the covariance in the data set (ventral shape–muscle; Fig. S4).

Cranial shape in both dorsal ($R^2 = 0.95$, $P = 0.005$) and ventral ($R^2 = 0.92$, $P = 0.005$) view was also correlated with bite force, as was mandible shape ($R^2 = 0.96$, $P < 0.001$) when taking into account all individuals. These results were largely maintained when analyzing data for males only (dorsal shape–bite force: $R^2 = 0.98$, $P = 0.009$; ventral shape–bite force: $R^2 = 0.92$, $P = 0.08$; mandible shape–bite force: $R^2 = 0.90$, $P = 0.009$), but not for females (all $P > 0.05$).

Discussion

Co-variation

Our results showed strong patterns of co-variation between the cranium and the mandible in the large omnivorous lizard, *T. merianae*. A single PLS axis explained most of the variation in the data set with 77 % of the co-variation between the mandible and the dorsal side of the cranium, and 84.6 % of the co-variation between the mandible and the ventral side of the cranium being explained. Interestingly, the co-variation between the mandible and the ventral side of the cranium was greater than between the mandible and the dorsal side of the cranium. Intuitively this makes sense as the ventral side of the cranium provides most of the attachment area for the jaw adductors that physically link both structures. Specifically, the pterygoids and the quadrate changed their position and shape, resulting in animals with shorter, more robust mandibles to have slightly posteriorly and laterally displaced quadrates. The pterygoids move inward and the jugal forward and outward resulting in a greater area available for the

jaw adductors. In addition the rostrum also shortens as the mandible shortens, decreasing the out-lever for biting and resulting in an overall design of the feeding system that appears optimized towards the generation of higher bite force. Individuals to the left of the first PLS axis that display this shape are all males, suggesting strong differences between the sexes (see below).

In contrast to our predictions, the cranial shape co-varied equally with absolute variation in muscle cross sectional area as did the mandible with muscle data explaining around 97–98 % of the variation in cranial shape. Similarly, mandibular shape was well explained by bite force (96 %) as was cranial shape (95 and 92 % for dorsal and ventral views respectively). The slight differences in the relationship between the ventral and dorsal side of the cranium with bite force suggests that the role of the ventral side of the cranium is to provide attachment area for the jaw adductors. The shape of the mandible and the dorsal side of the cranium may play an important role in resisting feeding forces, in addition to providing insertion sites for muscles given their very strong association with bite force. The muscle cross-sectional areas were strongly correlated to bite force as well, and explained 84 % of the variation in bite force. Interestingly, the muscle that most strongly varied in the overall data set was the m. pterygoideus pars lateralis. This muscle is known to hypertrophy in males of many sexually dimorphic species, including *Tupinambis* lizards (Herrel et al. 1999; Naretto et al. 2013). Yet, this muscle is poorly positioned to generate bite force and as such has been suggested to potentially play a role as a display structure (Herrel et al. 1999). The predominance of variation in this muscle may also explain why the ventral side of the cranium displayed the strongest co-variation with the mandible as this muscle attaches specifically on the pterygoid and ectopterygoid bones at the ventral side of the cranium.

Cranial regions not involved in biting showed little shape variation in our data set (e.g. orbits and nares; see Fig. 1). This suggests that these regions are not constrained by variation in jaw musculature. As such, their shape may have evolved for purposes such as the protection of sensory structures rather than to resist bite or feeding forces. However, this should be tested using finite element models. Given that validated MDA models have been established for this species (Gröning et al. 2013), this would be worth pursuing. Moreover, such models could be used to test the role of the pterygoideus muscle in bite force generation and may help us understand how cranial shape evolves in these lizards.

