Quantifying the effect of gape and morphology on bite force: biomechanical modelling and *in vivo* measurements in bats

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Summary

1. Maximum bite force is an important metric of feeding performance that defines the dietary ecology of many vertebrates.

2. In mammals, theoretical analyses and empirical studies suggest a trade-off between maximum bite force and gape at behavioural and evolutionary scales; *in vivo* bite force is expected to decrease at wide gapes, and cranial morphologies that enable high mechanical advantage are thought to have a lower ability to generate high bite forces at wide gapes, and vice versa. However, very few studies have confirmed these relationships in free-ranging mammals.

3. This study uses an ecologically diverse sample of bats to document the variation in bite force with respect to gape angle, and applies three-dimensional models of the feeding apparatus to identify the major morphological and biomechanical predictors of the gape-bite force relationship.

4. *In vivo* and model data corroborated that bite force decreases significantly at wide gapes across species, but there is substantial intraspecific variation in the data obtained from live bats. Results from biomechanical models, analysed within a phylogenetic framework, revealed that species with larger temporalis muscles, higher temporalis stretch factors and high mechanical advantages experience a steeper reduction in bite force with increasing gape. These trends are illustrated by short-faced durophagous frugivores.

5. The results from this study suggest that gape-mediated changes in bite force can be explained both by behavioural effects and cranial morphology, and that these links are relevant for functional analyses of mammal dietary ecology.

Key-words: bite performance, mechanical advantage, Phyllostomidae, skull

Introduction

Maximum bite force is a metric of whole-organism performance that is tightly linked to many aspects of the ecology of vertebrates, including diet (Verwaijen, Van Damme & Herrel 2002; Aguirre *et al.* 2003; Nogueira, Peracchi & Monteiro 2009; Santana, Dumont & Davis 2010; Becerra *et al.* 2014; Meyers & Irschick 2015), mating success (Lappin & Husak 2005), and roosting and burrowing habits (Dechmann, Santana & Dumont 2009; Becerra *et al.* 2014). In mammals, there are marked differences in maximum bite force among species that specialize on food items with different mechanical properties (Binder & Valkenburgh 2000; Norconk *et al.* 2009), and this variation in performance may enable dietary niche separation

within ecological communities (Aguirre *et al.* 2002). Interspecific differences in bite force are the product of evolutionary specializations of the feeding apparatus, such as changes in jaw muscle attachments, tension and cross-sectional areas (Herrel *et al.* 2008; Perry, Hartstone-Rose & Wall 2011; Hartstone-Rose, Perry & Morrow 2012), and skull and mandible shape (Ellis, Thomason & Kebreab 2009; Nogueira, Peracchi & Monteiro 2009; Slater, Dumont & Van Valkenburgh 2009; Cornette *et al.* 2013). Within species, or during individual feeding events, further variation in bite force can be introduced via differential recruitment of jaw adductors (De Gueldre & De Vree 1988; Spencer 1998; Ross *et al.* 2005), modifications in the position of the bite along the tooth row (Greaves 1991; Dumont & Herrel 2003; Clausen *et al.* 2008; Santana & Dumont 2009) and, importantly, changes in the degree of mandibular opening, or gape angle (Paphangkorakit &

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Osborn 1997; Dumont & Herrel 2003; Williams, Peiffer & Ford 2009; Ross & Iriarte-Diaz 2014).

The relationship between maximum bite force and gape angle is of particular interest for ecomorphology because changes in these performance traits can determine differential access to food items along a gradient of physical properties (i.e., hardness and size). For example, the ability of mammalian carnivores to generate large bite forces at wide gapes allows them to capture and kill large vertebrate prey (Christiansen & Adolfssen 2005), whereas durophagous fruit bats and ungulates can generate large bite forces at low gapes for maceration of plant material (Herring & Herring 1974; Dumont *et al.* 2009). Modelling and experimental studies suggest that there is a trade-off between maximum bite force and gape at both behavioural and evolutionary scales; bite force decreases as a mammal bites at gapes that are wider than the optimum due to an increase in muscle stretching (Herring & Herring 1974; Paphangkorakit & Osborn 1997; Dumont & Herrel 2003; Williams, Peiffer & Ford 2009), and morphologies that enable high mechanical advantage have decreased ability to generate high bite forces at wide gapes (Herring & Herring 1974; Dumont *et al.* 2009). Specifically, morphological specializations that maximize bite force include shorter jaws, jaw adductors with origins and insertions that confer a greater force output to input ratio, shorter and more pinnate muscle fibres, and larger muscle physiological cross-sectional areas (Herrel *et al.* 2008; Santana, Dumont & Davis 2010; Perry, Hartstone-Rose & Wall 2011; Hartstone-Rose, Perry & Morrow 2012). Conversely, cranial specializations for wider gapes include longer jaws, jaw adductors with longer muscle fibres, and larger masseter origin-insertion ratios (Herring & Herring 1974; Smith 1984; Taylor & Vinyard 2004).

