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RESEARCH ARTICLE

Bite force and cranial bone strain in four species of lizards

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ABSTRACT

In vivo bone strain data provide direct evidence of strain patterns in the cranium during biting. Compared with those in mammals, *in vivo* bone strains in lizard skulls are poorly documented. This paper presents strain data from the skulls of *Anolis equestris*, *Gekko gecko*, *Iguana iguana* and *Salvator merianae* during transducer biting. Analysis of variance was used to investigate effects of bite force, bite point, diet, cranial morphology and cranial kinesis on strain magnitude. Within individuals, the most consistent determinants of variance in bone strain magnitude were gauge location and bite point, with the importance of bite force varying between individuals. Inter-site variance in strain magnitude – strain gradient – was present in all individuals and varied with bite point. Between individuals within species, variance in strain magnitude was driven primarily by variation in bite force, not gauge location or bite point, suggesting that inter-individual variation in patterns of strain magnitude is minimal. Between species, variation in strain magnitude was significantly impacted by bite force and species membership, as well as by interactions between gauge location, species and bite point. Independent of bite force, species differences in cranial strain magnitude may reflect selection for different cranial morphology in relation to feeding function, but what these performance criteria are is not clear. The relatively low strain magnitudes in *Iguana* and *Uromastyx* compared with those in other lizards may be related to their herbivorous diet. Cranial kinesis and the presence or absence of postorbital and supratemporal bars are not important determinants of inter-specific variation in strain magnitude.

KEY WORDS: Feeding, Biting, Skull, Lepidosauria, Biomechanics

INTRODUCTION

Data on *in vivo* bone strain magnitude are crucial for understanding the relationships between animal behavior and skeletal design – form–function relationships. Bone tissue yields in the range of 4000 to 12,000 με (Currey, 2004), suggesting that skeletal form should be adapted to keep strain magnitude below these values, but attempts to identify more restrictive rules on maximum strain magnitude have been unsuccessful. The theory of dynamic strain similarity, that similar safety factors to failure are maintained 'by allometrically scaling the magnitude of the peak forces applied to them during vigorous locomotion' (Rubin and Lanyon, 1984), does not explain the diversity of safety factors in tetrapod limb bones associated with taxonomic, ontogenetic, physiological and functional diversity (Biewener, 1993; Blob et al., 2014; Kawano et al., 2016; Main and Biewener, 2004). In part, this reflects the fact that repetitive loading of bones during cyclic behaviors lowers the strain magnitude at which bone fails – human and bovine femoral bone loaded at 9000 με in tension fails after 10 cycles, but it will fail at only 6000 με after 10,000 cycles (Zioupos et al., 2001). The maximum strain magnitude to which limb bones should be adapted probably varies with the frequency with which a given behavior is employed, predicting variation in bone safety factors across taxa, ages, behaviors or – the focus of this study – functional systems.

To date, the majority of studies relating strain magnitude to skeletal design have focused on the limb skeleton, which has one predominant function – transmission, amplification and resistance of muscle and substrate reaction forces during locomotion (Biewener, 2003; Main and Biewener, 2004). In contrast, the bones of the skull perform many functions, suggesting that optimality criteria driving skull evolution may be more diverse than those driving limb bone shape. This diversity in skull function has been invoked to explain the wide variation in strain magnitude recorded from different parts of the skull during feeding. In mammals, the neurocranial and circumorbital skeletons experience much lower strain magnitudes during feeding than the zygomatic bone or mandible (Hylander et al., 1991b; Ross and Metzger, 2004). Strain magnitudes in the brow ridges of cercopithecine monkeys during feeding are absolutely low (always <500 με and usually <200 με), and lower than those recorded simultaneously elsewhere in the facial skeleton (Hylander et al., 1991b). Indeed, strain magnitudes in primate mandibles are 3.5–6.5 times higher than those recorded from the circumorbital region simultaneously or during similar behaviors (Ross and Metzger, 2004). These data contradict the idea that the facial skeleton is optimized for 'maximum strength with minimum material' during feeding, i.e. the bones of the brow ridges and other regions of the skull that experience low strain magnitudes during feeding could be significantly reduced in size, or their shapes changed, without compromising their strength during feeding (Hylander and Johnson, 1997; Hylander et al., 1991a; Ross and Metzger, 2004).

This conclusion may at first seem counter-intuitive: don't low bone strain magnitudes during feeding suggest that a structure is well designed, not poorly designed for resisting feeding forces? Hylander and colleagues noted several problems with this line of reasoning (Hylander and Johnson, 1997; Hylander et al., 1991b). It does not explain the diversity of strain magnitudes (and inferred safety factors) recorded from the facial skeleton during feeding: if low strained areas of the skeleton are well designed, must that mean that high strained areas are poorly designed? And if so, why has selection not provided mechanisms to reduce those strain magnitudes? After all, there are costs to moving bony structures during locomotion and feeding, costs to building them during development, and costs to maintaining and repairing them during life. Indeed, larger bones than necessary are not necessarily better as

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they can fail at lower stresses than smaller bones because their larger volume increases the probability that they accumulate microcracks that can grow into larger deficits that might cause bone yield or failure (Currey, 2002; Weibull, 1951).

Hence, if two regions of bone experience very different bone strain magnitudes during the same behavior, they are by definition not optimized for maximum strength with minimum material during that behavior, leading to the conclusion that either this optimality criterion is not equally important in the two bones or regions, or the experimenter has not captured the full range of behaviors generating strain in that bone (Gröning et al., 2013). Perhaps the most difficult strain data to collect *in vivo* are those associated with infrequent traumatic events, such as blows or bites during predation or intra-specific agonistic interactions, which have been hypothesized to be important determinants of skull design in primates (Carrier, 2011; Hylander and Johnson, 1997; Hylander et al., 1991b; Hylander and Ravosa, 1992). In the absence of *in vivo* strain data across the complete range of animal behaviors, and estimates of their frequency and ecological importance (Ross et al., 2016), some progress can be made by assuming that behaviors associated with relatively high strain magnitudes are likely to impose greater demands on skeletal design than behaviors associated with lower strain magnitudes – bone size and shape are expected to be more closely adapted to resist high strain than low strain magnitude loading regimes. This expectation applies not only across behaviors – different gaits (Biewener et al., 1983a, b; Blob and Biewener, 1999); biting versus chewing, licking and yawning (Hylander, 1981; Ross et al., 2016) – but also between different phases of the same behavior. For example, the shapes of limb bones are expected to be more closely adapted to dissipating forces associated with stance phase than swing phase of the gait cycle (Biewener, 2003), and mandible shape is expected to be adapted more to dissipating forces associated with the power stroke than with the opening phases of the gape cycle (Hylander et al., 1987).

Invoking this line of reasoning, skull bones that experience relatively low strain magnitudes during feeding must be designed to perform non-feeding functions, such as: insulating the visual system from unwanted displacements (Cartmill, 1972; Cartmill, 1980; Heesy, 2005; Ravosa et al., 2000b; Ross, 1995a, 1996); augmenting areas for muscle attachment (Ross, 1995b); providing a rigid framework to maintain the volume of the respiratory passages (Ross, 1995b; Ross, 2001; Ross and Metzger, 2004); and protecting the brain and sense organs from 'infrequent non-masticatory traumatic loads' (Carrier, 2011; Hylander and Johnson, 1992; Hylander and Johnson, 1997; Hylander et al., 1991a; Ravosa et al., 2000b). Areas such as the mammalian zygoma, which experiences high strain magnitudes during feeding, are expected to show closer matches between form and the mechanical demands of feeding (Behrents et al., 1978; Herring and Teng, 2000; Herring et al., 1996; Hylander et al., 1991b; Hylander and Ravosa, 1992; Lieberman et al., 2004; Ravosa, 1991; Ross, 2001, 2008; Ross et al., 2011; Ross and Hylander, 1996; Ross and Metzger, 2004; Thomason et al., 2001).