Sexual dimorphism

We predicted that the degree of co-variation would be greater in males compared to females given the strong

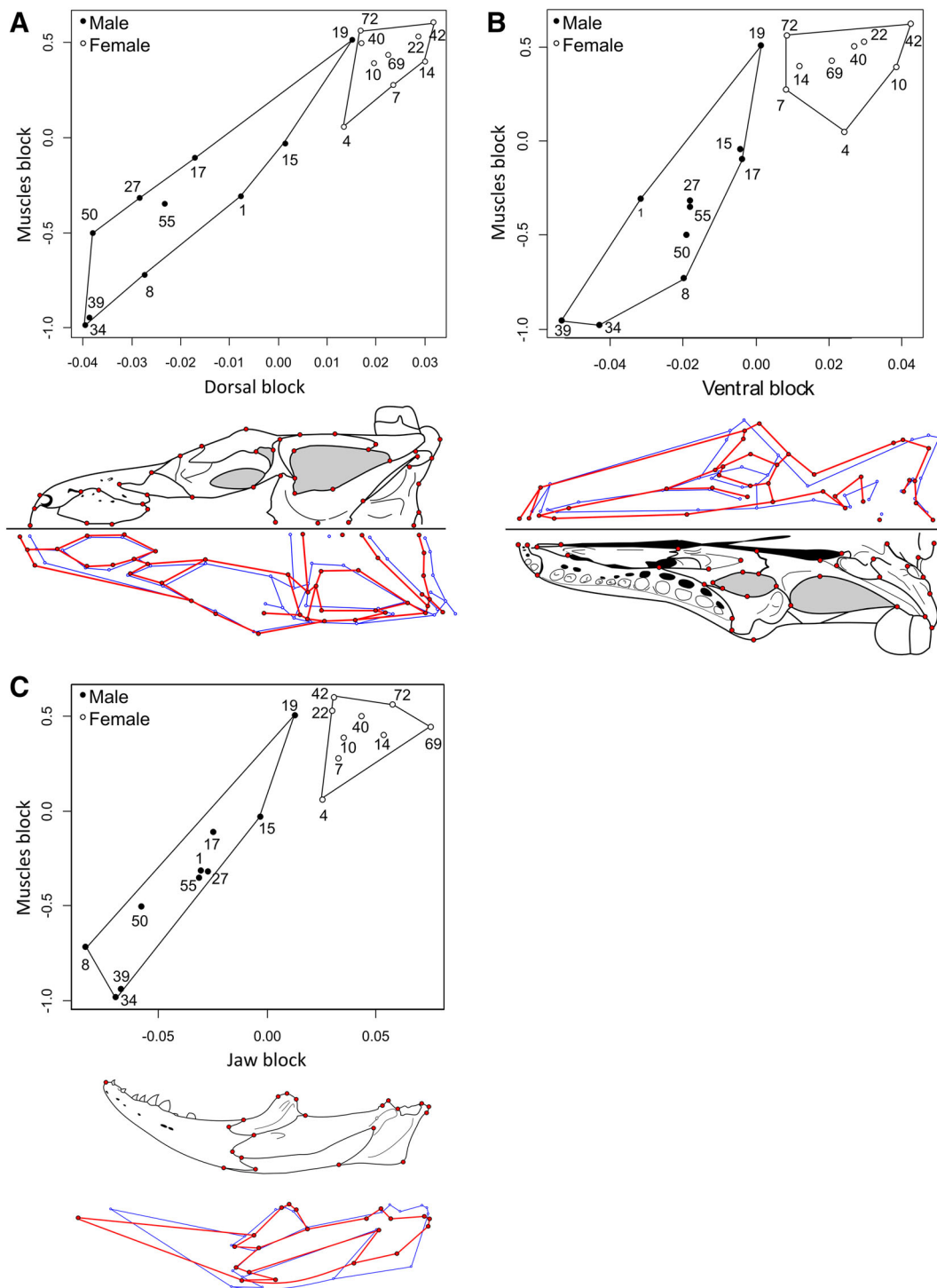


Fig. 3 Results of a two-block partial least squares analysis performed on data for both males and females. **a** covariation between the dorsal side of the skull and the muscle cross sectional areas; **b** covariation between the ventral side of the skull and the muscle cross sectional areas; **c** covariation between the mandible and the

muscle cross sectional areas. Illustrated are the landmarks taken on the cranium and mandible as well as the shapes representing the extremes along the first PLS axis (red positive; blue negative). Closed symbols represent males, open symbols females (Color figure online)

selection on biting in males (Naretto et al. 2013). Our results confirm this prediction. Whereas for males covariation between the cranium and mandible was strong