Very few studies have investigated the association between gape and maximum bite force in free-ranging mammals biting voluntarily (but see Dumont & Herrel 2003; Williams, Peiffer & Ford 2009), and even fewer have matched *in vivo* bite force measurements with modelling tools to determine how behavioural modulation and cranial specialization underlie these relationships. As a consequence, the combined effects of these factors on organismal performance and dietary ecology remain unclear. Here, I capitalize on the extraordinary ecological and morphological diversity of bats to document their variation in bite force with respect to changes in gape across a wide array of diets, and apply three-dimensional models of the feeding apparatus (Davis *et al.* 2010) to identify morphological and biomechanical traits affecting the magnitude of gape-driven bite force changes. Bats constitute an excellent system for this research because they possess highly diverse cranial morphologies and performance abilities within the relatively simple anatomical and functional template of mammals (Freeman 1998). This study focuses on Neotropical leaf-nosed bats (family Phyllostomidae), an adaptive radiation that has been relatively

well-studied in terms of their dietary ecology (e.g., Giannini & Kalko 2005; Da Silva, Gaona & Medellin 2008; Rex *et al.* 2011), cranial functional morphology (Freeman 2000; Santana, Dumont & Davis 2010; Dumont *et al.* 2014) and feeding behaviour plasticity (Dumont 1999; Santana & Dumont 2009). Phyllostomid bats include insectivorous, frugivorous, nectarivorous, sanguivorous, carnivorous and omnivorous species, and their diets encompass a wide range of physical properties (e.g., size and hardness) among and within dietary guilds (Gardner 1977; Dumont 2003). Cranial specializations in phyllostomids seem to match this gradient of food physical properties; durophagous species possess skulls and jaw adductors with a high mechanical advantage, whereas species that feed on liquid diets have a low mechanical advantage and reduced jaw musculature (Herrel *et al.* 2008; Santana, Dumont & Davis 2010). Frugivorous phyllostomids exhibit high levels of plasticity in their biting behaviours when compared to insectivorous and omnivorous species (Santana & Dumont 2009), but there have not been any quantifications of how gape varies during feeding events within and across species.

With a data set spanning a total of 28 bat species, I use comparative methods to test the hypothesis that bite force production is behaviourally influenced by gape, and that dietary specializations in skull morphology affect the magnitude of this gape-bite force relationship. I predict that, within a species, bite force will decrease at wide gapes (e.g., Herring & Herring 1974; Dumont & Herrel 2003), and the gape-driven reduction in bite force will be larger for species that have skull morphologies with a high mechanical advantage (durophagous species such as hard-fruit and vertebrate-eating phyllostomids; Dumont *et al.* 2009; Santana, Dumont & Davis 2010). Reductions in bite force at wider gapes are thought to be the result of a lower potential for muscle force production due to stretching (Anapol & Herring 1989), as the force-length relationship observed in single muscle fibres also appears to apply for whole muscles (Close 1972; maximal sarcomere force is linearly related to the amount of actin-myosin overlap, and sarcomere lengths away from the optimum in the force-length curve result in a decrease in the number of actin-myosin interactions and thus lower force production). Therefore, I also predict that gape-driven decreases in bite force will be explained by the amount of muscle stretching of the major jaw adductors (temporalis and masseter) at wide gapes, which is a function of skull and mandible architectures.