All of the examples given above come from mammals, predominantly primates: among non-avian reptiles, strain data have only been extensively sampled from the skull of *Alligator* (Metzger et al., 2005; Porro et al., 2013), with limited data from the crania of *Varanus* and *Uromastyx* (Porro et al., 2014; Smith and Hylander, 1985). Consequently, the magnitude and determinants of variation in strain magnitude across the crania of non-mammalian tetrapods are poorly understood, and we have little idea how the apparent principles of skull design in mammals might or might not apply to other tetrapod clades. This paper presents *in vivo* bone strain data from the frontal, parietal and maxilla bones of four species of lepidosaur: the insectivorous anole *Anolis equestris* Merrem 1820 (Dalrymple, 1980; Lister, 1981); the insectivorous gekkonid *Gekko gecko* (Linnaeus 1758); the herbivorous iguanid *Iguana iguana* (Linnaeus 1758); and the omnivorous teiid *Salvator* (previously *Tupinambis*) *merianae* Dumeril and Bibron 1839 (Colli et al., 1998). The data were used to address general questions regarding patterns of strain in non-mammalian crania. Do individuals in the same species share common patterns of strain magnitude? How do bite force, bite point, species-specific cranial morphology and cranial kinesis impact strain magnitude in lizard crania? What drives variation in strain magnitude within and between individuals and species?

We then addressed hypotheses regarding the effects of diet, cranial morphology and kinesis on lizard cranial strain magnitude. Herbivorous lepidosaurs, like *I. iguana* in our study, have light skulls, short snouts, large jaw elevator (adductor) muscles and high bite forces (Herrel et al., 2007; Metzger and Herrel, 2005; Stayton, 2006). Light skulls suggest that, when bite force is controlled for, the crania of our herbivorous species – *I. iguana* – might experience higher strains compared with those of the other species studied here. Arguing against this prediction, Porro et al. (2014) reported low strain magnitudes in the herbivorous lizard *Uromastyx*, suggesting that this might reflect adaptation for avoiding fatigue failure of repetitively loaded bone (see above), assuming that herbivorous lizards perform more feeding cycles per day than carnivorous or omnivorous lizards. There are no data that we know of that speak to the validity of that assumption, but Porro et al.'s (2014) hypothesis predicts that *I. iguana* should experience lower strains than the non-herbivorous species in our sample.

Our data also allowed us to make preliminary assessments of the impact of important variants in cranial architecture in lepidosaurs: the presence or absence of bars of bone and cranial kinesis. *Gekko gecko* displays streptostyly, mesokinesis and metakinesis, and lacks the supratemporal and postorbital bars possessed by the other three species (Herrel et al., 1999; Metzger, 2002); *Salvator* is streptostylic but not measurably mesokinetic (Barberena et al., 1970; Smith, 1980); *A. equestris* is streptostylic (A.H., personal observations); and *I. iguana* is akinetic (Throckmorton, 1976). The precise effects of these interspecific differences in cranial morphology and kinesis are difficult to predict because of uncertainty regarding deformation regimes in lizard crania. One possibility is that the absence of supratemporal and postorbital bars will result in higher strain magnitudes, either because there is less bone to absorb muscle and bite forces or because the cranium is less rigid overall. Kinesis might be expected to be associated with lower strain magnitudes because strain energy is dissipated in viscoelastic sutural tissues. This may be linked to large differences in strain magnitudes between frontal and parietal bones, on either side of the mesokinetic joint.

To control for effects of bite force and bite point, this study focused on strain magnitudes recorded during transducer biting. Although transducer biting is not normal feeding behavior, it does allow the effects of bite force to be controlled while testing hypotheses regarding the effects of species-specific morphology on variation in bone strain magnitude. Bite force impacts cranial bone strain magnitude through interactions with bite point, not only because the torques acting on the cranium change with bite point but also because of relationships between bite point and bite force. In mammals, variation in bite force with bite point has been shown to be described by the constrained lever model which predicts that, because of constraints against tensile forces in the biting side jaw joint, bite forces at the most posterior (distal) teeth are lower than

those in the middle of the postcanine tooth row (Greaves, 1978; Spencer, 1995; Spencer, 1998; Thompson et al., 2003). Similar predictions have been made for 'reptiles' (Druzinsky and Greaves, 1979). Here, we evaluated whether the constrained lever model applies to lepidosaurs and tested the hypothesis that there are significant interactions between bite point and bite force in their effects on bone strain magnitude in the lizard cranium.

In summary, this study used *in vivo* bone strain magnitude data collected simultaneously from multiple cranial sites, from multiple individuals from four lizard species with diverse cranial morphologies to address the following questions. (1) Controlling for bite force and bite point, do lizards show strain gradients – variation in strain magnitude – across the cranium during biting? (2) Do individuals in the same species share common patterns of variation in strain magnitude across the cranium when bite force and bite point are controlled statistically? (3) When bite force and bite point are controlled, what are the effects of diet, species-specific cranial morphology and cranial kinesis on variation in strain magnitude in lizard crania?

MATERIALS AND METHODS

Animal care

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago and the *S. merianae* experiments were approved by the University of Antwerp Ethics Committee. Four adult *A. equestris*, *G. gecko* and *I. iguana* were purchased through commercial dealers and housed in individual enclosures (152×61×61 cm) in the Carlson Animal Resources Center at the University of Chicago, USA. The housing room was maintained at appropriate ranges of ambient temperature (24–28°C) and humidity (50–80%). Each enclosure was equipped with a heat lamp to provide a basking spot (38°C) during the day and a UVB light to ensure proper vitamin D3 production. Animals were fed with crickets, worms, mice and/or fruit every other day and fresh water was provided daily. Three adult *S. merianae* were obtained through commercial dealers and housed in individual cages (120×80×80 cm) in a temperature-controlled room set at 25°C in the Functional Morphology Laboratory, Department of Biology, University of Antwerp, Belgium. A basking spot at higher temperature (45°C) was available, the animals were fed with mice and/or fruit twice weekly, and water was available *ad libitum*. Jaw length, a biomechanically relevant size variable for the feeding system, was measured from the tip of the retroarticular process to the tip of the jaw at the symphysis *in vivo* or from 3D reconstructions of CT scans of the animals post mortem (Table 1; Table S1).

Bone strain data

Bone strain data were recorded using stacked delta rosette strain gauges (SA-06-030WY-120, Micromeasurements, Raleigh, NC, USA) wired, insulated and gas sterilized using procedures described previously (Ross, 2001; Ross et al., 2011). Following anesthesia through intramuscular injection of a mixture of ketamine and dexmedetomidine (respectively 50 mg kg^{-1} and 200 µg kg^{-1} body mass) (Chai et al., 2009), <1 cm^2 of skin overlying each gauge site was removed, the periosteum elevated, the bone degreased with chloroform and the gauge bonded to the surface of the bone using cyanoacrylate adhesive. The lead wires were either tunneled under the skin to the nuchal region (*S. merianae*) or run outside the skin to the nuchal area where they were sutured to the skin (other taxa). Strain gauge sites are shown in Fig. 1 and Fig. S1 and include the mandible site in one *S. merianae*. After placement of EMG electrodes (EMG data not presented in this study), the animals were returned to temporary housing cages for at least 12 h prior to data recording. Instrumentation effects were tested using pairwise comparisons between bite forces of individual animals before and after placement of strain gauges and EMG electrodes.

Data collection

The animals were manually restrained and simultaneous bite force and bone strain data were collected while the animals bit on a calibrated bite force transducer described previously (Herrel et al., 1999). Bite point (anterior midline; anterior one-third of non-midline tooth row, middle third and posterior third on left and right sides) was recorded on video tape or on the voice track of a tape recorder. Voltage changes in the strain gauges were conditioned and amplified on Vishay 2310 bridge-amplifiers and the data acquired at 1 kHz through a National Instruments DAQ board run by MiDAS data acquisition software package (Xcitex, Cambridge, MA, USA) or the analog data collection module in a Vicon MX T40 system (Los Angeles, CA, USA), and were saved to a server for subsequent analysis.