(Fig. S1), in females only the co-variation between the mandible and the ventral side of the cranium was significant. Moreover, the amount of shape co-variation

explained was rather low (64 %) suggesting that the strong co-variation observed in males and in the overall data set is indeed driven by the jaw adductors through selection on biting. This is further supported by the strong correlations with bite force in males. In females, most of the variation in jaw muscles was situated in the deep pseudotemporalis whereas in males the lateral part of the m. pterygoideus drove much of the variation in the data set. Interestingly, the co-variation in cranial and mandibular shape observed in females corresponds to variation in the insertion site of the deep pseudotemporalis that is associated with a deeper mandibular corpus at the level of the coronoid, the insertion site of this muscle. This suggests again that muscles are driving patterns of co-variation, yet in a more localized way compared to what was observed in the overall data set.

When comparing data for the two sexes, differences in the importance of the lateral part of the m. pterygoideus appear to drive most of the sexual shape dimorphism (Fabre et al. in press). In the male data set, the first PCA axis for the muscles as well as the first PLS axis of co-variation between cranium and mandible appear to represent a size gradient ranging from the smallest male in our study (individual 19, see Figs. 2, S1) to the largest males we measured (individual 39). Thus growth patterns, at least up to sexual maturity appear to involve an increase in this muscle and a concordant change in shape of both the cranium and mandible. Interestingly, this muscle only contributes little to bite force in lizards in general (Herrel et al. 1998, 1999) and may thus serve other functions such as display, as it is known that lizards with wider heads typically win male–male contests (Herrel et al. 1999; Huyghe et al. 2005). Given that the size of this muscle fluctuates seasonally (Naretto et al. 2013) and is known to respond to fluctuating testosterone levels (Huyghe et al. 2010) it may indeed function as a sexual signal (see Herrel et al. 1999). A direct prediction following from our results would be that cranial and mandible may also show seasonal changes in shape, yet this remains to be tested.

In conclusion, our results demonstrate strong patterns of co-variation between the cranium and mandible that appear to be driven by the muscles bridging the two structures. Further, evidence for an important role of biting and muscle forces in driving this co-variation is provided by the strong co-variation observed in males but not females. Finally, our results suggest that parts of the cranium may respond to loading induced by biting and muscle forces, whereas others may play a role in providing attachment areas and in the protection of sensory structures. Clearly, the generality of these data remains to be tested and biomechanical models such as finite element models may be especially suited to explore some of the hypotheses put forward here.