Materials and methods

IN VIVO BITE FORCE MEASUREMENTS AT INCREASING GAPES

To determine the behavioural effect of gape on bite force in free-ranging bats, voluntary bite force measurements were collected from 17 species of phyllostomids and one morphologically

distinct, non-phyllostomid species (*Molossus sinaloe*, Molossidae) as an out-group (Table 1). Fieldwork was conducted at three localities in Costa Rica (Sarapiquí, La Selva Biological Station and Palo Verde Biological Station). Bats were captured in mist nets that were placed in the forest understory. Only adult males and adult non-pregnant, non-lactating females were used in this study. Shortly after capture, the bats' voluntary bite force was measured using a piezoelectric force transducer (Kistler, type 9203, range ±500 N, accuracy 0·01–0·1 N; Amherst, NY, USA) attached to a handheld charge amplifier (Kistler, type 5995; Herrel *et al.* 1999). The force transducer was mounted between two bite plates with the upper plate pivoting about a fulcrum provided by a micrometer head (Mitutoyo 150-802; Figure S1, Supporting information). The tips of the bite plates were covered with a layer of medical tape (3M Nexcare™ Absolute Waterproof First Aid Tape) to protect the bats' teeth from damage and to provide a non-skid surface. Bite force was measured at a deep bilateral bite position with the first molars on both sides of the mandible engaged in contact with the bite plates (Dumont 1999; Santana & Dumont 2009). To change the gape angle at which the bats bit, the distance between the bite plates was adjusted using the micrometer head. For each gape, the plates were set initially to an approximate distance depending on the bat's size and, upon a test bite, the distance was re-adjusted until the desired gape was achieved. Gapes were inspected visually, and corroborated through photographs taken during measurements for a subset of individuals. At least five measurements were taken per bat at each of four gape angles: 20°, 30°, 45° and 60°. These were presented to bats in a random order to reduce motivation effects. The maximum bite force measurement per gape angle was used for subsequent analyses. Only the results for 20° and 60° are presented here, since these produced the highest and lowest bite forces, respectively. Following bite force measurements, bats were weighed using a spring scale, and head dimensions (maximum head length, width, and height) were taken using digital calipers. Most bats were released at the site of capture, and a subset was kept as voucher specimens for morphological analyses. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Washington (Protocol # 4307-01).

MODELLING THE EFFECT OF GAPE ON BITE FORCE

Theoretical predictions of the effect of gape on bite force were estimated through the application of three-dimensional lever models of the feeding apparatus of 20 phyllostomid species (Table 2), which were selected to represent the major trends in morphological and dietary diversity in this family (Dumont *et al.* 2012). The models integrate data on 3D skull architecture, muscle attachment areas and muscle forces to generate biomechanical parameters, including each jaw adductor's applied force, moment arms, moments about the temporomadibular joint (TMJ) and bite force estimations. A full description of the bite force model is provided in Davis *et al.* (2010), including validation with *in vivo* bite force measurements.

To run the models, 3D representations of bat skulls and mandibles were generated from micro-Computed Tomography (μCT) scans of dry specimens (Skycan 1174 μCT scanner, Bruker micro-CT, Belgium), and coupled with published data on jaw adductor origin and insertion areas, and forces (Santana, Dumont & Davis 2010). Mammals have very fine control over the directionality and magnitude of occlusal forces, but these anatomical estimates of muscle forces are a necessary approximation due to the lack of published electromyography data for phyllostomids. For each species, models were run at gape angles of 20° and 60° (Fig. 1a), which were achieved by manually moving the mandible to the desired position in Geomagic Studio (3D Systems, Rock Hill, SC, USA). To overcome interspecific variation in cranial and dental morphologies and to match field measurements as closely as possible, the anterior-most cusp of the upper and lower first molars were used as reference points to set the gape angles during model simulations. A Uniform Traction algorithm was used in the bite force models, accounting for negative moments about the TMJ caused by muscle forces (Davis *et al.* 2010).