Bite force data analysis

To assess whether our subjects are representative of their wider populations, bite forces recorded at the anterior midline bite point in the experimental animals were compared with those collected using the same methods from non-experimental, conspecific, captive *A. equestris*, *I. iguana*, *G. gecko* and *S. merianae* housed in: Prague, Czech Republic; Miami, FL, USA; Paris, France; the University of Antwerp, Belgium; and the University of Tulane, New Orleans, LA, USA (Fig. 2A). *Salvator merianae* data were also collected from 125 semi-wild animals in a conservation breeding program (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, reg. 1-35-94-1088-8) in the Jacarezário, Universidade Estadual Paulista (Rio Claro, São Paulo, Brazil). There, the lizards were kept in groups of 5–10 individuals in outdoor pens (5 m×10 m or 2 m×2 m) with free access to water, ground shelters, and shaded and sunny areas for thermoregulation. In spring and summer, the animals were fed three times a week with ground beef, fruits and/or 1 day old chickens.

The simple lever model predicts that bite forces will increase at progressively posterior bite points, whereas the constrained lever model predicts that bite forces will increase as the bite point moves posteriorly, except at the most posterior bite points, where they will decrease. To determine which of these models best explains bite force distribution along the toothrow, a Jonckheere–Terpstra (Jonckheere, 1954; Terpstra, 1952) test for an ordered difference in bite force medians was used, within each species and on each side of the tooth row.

Bone strain data analysis

Custom-written software (IGOR Pro 4.0, WaveMetrics, Inc., Lake Oswego, OR, USA) was used to convert the strain data from volts to microstrain ($\mu\varepsilon=10^{-6}$ $\Delta L/L$, where L is length) using shunt calibration files recorded during the experimental sessions, and to calculate the magnitudes of maximum (ε_1) and minimum (ε_2) principal strains (Hibbeler, 2000). The peak magnitudes of these variables during each bite were extracted to IBM SPSS Statistics for Windows (version 24, IBM Corp., Armonk, NY, USA) for statistical analysis.

Univariate ANOVA were used to investigate the factors driving variance in bone strain magnitude at different sites in the cranium in the four lizard species. Separate models were calculated: across gauge locations and bite points within each individual; across gauge locations, bite points and individuals within each species;

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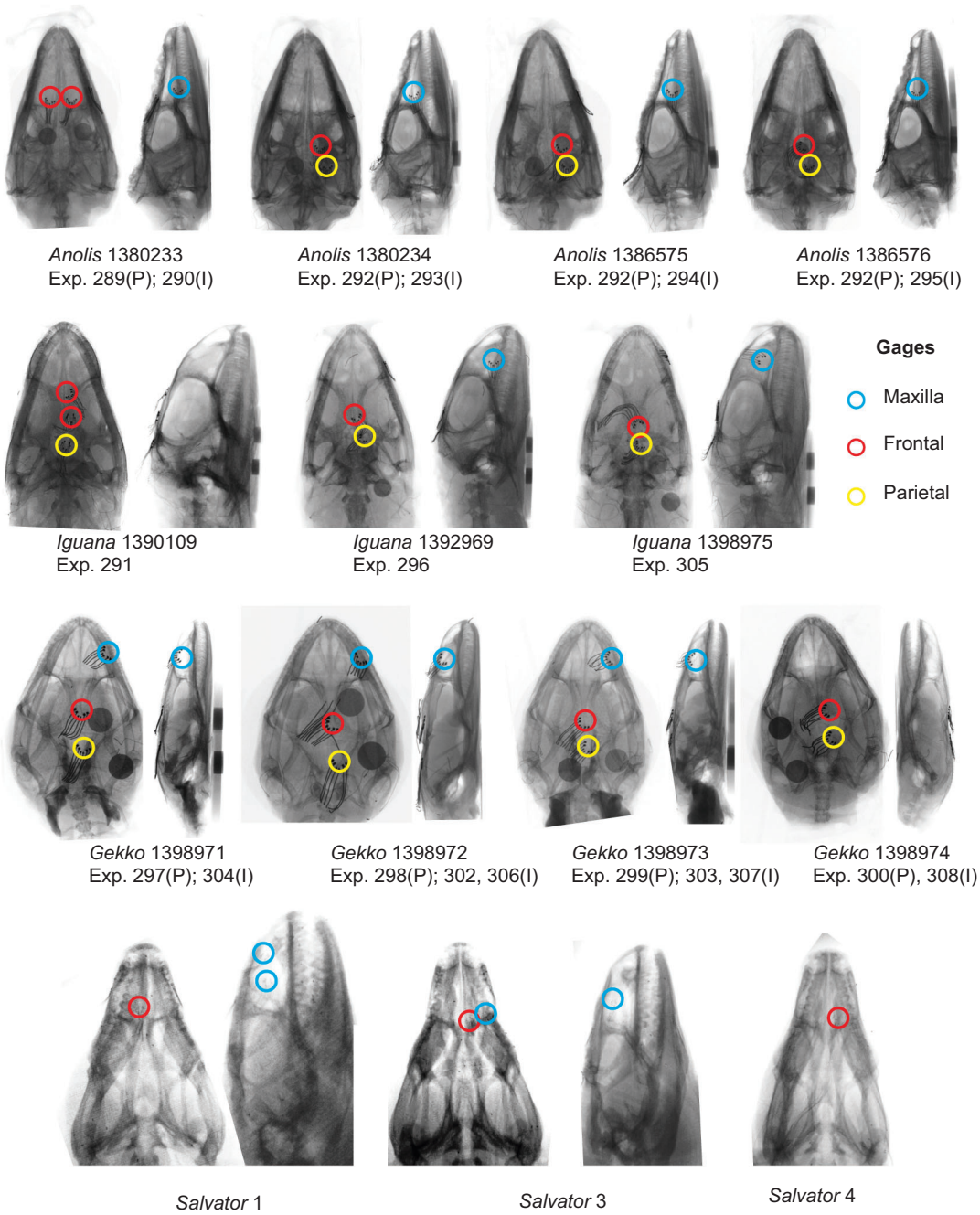

Fig. 1. Strain gauge locations in all experimental individuals. Radiographs of *Anolis equestris*, *Iguana iguana* and *Gekko gecko* were made post mortem; *Salvator merianae* radiographs are stills from videoradiographic sequences made during the recording sessions. Images not to scale: lower jaw lengths are given in Table 1; Table S1. Exp. indicates experiment numbers, before instrumentation (P) or after the animals were instrumented (I) with gauges and EMG electrodes. Dorsoventral views are from the top so that the animal's right is to the right.

and then across all factors, including species membership. Species membership was treated as a random factor, i.e. the set of species from the clade Lepidosauria was randomly chosen with respect to the hypotheses. Because of the wide diversity in size and cranial design, the long branches joining them, and the fact that only four species were sampled, the species were assumed to be independent, i.e. phylogeny was not taken into account. Bite point (seven 'levels' or locations: anterior midline; anterior, middle and posterior on left and right sides) was treated as a fixed factor because it has precisely defined locations replicated across individuals, because bite point effects apply only to those locations (assuming we sampled the tooth row densely enough), and because variation in bite point within each of the seven locations is random. Gauge location was also treated as a fixed factor, with three locations (parietal, frontal, maxilla) because similar/homologous sites were sampled across species, and because we assume there is no variation in gauge sites between individuals (Doncaster and Davey, 2007). The validity of this assumption depends on the level of precision one is prepared to accept: certainly, the gauges were placed on homologous