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References

- Anderson, R., McBrayer, L. D., & Herrel, A. (2008). Bite force in vertebrates: Opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biological Journal of the Linnean Society*, 93, 709–720.
- Barel, C. D., Anker, G. C., Witte, F., Hoogerhoud, R. J., & Goldschmidt, T. (1989). Constructional constraint and its ecomorphological implications. *Acta Morphologica Neerlandica Scandinavia*, 27, 83–109.
- Baylac, M. (2012). Rmorph: A R geometric and multivariate morphometrics library. Available from the author: baylac@mnhn.fr.
- Chen, S., Krinsky, B. H., & Long, M. (2013). New genes as drivers of phenotypic evolution. *Nature Reviews Genetics*, 14, 645–660.
- Clabaut, C., Herrel, A., Sanger, T., Smith, T., & Abzhanov, A. (2009). Development of beak polymorphism in the African Seedcracker, *Pyrenestes ostrinus*. *Evolution and Development*, 11, 636–646.
- Cox, C. L., & Rabosky, A. R. D. (2013). Spatial and temporal drivers of phenotypic diversity in polymorphic snakes. *American Naturalist*, 182, E40–E57.
- Currey, J. D. (2002). *Bones, structure and mechanics* (p. 436). Princeton: Princeton University Press.
- Currey, J. D. (2003). The many adaptations of bone. *Journal of Biomechanics*, 36, 1487–1495.
- Development Core Team, R. (2010). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4), 1–20.
- Escoufier, Y. (1973). Le traitement des variables vectorelles. *Biometrics*, 29, 751–760.
- Fabre, A.-C., Cornette, R., Huyghe, K., Andrade, D. & Herrel, A. (in press). Linear versus geometric morphometric approaches for the analysis of head shape dimorphism in lizards. *Journal of Morphology*. doi:10.1002/jmor.20278.
- Fulton, C. J., Binning, S. A., Wainwright, P. C., & Bellwood, D. R. (2013). Wave-induced abiotic stress shapes phenotypic diversity in a coral reef fish across a geographical cline. *Coral Reefs*, 32, 685–689.
- Goswami, A., & Polly, P. D. (2010). Methods for studying morphological integration and modularity. In J. Alroy J & G. Hunt (Eds.), *Quantitative Paleontology* (pp. 213–243). The Paleontological Society Papers.
- Gould, S. J. (2002). *The structure of evolutionary theory*. Cambridge: Harvard University Press.
- Gröning, F., Jones, M. E. H., Curtis, N., Herrel, A., O'Higgins, P., Evans, S. E., et al. (2013). The importance of accurate muscle modelling for biomechanical analyses: A case study with a lizard. *Journal of the Royal Society, Interface*, 10, 20130216.