Maximum bite force at each gape was calculated by adding the moments estimated for all the jaw adductor muscles (temporalis, masseter, lateral and medial pterygoid), dividing this quantity by the out-lever, and multiplying by two to account for bilateral biting (Davis *et al.* 2010; Santana, Dumont & Davis 2010). The out-lever was measured in Geomagic Studio as the perpendicular distance from the centroid of the planar occlusal

Table 1. *In vivo* bite force measurements (in Newtons) at a low (20°) and wide (60°) gapes for species included in this study. Average maximum bite force and average percentage reduction in bite force were calculated using individual data prior to averaging

Family	Species	Sample size (male/female)	Mass (g)	Average bite force (N)			% Bite force reduction
				20° gape	60° gape	Maximum	
Molossidae	*Molossus sinaloe*	2 (1/1)	21·0 ± 2·8	10·33 ± 1·53	7·68 ± 0·14	11·21 ± 0·29	23·47 ± 14·29
Phyllostomidae	*Artibeus jamaicensis*	2 (1/1)	48·5 ± 9·2	17·34 ± 3·17	6·40 ± 0·28	17·34 ± 3·17	63·79 ± 4·98
	Artibeus lituratus	1 (–/1)	73	32·00	19·40	32·00	39·38
	Carollia castanea	5 (5/–)	12·0 ± 1·9	4·03 ± 1·04	2·32 ± 0·83	4·08 ± 0·73	40·32 ± 6·05
	Centurio senex	1 (–/1)	22	4·10	2·60	4·10	36·59
	Chiroderma villosum	1 (1/–)	20	10·64	2·10	10·64	80·26
	Dermanura watsoni	2 (1/1)	13·5 ± 4·9	6·02 ± 0·87	1·41 ± 1·59	6·22 ± 0·59	65·43 ± 13·05
	Lampronycteris brachyotis	1 (1/–)	11	7·60	4·80	7·60	36·84
	Lophostoma brasiliense	11 (5/6)	8·9 ± 1·8	6·73 ± 2·59	5·95 ± 0·85	6·98 ± 2·53	35·56 ± 5·39
	Lophostoma silvicolum	1 (1/–)	34	28·40	22·60	28·40	20·42
	Micronycteris hirsuta	2 (2/–)	14·3 ± 0·4	11·82 ± 1·39	8·41 ± 3·50	11·82 ± 1·39	30·12 ± 21·39
	Micronycteris microtis	4 (3/1)	7·3 ± 0·6	5·01 ± 0·76	3·61 ± 1·59	5·15 ± 0·51	38·29 ± 34·08
	Mimon crenulatum	2 (2/–)	14·5 ± 0·7	9·05 ± 2·70	4·61 ± 0·18	9·72 ± 1·75	36·80 ± 10·49
	Phyllostomus hastatus	6 (2/4)	126·5 ± 9·4	21·65 ± 8·04	14·83 ± 3·79	22·75 ± 8·42	43·42 ± 17·56
	Platyrrhinus helleri	1 (1/–)	15·8	11·50	6·95	11·50	39·57
	Tonatia saurophila	5 (2/3)	29·2 ± 4·4	18·25 ± 6·81	12·72 ± 3·78	18·80 ± 7·50	25·51 ± 8·81
	Trachops cirrhosus	5 (4/1)	27·8 ± 2·6	12·92 ± 4·82	6·21 ± 2·69	13·27 ± 4·68	40·59 ± 24·52
	Uroderma bilobatum	2 (1/1)	15·5 ± 0·7	5·75	4·33	5·75	24·69

Table 2. Modelled bite forces, their reduction from low to wide gapes, and morphological and biomechanical predictors across the species studied. Molar bite force estimates are presented as a percentage of the model input (total muscle) force. Within each diet, species are arranged from lowest to highest reduction in bite force. Proportions of the total moment about the temporomadibular joint (TMJ) and stretch factors for the major jaw adductors were generated from 3D models of skulls