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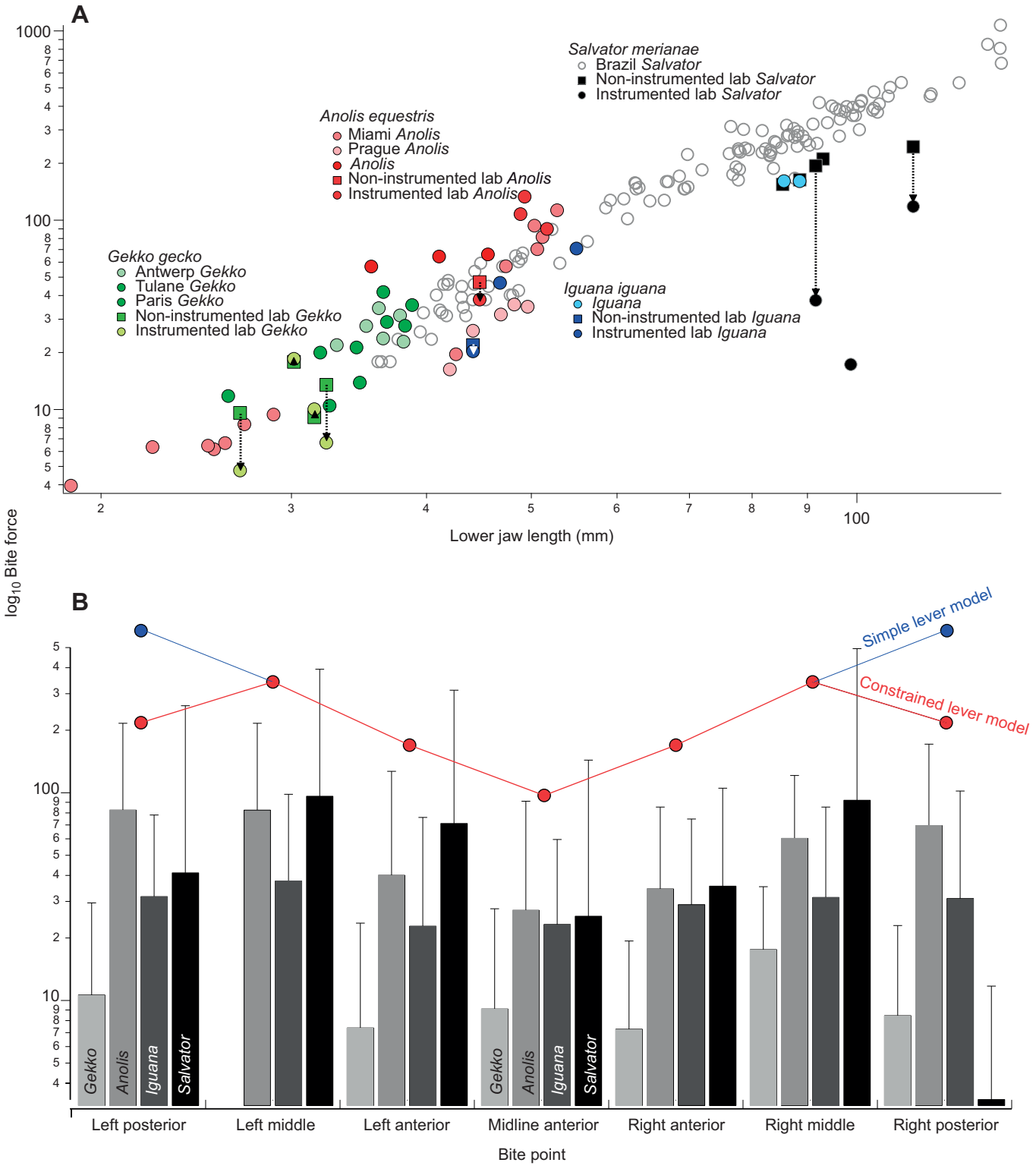

Fig. 2. Bite force data from experimental animals and conspecifics. (A) Maximum bite force (in N) at the anterior bite point plotted against lower jaw length, colored by species. Dashed arrows indicate intra-individual decreases in bite force associated with instrumentation. (B) Bite force (N) by species and bite point. Bars represent species means; whiskers are species maxima. Red (and blue) lines and markers illustrate the rank order of bite force predicted by the simple (red and blue) and constrained (red) lever models. Two models were run for the left and right sides separately: one predicting that posterior bite points would be associated with bite forces higher than middle bite points (as predicted by a simple lever model); and one predicting that posterior bite point forces would fall below middle but above anterior bite forces (constrained lever model).

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Table 1. Tests of instrumentation effects

			Pre-instrumentation bite force (N)				Post-instrumentation bite force (N)				Instrumentation effect		Significance	
Species	ID	Bite point	*n*	Mean	Max.	s.d.	*n*	Mean	Max.	s.d.	Absolute (N)	% Pre-instrumented	*F*	*P*
Anolis equestris	1386575	Anterior midline	4	36.31	47.07	7.894	13	26.39	38.07	8.959	−9.93	−27.33	27.451	0.000
Jaw length (mm)	44.79	L anterior	4	65.73	67.13	2.281	13	35.04	47.87	6.963	−30.69	−46.70		
		L middle	6	94.50	117.78	16.117	1	60.59	60.59		−33.91	−35.88		
		R anterior	3	62.95	65.38	2.143	8	32.76	47.45	15.187	−30.19	−47.96		
		R posterior	1	87.24	87.24		8	71.61	101.61	19.276	−15.63	−17.92		
Gekko gecko	1398971	Anterior midline	5	11.33	13.50	1.777	10	5.58	6.68	0.988	−5.75	−50.73	101.017	0.000
Jaw length (mm)	32.31	L anterior	6	15.16	17.67	1.850	21	7.11	12.70	2.659	−8.05	−53.08		
		L posterior	6	13.08	14.56	1.277	11	7.59	12.54	3.120	−5.50	−42.01		
		R anterior	3	11.95	13.79	1.861	7	6.78	17.05	1.789	−5.17	−43.28		
		R posterior	5	15.73	17.05	0.888	15	9.09	14.46	3.635	−6.65	−42.25		
Gekko gecko	1398972	L anterior	4	7.09	9.59	2.539	5	4.13	4.76	0.566	−2.96	−41.70	13.470	0.003
Jaw length (mm)	26.89	R posterior	1	9.21	9.21		5	4.82	5.19	0.326	−4.39	−47.68		
Gekko gecko	1398973	Anterior midline	4	12.10	17.84	6.568	6	15.33	18.52	2.510	3.23	26.65	0.238	0.628
Jaw length (mm)	30.16	L anterior	12	14.48	19.05	5.080	2	11.51	16.15	6.568	−2.97	−20.52		
		L posterior	10	19.00	24.96	4.512	6	14.50	18.84	3.387	−4.50	−23.67		
		R posterior	4	8.18	15.54	5.151	2	10.91	12.56	2.335	2.73	33.31		
Gekko gecko	1398974	Anterior midline	2	6.31	9.10	3.951	2	7.99	10.03	2.884	1.67	26.53	16.017	0.001
Jaw length (mm)	31.50	L posterior	5	18.91	20.74	1.128	6	13.41	16.35	1.918	−5.49	−29.06		
		R anterior	1	15.15	15.15		3	9.16	12.03	4.202	−5.99	−39.55		
		R posterior	1	13.43	13.43		5	9.85	11.45	1.178	−3.58	−26.65		
Iguana iguana	1398975	Anterior midline	2	20.44	21.89	2.051	1	20.28	20.28		−0.17	−0.81		
Jaw length (mm)	44.16	L anterior	10	18.99	34.26	12.346	7	23.46	34.40	11.613	4.46	23.50	0.117	0.735
		L posterior	8	19.98	30.86	8.217	2	29.04	29.14	0.148	9.06	45.34		
		R posterior	7	33.00	45.99	10.809	4	17.66	35.18	11.783	−15.34	−46.48		

F-values are result of tests of the effect of instrumentation based on linearly independent, pairwise comparisons among estimated marginal means. L, left; R, right.

bones in very similar places. The effects of this variation will emerge at the level of inter-individual variation within species. The degree to which the gauge sites are 'homologous' across species is debatable.