- Hallgrímsson, B., Jamniczky, H., Young, N. M., Rolian, C., Parsons, T. E., Boughner, J. C., et al. (2009). Deciphering the Palimpsest: Studying the relationship between morphological integration and phenotypic covariation. *Evolutionary Biology*, 36, 355–376.
- Herrel, A., Aerts, P., & De Vree, F. (1998). Ecomorphology of the lizard feeding apparatus: A modelling approach. *Netherlands Journal of Zoology*, 48(1), 1–25.
- Herrel, A., Andrade, D. V., de Carvalho, J. E., Brito, A., Abe, A., & Navas, C. (2009). Aggressive behavior and performance in the tegu lizard *Tupinambis merianae*. *Physiological and Biochemical Zoology*, 82, 680–685.
- Herrel, A., McBrayer, L. D., & Larson, P. M. (2007). Functional basis for intersexual differences in bite force in the lizard *Anolis carolinensis*. *Biological Journal of the Linnean Society*, 91, 111–119.
- Herrel, A., Spithoven, L., Van Damme, R., & De Vree, F. (1999). Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses. *Functional Ecology*, 13(3), 289–297.
- Hulsey, C. D., Mims, M. C., & Streelman, J. T. (2007). Do constructional constraints influence cichlid craniofacial diversification? *Proceedings of the Royal Society B*, 274, 1867–1875.
- Husak, J. F., Lappin, A. K., Fox, S. F., & Lemos-Espinal, J. A. (2006). Bite-force performance predicts dominance in male venerable collared lizards (*Crotaphytus antiquus*). *Copeia*, 2006, 301–306.
- Huyghe, K., Herrel, A., Adriaens, D., Tadić, Z., & Van Damme, R. (2009). It 's all in the head. Morphological basis for differences in bite force among colour morphs of the Dalmatian wall lizard. *Biological Journal of the Linnean Society*, 96, 13–22.
- Huyghe, K., Husak, J. F., Moore, I. T., Vanhooydonck, B., Van Damme, R., Molina-Borja, M., et al. (2010). Effects of testosterone on morphology, performance and muscle mass in a lizard. *Journal of Experimental Zoology*, 313A, 9–16.
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., & Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology*, 19, 800–807.
- Kaliontzopoulou, A., Carretero, M. A., & Llorente, G. A. (2008). Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: Joining linear and geometric morphometrics. *Biological Journal of the Linnean Society*, 93, 111–124.
- Klingenberg, C. P. (2009). Morphometric integration and modularity in configurations of landmarks: Tools for evaluating a priori hypotheses. *Evolution and Development*, 11, 405–421.
- Klingenberg, C. P., & Marugan-Lobon, J. (2013). Evolutionary covariation in geometric morphometric data: Analyzing integration, modularity, and allometry in a phylogenetic context. *Systematic Biology*, 62, 591–610.
- Losos, J. B., Arnold, S. J., Bejerano, G., Brodie, E. D. I. I., Hibbett, D., Hoekstra, H. E., et al. (2013). Evolutionary biology for the 21st century. *PLoS Biology*, 11, e1001466. doi:10.1371/journal.pbio.1001466.
- Mendez, J., & Keys, A. (1960). Density and composition of mammalian muscle. *Metabolism*, 9, 184–188.
- Monteiro, L. R., & Abe, A. S. (1997). Allometry and morphological integration in the of *Tupinambis merianae* (Lacertilia: Teiidae). *Amphibia-Reptilia*, 18, 397–405.
- Murray, P. D., & Drachman, D. B. (1969). The role of movement in the development of joints and related structures: The head and neck in the chick embryo. *Journal for Embryology and Experimental Morphology*, 22, 349–371.
- Naretto, S., Cardozo, G., Blengini, C. S., & Chiaraviglio, M. (2013). Sexual selection and dynamics of jaw muscle in *Tupinambis* lizards. *Evolutionary Biology*. doi:10.1007/s11692-013-9257-0.
- Pontzer, H., Lieberman, D., Momin, E., Devlin, M., Polk, J., Hallgrímsson, B., et al. (2006). Trabecular bone responds with high sensitivity to load orientation in the knee. *Journal of Experimental Biology*, 209, 57–65.
- Renaud, S., Auffray, J-F. & de la Porte, S. (2010). Epigenetic effects on the mouse mandible: common features and discrepancies in remodeling due to muscular dystrophy and response to food consistency. *BMC Evolutionary Biology* 10, p 28 <http://www.biomedcentral.com/1471-2148/10/28>.
- Rohlf, F. J., & Corti, M. (2000). Use of two-block partial least-squares to study covariation in shape. *Systematic Biology*, 49, 740–753.
- Rohlf, F. J., & Slice, D. E. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, 39, 40–59.
- Ross, C. F. (2001). *In vivo* function of the craniofacial haft: The interorbital “pillar”. *American Journal of Physical Anthropology*, 116, 108–139.
- Schwenk, K. (2000). *Feeding: Form, function and evolution in tetrapod vertebrates* (p. 537). San Diego: Academic Press.
- Seilacher, A. (1970). Arbeitskonzept zur konstruktions-morphologie. *Lethaia*, 3, 393–396.
- Sharir, A., Stern, T., Rot, C., Shahar, R., & Zelzer, E. (2011). Muscle force regulates bone shaping for optimal load-bearing capacity during embryogenesis. *Development*, 138, 3247–3259.
- Slizewski, A., Schönau, E., Shaw, C., & Harvati, K. (2013). Muscle area estimation from cortical bone. *Anatomical Record*, 296, 1695–1707.
- Verwajen, D., Van Damme, R., & Herrel, A. (2002). Relationships between head size, bite force, prey handling efficiency and diet in two sympatric lacertid lizards. *Functional Ecology*, 16, 842–850.
- Zelditch, M. (2004). *Geometric morphometrics for biologists: A primer*. Amsterdam: Elsevier.