Diet	Species	Bite force as % model input force		Bite force reduction	Proportion of total moment about TMJ		Stretch factor at 60° gape	
		20°	60°		Temporalis	Masseter	Temporalis	Masseter
Fruit	*Uroderma bilobatum*	39·78	23·05	16·72	69·45	14·62	0·68	1·68
	Enchisthenes hartii	42·94	26·08	16·85	78·01	8·84	0·48	1·19
	Carollia perspicillata	39·57	22·47	17·10	67·53	17·29	1·13	0·54
	Vampyressa pusilla	47·03	28·49	18·54	74·79	4·36	1·65	1·04
	Artibeus jamaicensis	49·34	29·18	20·15	74·48	15·21	0·52	1·79
	Sturnira lilium	44·50	23·80	20·70	73·07	11·38	1·18	1·38
	Sphaeronycteris toxophyllum	55·05	30·50	24·54	85·18	5·57	0·73	0·49
	Centurio senex	57·01	31·97	25·03	89·05	5·81	1·63	1·20
Nectar	*Lonchophylla robusta*	29·84	15·15	14·69	67·49	16·01	0·54	0·55
	Glossophaga soricina	37·28	20·15	17·13	89·02	5·41	1·17	1·73
	Anoura geoffroyi	39·61	22·17	17·44	70·09	10·84	0·78	0·51
Omnivory	*Phyllostomus hastatus*	39·43	23·93	15·50	76·69	12·14	0·74	2·61
	Phylloderma stenops	44·95	27·67	17·29	70·41	18·16	0·97	2·28
Insect	*Mimon crenulatum*	33·63	15·96	17·67	76·38	13·09	0·52	0·98
	Lophostoma silvicolum	43·96	25·74	18·22	78·79	13·82	0·44	0·64
	Tonatia saurophila	47·57	29·04	18·54	73·94	13·94	0·46	1·00
	Micronycteris hirsuta	45·61	26·12	19·49	77·14	12·50	0·87	0·71
	Lophostoma brasiliense	45·87	24·68	21·19	78·55	13·25	1·27	2·15
Insect + Vertebrates	*Trachops cirrhosus*	40·02	20·30	19·72	81·93	8·97	0·68	2·28
Blood	*Desmodus rotundus*	42·28	20·61	21·66	70·88	10·75	2·20	1·28

surface of the first upper molar to the centroid of the mandibular fossa on the same side of the skull. Mechanical advantage was calculated for each species at 20° and 60° gapes as the ratio of the total predicted output (bite) force to the input (muscle) force (Table 2). The relative contributions of the temporalis and masseter to bite force at these gapes were calculated as the proportion of their moments about the TMJ in relation to the total moment estimated by the model.

A rough measurement of cranial form was estimated by measuring, from 3D models in Geomagic Studio, the ratio between skull length (distance from the first pair of upper incisors to the most posterior point of the occipital bone) and skull width (distance at the broadest part of the zygomatic arches). Stretch factors for the masseter (Herring & Herring 1974) and temporalis (Dumont *et al.* 2009) muscles were calculated from measurements performed on lateral views of the 3D skull and mandible models of the 20 phyllostomid species, with the mandible positioned in molar occlusion (Fig. 1b). Muscle attachment areas defined on these models (derived from dissections; Santana, Dumont & Davis 2010) were used as guidelines to measure the distances between: (a) the muscle's origin and the TMJ, (b) the muscle's insertion and the TMJ and (c) the angle between a and b. For the masseter, these distances were measured from the centre of the TMJ to the most anterior point of the attachment area in the zygomatic arch (origin–TMJ distance, a_m, Fig. 1b), and to the tip of the angular process (insertion–TMJ distance, b_m). For the temporalis, origin–TMJ distance (a_t) was measured from the centre of the TMJ to the most posterior point of the attachment area in the sagittal crest, and insertion–TMJ distance (b_t) from the TMJ to the tip of the coronoid process. These measurements were then used to calculate muscle stretch factors for the masseter and temporalis at a wide gape of 60° using the equation outlined by Herring & Herring (1974) (Fig. 1b). This study posed that, at stretch factors >1, the wide gape stretches the muscle beyond the length at which it

can generate its maximum force (Herring & Herring 1974). Since the optimal gape for force production is unknown for the species studied and for bats in general, the muscle stretch values presented here are primarily for comparative purposes.