Both bite force and animal size (jaw length) are plausible covariates of inherent interest (Doncaster and Davey, 2007): bite force is a covariate of muscle forces and joint reaction forces; and jaw length is not only an indicator of the size of the skeleton resisting these forces but also a covariate of muscle size (Metzger and Herrel, 2005). The effects of kinesis and herbivory on cranial strain magnitude were estimated by comparing measured principal strain magnitudes and estimated marginal means from the ANOVA, which remove the effects of bite force (as a covariate) and bite point (random effect). Frontal and parietal gauge sites were compared to assess the effects of mesokinesis; strains from *I. iguana* were compared with those recorded from other, non-herbivorous taxa to test for effects of diet and its associated suite of skull modifications. Type III sums of squares were used because of the interactions between factors. Interaction terms were included in the models: interaction effects are represented with a multiplication sign (×), e.g. gauge location × species interaction effects. ANOVA were run in SPSS using the General Linear Models menu.

RESULTS

Bite force is affected by instrumentation, size and bite point

The bite forces of the *A. equestris* and *G. gecko* subjects (Table S2) fell within the range of values obtained from non-experimental animals but the bite forces of the captive *S. merianae* were lower than those of similarly sized semi-wild animals (Fig. 2A). Our experimental *I. iguana* individuals were smaller than the non-experimental animals for which data were available, making meaningful comparisons impossible. The effects of instrumentation (strain gauge and EMG electrode placement) on bite force magnitude were estimated in one *A. equestris*, one *I. iguana* and four *G. gecko* using a univariate ANOVA. After controlling for bite point, there were significant decreases in bite force associated with instrumentation in all individuals except one *G. gecko* and the *I. iguana* (Table 1).

In all species, an independent samples test rejected the null hypothesis that bite force is the same at all bite points in the tooth row (Table 2, Fig. 2B; Fig. S2). Jonckheere–Terpstra tests for an ordered difference in bite force medians revealed that there was a significant effect of bite point on bite forces on both sides in all species except for right bites in *G. gecko* and *I. iguana*. The constrained lever model predicted rank order of bite force magnitude on both left and right sides in *G. gecko* and *A. equestris*, and on left sides in *I. iguana* and *S. merianae*, whereas the simple lever model only predicted rank order of bite forces in *A. equestris* (both sides), *G. gecko* (left side) and *I. iguana* (left side). These results suggest that either model may apply to lepidosaurs, they confirm the effects of bite point on bite force (and plausibly bone strain magnitude) and they necessitate the inclusion of bite point as a factor in the ANOVA analyses reported below. Bivariate correlations between $\log_{10}$ bite force and $\log_{10}$ jaw length between individuals within species were not significant, but across all individuals and bite points both mean and maximum bite forces were correlated with jaw length at $P<0.007$ (r_{mean}, 0.825; r_{max}, 0.885) (Fig. S2). These analyses suggest that animal size and bite force were correlated, so bite force was included in our analyses as a covariate and jaw length was excluded.

Gauge location and bite point drive variance in cranial bone strain magnitude within individuals

Strain magnitude data for all individuals are given in Table S2. Within each individual, ANOVA was used to test for effects of gauge location, bite point and bite force on mean ε_1 and ε_2 magnitude separately. In one *G. gecko* (1398975), one *I. iguana*

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Table 2. Summary of Jonckheere–Terpstra tests for ordered differences in bite force

Species	Rank order model	Left bites	Right bites
Anolis equestris	Bite point effect	<0.001	<0.001
	Simple lever	<0.001	<0.001
	Constrained lever	<0.001	<0.001
Gekko gecko	Bite point effect	0.003	n.s.
	Simple lever	0.008	n.s.
	Constrained lever	0.008	0.007
Iguana iguana	Bite point effect	<0.000	n.s.
	Simple lever	<0.001	n.s.
	Constrained lever	<0.001	n.s.
Salvator merianae	Bite point effect	<0.000	0.008
	Simple lever	n.s.	n.s.
	Constrained lever	<0.001	n.s.

P-values indicate the probability that the null hypothesis for the rank order model is correct: bite point effect – that median bite forces are the same across bite points; simple lever – that bite forces are the same across simple lever model ranks; constrained lever – that bite forces are the same across constrained lever model ranks.

(1392969), two *A. equestris* (1386575, 1386576) and one *S. merianae* (no. 3), bite force was not a significant covariate with either mean ε_1 or mean ε_2 magnitude. In two *S. merianae* (nos 1 and 4), bite force was not a significant covariate with mean ε_2 magnitude but it was with mean ε_1 magnitude. In the rest of the individuals (two *A. equestris*, two *I. iguana*, two *G. gecko*), bite force was a significant covariate with both mean ε_1 and mean ε_2 magnitude. In most individuals, bite point had a significant impact on both mean ε_1 and mean ε_2 magnitude, the exceptions being two *S. merianae*, one *A. equestris* and one *G. gecko*, in which bite point affected mean ε_1 but not mean ε_2 magnitude. In most individuals, gauge location also had a significant effect on both mean ε_1 and mean ε_2 magnitude, and in all individuals it had an effect on either mean ε_1 or mean ε_2 magnitude. Bite point×gauge site interactions were significant in all individuals except those in which gauge site or bite point effects alone were not significant.

In summary, within individuals, the most consistent determinants of variance in cranial bone strain magnitude were strain gauge location, bite point and their interaction, with the importance of bite force varying between individuals. These results reveal that inter-site variance in bone strain magnitude – strain gradient – was present in all the individuals studied here, and the nature of this gradient varied with bite point.

Bite force drives variance in cranial bone strain magnitude between individuals within species

Within each species, ANOVA were used to model the effects of individual, gauge location, bite point and bite force on mean ε_1 and ε_2 magnitude separately. Within all species, individual was not a significant factor and bite force was a significant covariate of both mean ε_1 and mean ε_2 magnitude. In *G. gecko* and *I. iguana*, gauge location did not affect inter-individual variation in either mean ε_1 or mean ε_2 magnitude, and in *A. equestris* and *S. merianae*, gauge location only had a significant effect on inter-individual variation in mean ε_1 magnitude. Bite point had a significant effect only on ε_1 magnitude in *S. merianae* and ε_2 magnitude in *A. equestris*. Thus, the most consistent determinant of variance in cranial bone strain magnitude within species was bite force, not individual, gauge location or bite point. These results reveal that the strain gradients documented in the previous section are consistent across individuals within species.

Species membership, bite force and diet, but not kinesis, drive variance in cranial bone strain magnitude between species

ANOVA was used to model the effects of species membership, bite force (covariate), bite point and gauge location on mean ε_1 and ε_2 magnitude (Table 3). Mean ε_1 and ε_2 magnitude was significantly impacted by species membership, bite force, gauge location×species interaction effects, and bite point×gauge location×species interaction effects. ε_1 magnitude was also impacted by bite point×gauge interactions. Independently, bite

Table 3. ANOVA models of determinants of $\log_{10}$ principal strain magnitude in *G. gecko*, *A. equestris*, *I. iguana* and *S. merianae*

		$\log_{10}\varepsilon_1$						$\log_{10}\varepsilon_2$					
Source		Type III SoS	*f*	Mean square	*F*	*P*	ηp^2	Type III SoS	*f*	Mean square	*F*	*P*	ηp^2
Intercept	Hypothesis	278.13	1.00	278.13	204.648	0.000	0.984	183.09	1.00	183.09	48.797	0.005	0.939
	Error	4.49	3.31	1.36				11.84	3.16	3.75			
$\log_{10}$ Bite force	Hypothesis	10.63	1.00	10.63	110.294	0.000	0.079	13.25	1.00	13.25	114.254	0.000	0.090
	Error	123.61	1283.00	0.10				133.92	1155.00	0.12			
Bite point	Hypothesis	3.67	6.00	0.61	2.455	0.053	0.376	5.11	6.00	0.85	1.157	0.395	0.391
	Error	6.10	24.50	0.25				7.95	10.81	0.74			
Gauge	Hypothesis	21.10	5.00	4.22	2.704	0.149	0.729	31.88	3.00	10.63	2.484	0.202	0.655
	Error	7.85	5.03	1.56				16.81	3.93	4.28			
Species	Hypothesis	20.78	3.00	6.93	5.479	0.045	0.758	54.00	3.00	18.00	8.523	0.023	0.843
	Error	6.64	5.25	1.26				10.06	4.76	2.11			
Bite point×gauge	Hypothesis	24.42	25.00	0.98	2.393	0.018	0.712	17.07	18.00	0.95	1.388	0.236	0.548
	Error	9.88	24.20	0.41				14.10	20.63	0.68			
Bite point×species	Hypothesis	6.80	17.00	0.40	1.017	0.474	0.411	8.88	11.00	0.81	1.244	0.320	0.393
	Error	9.74	24.78	0.39				13.68	21.08	0.65			
Gauge×species	Hypothesis	9.38	5.00	1.88	6.332	0.000	0.505	17.33	4.00	4.33	9.377	0.000	0.597
	Error	9.18	31.00	0.30				11.71	25.35	0.46			
Bite point×gauge×species	Hypothesis	10.67	22.00	0.48	5.032	0	0.079	14.80	20.00	0.74	6.383	0.000	0.100
	Error	123.61	1283.00	0.10				133.92	1155.00	0.12			

Species, random factor; bite point and gauge location, fixed factors; bite force, covariate. ε_1 and ε_2, maximum and minimum principal strain; SoS, sum of squares; ηp^2, partial eta squared.

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Table 4. Species-level estimated marginal means of principal strain magnitude across all gauge sites from ANOVA in Table 3

	ε_1 estimated marginal means					ε_2 estimated marginal means				
Species	Mean (με)	$\log_{10}$ Mean[a]	s.d.	Lower bound	Upper bound	Mean (με)	$\log_{10}$ Mean[b]	s.d.	Lower bound	Upper bound
Anolis equestris	360.6	2.557	0.031	2.496	2.619	463.4	2.666	0.035	2.598	2.734
Gekko gecko	457.1	2.66	0.036	2.590	2.730	482.0	2.683	0.039	2.606	2.760
Iguana iguana	157.8	2.198*	0.016	2.167	2.230	90.6	1.957‡	0.018	1.922	1.991
Salvator merianae	452.9	2.656	0.039	2.580	2.733	157.0	2.196‡	0.139	1.923	2.469

[a]Covariates evaluated at $\log_{10}$ bite force=1.36. *Pairwise comparisons: *I.iguana* ε_1 estimated marginal mean differs from all others at $P<0.001$.
[b]Covariates evaluated at $\log_{10}$ bite force=1.33. ‡Pairwise comparisons: *I. iguana* and *S. merianae* ε_2 estimated marginal means differ from those of *A. equestris* and *G. gecko* at $P<0.001$.

point and gauge location did not significantly affect strain magnitude.

The estimated marginal means from this ANOVA (Table 4) falsify the hypothesis that our herbivorous species (*I. iguana*) experiences higher strains than the other taxa. Indeed, the reverse is true. Pairwise comparisons reveal that, controlling for bite force and bite point, *I. iguana* has significantly lower, not higher, estimated marginal mean ε_1 strain magnitudes than the other three species, and significantly lower mean ε_2 strain magnitudes than *A. equestris* and *G. gecko*. Nor do the data support the hypothesis that absence of the supratemporal and postorbital bars is associated with higher cranial strain magnitudes or the presence of kinesis is associated with lower strain magnitudes: *G. gecko* (highly kinetic) and *A. equestris* (akinetic) do not differ from each other in estimated principal strain marginal means, but both experience significantly higher estimated mean ε_2 strain magnitudes than *I. iguana* (akinetic) and *S. merianae* (minimally streptostylic and not measurably mesokinetic).

The effect of mesokinesis on cranial strain magnitude was also tested by comparing marginal means from an ANOVA of principal strain magnitudes at frontal and parietal gauge sites, accounting for the effects of bite force (as a covariate) and bite point (random effect) (Table 5). There was no effect of the presence of mesokinesis on the ratios of principal strains at the frontal gauge site to that at the parietal gauge site. In the akinetic *I. iguana* – the largest species – principal strains in the frontal bone were 9–14 times larger than those in the parietal bone, whereas in both the akinetic *A. equestris* and the highly kinetic *G. gecko*, strains in the frontal bone were 1.5–2.6 times larger than those in the parietal bone. Hence, principal strain magnitudes are always several times higher in the frontal than in the parietal, regardless of the presence or absence of mesokinesis between frontal and parietal bones.

DISCUSSION

The data presented here have some limitations: not all gauge combinations were recorded from all animals, manual restraint of *S. merianae* appears to have deformed the cranium, requiring us to exclude some of the data; the data are from transducer biting, not feeding; and instrumentation may have resulted in lower bite forces in some individuals. Nevertheless, these data represent a significant advance in our understanding of *in vivo* cranial function of lizards during feeding.

Bite force data may corroborate the constrained lever model in lepidosaurs

The distribution of bite forces across bite points in these lepidosaurs is explained at least as well, and in some cases better, by the constrained lever model than by the simple lever model. The constrained lever model proposes that balancing side (non-biting side) muscle activity must be reduced during biting at the most posterior bite points in order to avoid tensile forces in the biting side jaw joint (Druzinsky and Greaves, 1979; Greaves, 1978; Spencer, 1995; Spencer, 1998; Thompson et al., 2003). This model predicts lower bite forces at the most posterior (more distal) teeth than in the middle of the postcanine tooth row, a prediction broadly consistent with the data presented here. Corroboration of this hypothesis in lepidosaurs would suggest that, as in mammals, models of muscle recruitment during biting by lepidosaurs should take into account effects of both bite point and joint reaction forces (Curtis et al., 2010; Shi et al., 2012). It also implies that, if the constrained lever model applies broadly across amniotes, then sensorimotor mechanisms modulating bite point-specific muscle recruitment might also be similarly distributed. In mammals, sensory afferents from muscle spindles and the periodontal ligament connecting the teeth to the mandible are essential for feed-forward and feed-back

Table 5. Estimated marginal means from ANOVA of principal strain magnitude at frontal and parietal gauge sites across *G. gecko*, *A. equestris* and *I. iguana*

Species	Principal strain	Gauge sites	Mean (με)	Frontal:parietal ratio	Mean ($\log_{10}$)	s.e.	Lower bound	Upper bound
Gekko gecko	ε_1	Frontal	727.8	2.1	2.86	0.064	2.737	2.987
		Parietal	343.6		2.54	0.064	2.411	2.661
	ε_2	Frontal	–709.6	2.6	2.85	0.072	2.710	2.992
		Parietal	–273.5		2.44	0.072	2.297	2.578
Anolis equestris	ε_1	Frontal	445.7	1.8	2.65	0.055	2.540	2.757
		Parietal	252.3		2.40	0.063	2.278	2.525
	ε_2	Frontal	–538.3	1.5	2.73	0.062	2.609	2.853
		Parietal	–356.5		2.55	0.071	2.413	2.691
Iguana iguana	ε_1	Frontal	389.9	9.1	2.59	0.027	2.538	2.645
		Parietal	42.7		1.63	0.028	1.576	1.685
	ε_2	Frontal	–306.9	14.1	2.49	0.031	2.427	2.548
		Parietal	–21.8		1.34	0.031	1.277	1.398

Species, random factor; bite point and gauge location, fixed factors; bite force, covariate.

regulation of bite force, respectively (Komuro et al., 2001; Ottenhoff et al., 1992a, b; Trulsson, 2006): the roles of spindle afferents and afferents in intra-cranial, intra-mandibular and craniomandibular joints for modulation of bite force in lepidosaurs and *Caiman* (McIntosh et al., 2002) remain to be evaluated.