STATISTICAL ANALYSES

All variables had a strong phylogenetic signal (Pagel's λ not significantly different from 1; Pagel 1999), proving the need to account for evolutionary relationships in these comparative analyses. To identify the morphological and biomechanical factors underlying the relationship between bite force and gape across species, phylogenetic generalized least-squares (PGLS) regressions were conducted using a Brownian motion model and a pruned version of a recent species-level phylogeny (Dávalos *et al.* 2012). Reductions in bite force with increasing gape were calculated and scaled for each species as follows: (i) for *in vivo* measurements: bite force reduction equalled the average maximum bite force measured at a 20° gape minus the average maximum bite force measured at a 60° gape, expressed as a percentage of the maximum bite force measured across individuals and gapes for each species (Table 1; similar to or higher than values in Santana & Dumont 2009 and Santana, Dumont & Davis 2010; which measured bite forces at a 30° gape and other bite positions) and (b) for 3D model results: bite force reduction for each species equalled the maximum bite force estimated at a 20° gape minus the maximum bite force estimated at a 60° gape, expressed as a percentage of the total input (muscle) force applied to the model. These scaled bite force changes were used as the response variable in PGLS regressions, and morphological traits (temporalis and masseter stretch factors, head length:width ratio) and biomechanical variables (mechanical advantage at 20° or 60° gape, proportion of total moment by temporalis or masseter) were used as predictor variables. Additionally,

Fig. 1. A subsample of three-dimensional models of bat skulls across diets showing (a) morphological diversity and jaw aperture at the two gape angles modelled (fruit: *Centuro senex*; nectar: *Lonchophylla robusta*; insect: *Micronycteris hirsuta*; blood: *Desmodus rotundus*) and (b) skull measurements and the equation used to calculate jaw adductor stretch factors (from Herring & Herring 1974), with t: temporalis, m: masseter, a: distance between the temporomandibular joint (TMJ) and the origin of the muscle, b: distance between the TMJ and the insertion of the muscle, Φ: angle between a and b in closed jaw position, θ: jaw opening angle (60°), l: length of muscle, and L: length of stretched muscle. The ratio L/l is the stretch factor of the muscle.

$$(L/l)^2 = \frac{1 - [2ab \cos(\theta + \Phi)] / (a^2 + b^2)}{1 - (2ab \cos \Phi) / (a^2 + b^2)}$$
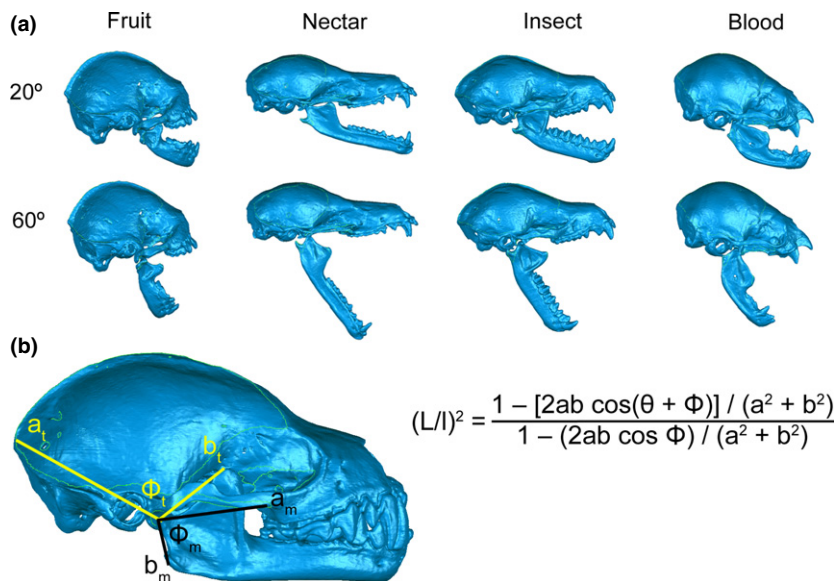
Fig. 2. Intraspecific variation in the bite force response to increasing gape across bat species. Within each diet, species are arranged from lowest to highest range of variation.
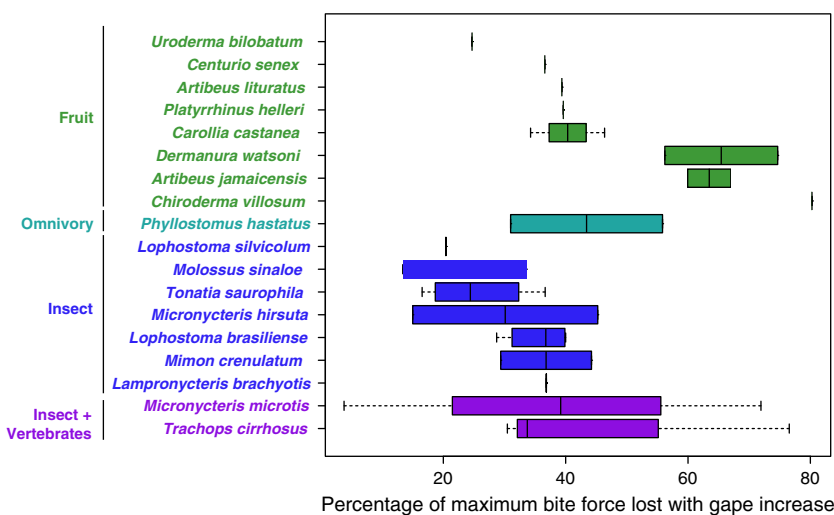
phylogenetic ANOVAs were conducted to explore the relationship between the scaled, gape-driven change in bite force and diet type (e.g., insectivorous, frugivorous, nectarivorous; Table 2) or diet hardness (Santana, Dumont & Davis 2010; Santana, Grosse & Dumont 2012; Table S1) in phyllostomids.