An alternative explanation for the decrease in bite force at the most posterior bite points is the effect of gape distance on the part of the jaw elevator muscle length–tension curves. The bite force transducer plates were a constant distance apart in all trials, so that, depending on the axis of rotation of the jaw, or jaw/quadrate system, at more posterior bite points the jaw elevator muscles may have been most highly stretched. It is possible that this stretched the muscles beyond the optimal region of their length–tension curves, resulting in lower maximum bite forces. Future studies of the effects of bite point on bite force should control for this effect to determine whether gape effects or the constrained lever model best explain the lower bite forces at the most posterior bite points. Investigation of the location of the axis of rotation of lizards would be of interest in this regard (cf. Iriarte-Diaz et al., 2017).

Determinants of strain magnitude in lepidosaur crania

We asked whether, when bite force and bite point are statistically controlled, lizards show strain gradients – variation in strain magnitude – across the cranium during biting. If lizard crania were optimized for maximum strength during feeding with minimum material, then strain magnitude would be fairly uniform across the cranium during feeding, and the crania of all species would experience similar strain magnitudes during feeding. Of course, biting on different regions of the toothrow must necessarily be associated with different strain magnitudes in different parts of the cranium because torques and compressive, tensile and shearing forces vary with bite point. However, once these factors are taken into account (by eliciting powerful bites across a range of bite locations *in vivo*), if the crania are optimized for maximum strength during feeding with minimum material, there should not be strain gradients – differences in strain magnitude across gauge locations. In fact, within individuals, the most consistent determinants of variance in bone strain magnitude during transducer biting are strain gauge location, bite point and gauge location×bite force interactions: cranial bone strain magnitudes vary across the cranium, i.e. there are strain 'gradients'.

We also asked whether individuals in the same species share common patterns of variation in strain magnitude across the cranium when bite force and bite point are statistically controlled. Indeed, between individuals, within species, the most consistent determinant of variance in cranial bone strain magnitudes was bite force, not gauge location, individual or bite point. The importance of bite force in driving differences in bone strain magnitude between individuals (and species, see below) is not surprising: higher bite forces exert larger compressive, tensile and shearing forces at gauge sites, as well as larger bending and twisting moments about the gauge sites, and must also be associated with higher muscle and joint reaction forces. Moreover, the fact that gauge location is not a significant determinant of inter-individual variation in strain magnitude suggests that the patterns of strain recorded from these individuals – including the variation between gauge locations – are consistent representations of species-specific patterns.

Finally, we asked what effect diet, species-specific cranial morphology and cranial kinesis have on variation in strain magnitude in lizard crania, when bite force and bite point are controlled. Between species, *in vivo* bone strain magnitudes in the cranium were significantly impacted by bite force and species membership independently, as well as by gauge location×species, gauge location×bite point (ε_1 magnitude), and gauge location×bite point×species interaction effects. Bite point and gauge location did not significantly affect interspecific variation in strain magnitude independent of these interaction effects. Species-level differences in cranial strain magnitude (independent of bite force) argue against the idea that selection designs all lizard crania to a common optimality criterion of maximum strength during feeding for minimum material. This interspecific variation may reflect selection for different cranial morphology:feeding function relationships – different cranial 'designs' – but what the specific performance criteria might be is not clear. *Iguana iguana* displayed lower overall ε_1 strain magnitudes than those of the other three species, and *I. iguana* and *S. merianae* displayed lower ε_2 strain magnitudes than those of *A. equestris* and *G. gecko* (Table 4). The only other species-level effects were species×bite force and species×bite force×bite point interaction effects.

Possible species-level effects on strain magnitude include the presence of supratemporal and postorbital bars, as well as varying degrees of kinesis. Previous bone strain studies of kinesis in *Varanus exanthematicus* used single-element gauges to measure strain across the top of the frontoparietal suture (mesokinetic hinge joint) and rosette gauges to record strain of several hundred microstrain from the frontal bone rostral to the joint during feeding sequences (Smith and Hylander, 1985). In the present study, strains recorded from the frontal and parietal bones on either side of the frontoparietal suture were recorded during transducer biting, not feeding. Strain magnitudes were uniformly higher in the frontal than in the parietal bone, often by two orders of magnitude, regardless of the presence or absence of mesokinesis, or supratemporal or postorbital bars (Table 5). This suggests that the distribution of strain magnitudes in the roof of the lepidosaur cranium is not significantly different between those animals with and without mesokinesis, arguing against the hypothesis that kinetic crania have lower frontal and parietal stress and strain magnitudes.

Another possible source of interspecific variation in strain magnitude is dietary effects. Herbivorous lepidosaurs have lighter skulls, shorter snouts, larger jaw elevator muscles and greater bite forces (Herrel et al., 2007; Metzger and Herrel, 2005; Stayton, 2006). We hypothesized that this might predict higher strains in the crania of our herbivorous species – *I. iguana* – than the other species, but the opposite was true: *I. iguana* had the lowest strains of all the species. Low strains may represent increased resistance to fatigue effects in *I. iguana* crania, as suggested for *Uromastyx* (Porro et al., 2014), a hypothesis that would be supported if *I. iguana* are shown to chew more frequently than non-herbivorous lepidosaurs. Fatigue effects explain strain magnitudes in a wide variety of situations (reviewed by Ross and Metzger, 2004), so their importance for lepidosaur cranial form would not be surprising. Interactions between diet and overall size (see below) would not be unexpected – herbivorous lizards might both chew more and be larger. However, size-related effects on diet and feeding behavior, including the number of chewing cycles per day, are certainly complex in mammals (Ross et al., 2009), and better data are needed before fatigue effects on lizard cranial form can be advocated.

Salvator merianae and *I. iguana* are larger than *A. equestris* and *G. gecko*, and it is possible that overall cranial size impacts strain magnitude in the cranium. Ravosa et al. (2000a) noted a negative allometry of peak principal strains in pairwise comparisons within cercopithecine primates (*Macaca* and *Papio*) and galagos (*Otolemur crassicaudatus* and *Otolemur garnetti*), a pattern replicated in the lemurids *Eulemur fulvus* and *Varecia variegata*

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Table 6. Summary of *in vivo* cranial bone strain data from tetrapod frontal bones