Results

THE EFFECT OF GAPE ON BITE FORCE

In a comparative study spanning a total of 28 species, bite forces significantly decreased when the mandibular opening increased from a narrow (20°) to a wide (60°) gape. Voluntary, maximum bite forces measured across 18 species of wild caught bats exhibited a significant reduction when the bats' gapes were changed from 20° to 60° (Table 1; paired *t*-test, $t = 6.89$, $P = 2.614 \times 10^{-7}$, d.f. = 26). Across species, the decrease in bite force ranged from 20·42% (*Lophostoma silvicolum*, an insectivore) to

80·26% (*Chiroderma villosum*, a durophagous frugivore/seed predator) of their maximum bite forces. *In vivo* experiments also exposed a substantial amount of inter-individual variation in bite force reduction (Fig. 2). This variation was observed at various degrees across species with similar sample sizes (e.g., 3·77–71·92% reduction in bite force in *Micronycteris microtis*, and 34·26–46·36% in *Carollia castanea*, both with $N = 3$; Table 1).

The trend of a sizable decrease in bite force with increasing gape was further confirmed by the results of biologically informed, 3D lever models of the feeding apparatus of 20 phyllostomid species (Table 2). These models predicted a significant reduction in bite force with increasing gape (paired *t*-test, $t = 8.221$, $P = 1.116 \times 10^{-7}$, d.f. = 19), which ranged from 14·69% (*Lonchophylla robusta*, a nectarivore) to 25·03% (*Centurio senex*, a durophagous frugivore) of the total muscle force that served as input to their respective 3D models.

MORPHOLOGICAL AND BIOMECHANICAL PREDICTORS
OF GAPE-DRIVEN BITE FORCE CHANGES

Cranial morphology and function were significantly associated with the magnitude of gape-driven reductions in bite force across species. Short-faced species tended to experience a larger reduction in bite force with increasing gape, but this relationship was not statistically significant for the sample examined (PGLS $\beta = -2.83 \pm 1.48$, $t = -1.91$, $P = 0.072$, d.f. = 20). Nevertheless, species with a higher mechanical advantage in their feeding apparatus (calculated at a 20° gape) experienced greater reductions in bite force in response to increasing gape (PGLS $\beta = 34.43 \pm 6.96$; $t = 4.94$, $P = 0.0001$, d.f. = 20). This relationship was still positive but marginally significant when mechanical advantage was calculated at a 60° gape (PGLS $\beta = 26.77 \pm 13.46$, $t = 1.99$, $P = 0.062$, d.f. = 20).

Species in which the temporalis produced a larger proportion of the moment about the temporomandibular joint also experienced a larger gape-driven reduction in bite force (PGLS $\beta = 0.20 \pm 0.08$, $t = 2.46$, $P = 0.024$, d.f. = 20), as did those with a higher stretch factor for this muscle (PGLS $\beta = 2.43 \pm 1.04$, $t = 2.33$, $P = 0.032$, d.f. = 20). Conversely, the masseter's stretch factor and moment, diet type and diet hardness were not significantly associated with the magnitude of bite force reduction across species ($P > 0.05$).