				ε_1 (με)		ε_2 (με)		γ_{max} (με)	
Species (reference)	Individual	Gauge location	Behavior	Mean	Max.	Mean	Max.	Mean	Max.
Papio anubis	1	Dorsal interorbital	Mastication	161	215	–51	–68	212	283
(Hylander et al., 1991a,b)			Incision	167	230	–42	–60	209	290
Macaca fascicularis	5A	Dorsal interorbital	Mastication	292	462	–119	–189	411	651
(Hylander et al., 1991a,b)			Incision	235	298	–68	–88	303	386
	6	Dorsal interorbital	Mastication	148	210	–37	–52	185	263
			Incision	216	311	–49	–75	266	386
	2A	Dorsal interorbital	Mastication	133	227	–72	–120	204	347
			Incision	189	270	–70	–98	259	369
	4	Dorsal interorbital	Mastication	51	91	–21	–35	72	126
			Incision	62	86	–23	–32	85	118
Aotus trivirgatus	1	Dorsal interorbital	Mastication	168	356	–145	–307	313	654
(Ross and Hylander, 1996)			Incision	78	105	–177	–245	255	346
	2	Dorsal interorbital	Mastication	35	114	–105	–194	140	248
			Incision	81	89	–168	–340	216	425
Otolemur garnetti	1	Dorsal interorbital	Mastication	361	587	–383	–634	745	1221
Otolemur crassicaudatus	2	Dorsal interorbital	Mastication	183	378	–315	–698	498	1076
(Ravosa et al., 2000a,b)	3	Dorsal interorbital	Mastication	312	354	–209	–235	642	918
Eulemur fulvus	Ba (exp 76)	Dorsal interorbital	Mastication	62	110	–69	–147	130	256
(C.F.R., unpublished)	Be (exp 78)	Dorsal interorbital	Mastication	34	104	–40	–145	73	225
	Ma (exp 79)	Dorsal interorbital	Mastication	44	109	–53	–136	96	237
Varecia variegata	D (Exp 97)	Dorsal interorbital	Mastication	23	88	–44	–156	65	242
(C.F.R., unpublished)	B (exp 94)	Dorsal interorbital	Mastication	21	39	–137	–369	148	393
Sus scrofa	147	Frontal	Mastication	124		–74		198	
(Herring and Teng, 2000)	154	Frontal	Mastication	21		–27		48	
	157	Frontal	Mastication	47		–58		105	
	158	Frontal	Mastication	52		–31		83	
	162	Frontal	Mastication	18		–25		43	
	164	Frontal	Mastication	30		–66		96	
	165	Frontal	Mastication	54		–69		123	
Procavia capensis	H2	Dorsal interorbital	Mastication	273		–159		432	
(Lieberman et al., 2004)	H3	Dorsal interorbital	Mastication	16		–231		247	
Ovis[3,4]	1	Frontal	Mastication	515	635	–469	–575	984	1210
(Thomason et al., 2001)	2	Frontal	Mastication	271	356	–115	–358	386	714
	3	Frontal	Mastication	351	492	–235	–339	586	831
	4	Frontal	Mastication	412	504	–325	–412	737	916
	5	Frontal	Mastication	227	345	–210	–312	437	657
	2	Maxilla	Mastication	711	955	–29	–147	740	1102
	3	Maxilla	Mastication	204	257	–170	–379	374	636
	4	Maxilla	Mastication	479	593	–32	–49	511	642
	5	Maxilla	Mastication	280	424	–96	–143	376	567
Alligator	e64	Frontal	Biting	631	1388	–216	–391	858	1778
(Metzger et al., 2005)									
Anolis equestris	1380233	Frontal	Biting	379	582	–306	–939	681	1368
(This study)	1380234	Frontal	Biting	993	2091	–682	–1386	1675	3458
	1386575	Frontal	Biting	475	742	–863	–1266	1307	1762
	1386576	Frontal	Biting	842	1321	–1195	–2303	2036	3624
Gekko gecko	1398971	Frontal	Biting	739	1887	–692	–1460	1431	3246
(This study)	1398972	Frontal	Biting	662	817	–634	–808	1295	1550
	1398973	Frontal	Biting	424	575	–419	–619	840	1103
	1398974	Frontal	Biting	940	2063	–842	–1208	1774	2987
Iguana	1390109	Frontal	Biting	457	1188	–384	–930	841	2037
(This study)	1392969	Frontal	Biting	517	845	–492	–822	1008	1393
	1398975	Frontal	Biting	451	886	–295	–572	744	1130
Salvator merianae	1	Frontal	Biting	1004	1998	–278	–809	1256	2807
(This study)	3	Frontal	Biting	454	777	–218	–359	668	1135
	4	Frontal	Biting	231	521	–287	–1226	509	1738

[1]For each individual we used the experiment with the largest values; within experiments, the cycle with the largest value of ε_1 was chosen. [2]Strain magnitudes in these regions were even lower than those in the dorsal interorbital region. [3]Row with the largest ε_1 or ε_2 value. [4]Thomason et al. (2001) used single element gauges, so mandibular ε_1 and ε_2 underestimate principal strains.

(Ross, 2008) (C.F.R., unpublished data). Allometry of circumorbital strain magnitudes could be due to allometry of the external forces acting on the cranium, allometry in optimality criteria (i.e. differences in the optimality criteria influencing form at different body sizes) or allometry of the extent to which optimality criteria actually matter for cranial design (Ross and Metzger, 2004). Choosing between these explanations will require more data than are currently available, especially from studies of taxa with a wider range and finer gradation of diets.

Notably, gauge location and bite point do not drive interspecific variation in cranial strain magnitude independently of interaction effects with bite point and/or species membership, and bite point does not impact strain magnitude variation independently of gauge location. This suggests that strain magnitude is influenced by species-specific factors other than cranial morphology, such as patterns of muscle recruitment and associated joint reaction forces (Porro et al., 2011). Analysis of EMG data collected during these experiments will be of interest.

Strain magnitudes in the lepidosaur parietal and frontal are similar to or higher than those in the maxilla

Our results reveal that the strain magnitudes in the frontal and parietal bones of the lizards studied here are usually higher than those in the maxilla. The presence of high biting strains in the parietal and frontal bones indirectly overlying the braincase suggests that the morphology (size and shape) of these bones may be more optimized for maximum strength with minimum material during feeding than are the bones of the braincase in mammals (Table 6). Available data indicate that the calvarial bones of mammals experience lower strains than the facial skeleton (Behrents et al., 1978; Herring and Teng, 2000; Thomason et al., 2001), suggesting that the calvarial strength needed to protect their relatively enlarged brains against infrequent impact loads exceeds that necessary for a feeding system optimized for maximum strength with minimum material. In support of this hypothesis, it is noteworthy that mammals with relatively smaller brains (*Ovis*) experience higher strain magnitudes in the calvaria during feeding (ε_1 up to 635 με) (Thomason et al., 2001) than primates (Behrents et al., 1978), reflecting an increased influence of feeding system design criteria on the calvaria.

The *Alligator* cranium may also be less well optimized for maximum strength with minimum material, as some parts of the cranium appear to experience higher strains than others (Metzger et al., 2005; Ross and Metzger, 2004). It is possible that the aquatic habits of *Alligator* alleviate selective pressure to minimize skull mass, or maybe the need to quickly move the head laterally to capture prey in an aquatic environment imposes other design constraints on the cranium (Busbey, 1995; Metzger et al., 2005). Certainly, the cross-sectional shape of the snout is not optimal for resisting bending moments associated with high-magnitude bite forces, suggesting that the mechanical needs of moving through an aquatic environment trump those associated with resisting biting stresses (Erickson et al., 2012). To compensate for this sub-optimal cross-sectional shape, alligators exhibit an extensive hard palate and overlapping scarf joints between some of the bones (Busbey, 1989, 1995). However, it is important to note that the majority of the cranial bone strain data from *Alligator* (Metzger et al., 2005; Porro et al., 2011) and lizards (presented here) were collected during transducer biting, whereas the majority of mammal data were collected during feeding. Strong conclusions about the biological significance of differences in strain magnitude between these clades must await better controlled experiments.

Conclusions

In vivo bone strain data from the crania of four species of lizards reveal that, as in mammals and alligators, bone strain magnitude varies across the cranium of lepidosaurs. Although the mammal data were collected primarily during feeding, and those from alligators and lizards were mostly collected during transducer biting, the regional variability in bone strain magnitude indicates that cranial design in tetrapod skulls is not dominated by the criterion of maximum strength with minimum material during feeding. The data presented here also suggest that there are species-specific patterns of variation in cranial bone strain magnitude that are not obviously related to patterns of cranial kinesis, or to the presence or absence of postorbital and supratemporal bars, and future work should consider these features in the context of overall cranial architecture. Strain magnitudes are larger in the frontal than in the parietal bone, and are usually larger in the frontal bone than in the maxilla. This may reflect differences in optimality criteria between lizard and mammal crania, but more data are needed to confirm these clade-level differences.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.F.R., L.B.P., A.H., S.E.E., M.J.F.; Methodology: C.F.R., L.B.P., A.H., S.E.E., M.J.F.; Software: C.F.R.; Validation: C.F.R.; Formal analysis: C.F.R.; Investigation: C.F.R., L.B.P., A.H., S.E.E., M.J.F.; Resources: C.F.R., A.H., S.E.E., M.J.F.; Data curation: C.F.R., M.J.F.; Writing - original draft: C.F.R.; Writing - review & editing: C.F.R., L.B.P., A.H., S.E.E., M.J.F.; Visualization: C.F.R., L.B.P.; Supervision: C.F.R., S.E.E., M.J.F.; Project administration: C.F.R., S.E.E., M.J.F.; Funding acquisition: S.E.E., M.J.F.

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Supplementary information

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