Discussion

The results from this study support the prediction that wide gape angles negatively affect bite force production in mammals, corroborating previous trends described by more taxonomically restricted comparisons (Dumont & Herrel 2003; Williams, Peiffer & Ford 2009), estimations from models of the mammalian skull (Herring & Herring 1974), and findings in other vertebrate groups (Gidmark et al. 2013). Although data on jaw adductor tension has not been collected for bats, the association between bite force and gape could be broadly explained by the length-force relationship of skeletal muscle; maximum bite force is expected to be produced at intermediate jaw adductor (sarcomere) lengths and to decrease when they are excessively stretched (Ramsey & Street 1940). Still, the underlying physiological mechanisms of the gape-bite force relationship are more complex; whole muscles generate passive tension in their connective tissues, and this could result in changes in the optimum and extent of plateaus in the length-force curves (e.g., connective tissues may allow for greater sarcomere shortening; Rack & Westbury 1969). Furthermore, differences in sarcomere lengths across species and among muscle fibres within a muscle can also impact length-force relationships (Rassier, MacIntosh & Herzog 1999; Eng et al. 2009). Data on muscle fibre length exist for several of the species studied here (Herrel et al. 2008; Santana, Dumont & Davis 2010), but these were not significantly associated with the reduction in bite force at

wide gapes (results not shown). This highlights the need for in-depth analyses of jaw adductor physiology in bats.

Muscle force is also expected to decrease when jaw adductors are shortened in relation to their optimal contraction length, but this phenomenon was not captured by the experiments conducted here; maximum bite force was achieved by all species at the lowest gape angle measured (20°), and it dropped sharply with increasing gapes. Presumably, gape angles somewhere below 20° would result in lower in vivo bite forces, but further studies are required to verify this assumption.

Within species, in vivo bite force measurements revealed ample variation in the response of individual bats to experimental increases in gape. Previous work on individual bite force modulation in bats and other vertebrates has demonstrated that, within the same biting style, subjects need to fully recruit all of their jaw adductor muscles to generate maximum bite forces, and thus partial or differential recruitment leads to bite force variation (De Gueldre & De Vree 1988; Dessem 1989; McBrayer, White & McEachran 2002; Korff & Wainwright 2004). Human studies have also shown that individuals modulate their adductor forces potentially to minimize joint and dental loads (Orchardson & MacFarlane 1980; Nickel et al. 2003), thus maximum voluntary bite forces are also constrained by intra-oral afferent activity (Morneburg et al. 2014). Along with inter-individual differences in motivation to bite, cranial anatomy, and sample sizes, the aforementioned factors likely underlie some of the variation in gape-driven bite force responses seen in this study. Recent work on food-driven bite force plasticity (Anderson, Renaud & Rayfield 2014) also highlights the possibility that the variation in bite force response could be related to differences in the diversity of dietary items experienced by individuals and species. If this were the case, species with more physically diverse diets would be expected to exhibit the largest variation in bite force response. Although individual dietary data are not available for the animals measured, the species with the widest bite force response variability in the dataset is M. microtis, an animalivorous bat that has an unusually varied diet and high feeding behaviour plasticity when compared to closely related species (Kalka & Kalko 2006; Santana et al. 2011a). In sharp contrast, the species with the lowest variation in gape-driven bite force response is C. castanea, a frugivore that specializes on fruits of the plant genus Piper (Fleming 1991). The Piper species included in C. castanea's diet have fruits that are similar in their cross-sectional size, thus it is unlikely that individual bats experience much pressure for variation in gape across dietary items. Further dietary studies at the individual level are necessary to elucidate the ecological underpinnings of intraspecific bite force responses to changing gape.

For the first time, this study was able to identify which and how morphological and biomechanical aspects of the feeding apparatus explain gape-driven changes in bite force across bat species. As in many other generalized mammals, the temporalis muscle is the largest jaw adductor in bats

(Storch 1968; Herrel *et al.* 2008). Comparative analyses revealed that an increase in the relative importance of this muscle for bite force production is associated with sharper decreases in bite force at wide gapes. Mathematical and muscle physiology predictions (Ramsey & Street 1940; Herring & Herring 1974) were also confirmed; high temporalis stretch factors result in steeper reductions in bite force. Conversely, the masseter's stretch factor was not significantly associated with the observed decrease in bite force. At wide gapes, however, most of the species in the sample still have masseter stretch factor values that fall below the range of those estimated for the generalized mammal condition (1·5–2·0; Table 2; Herring & Herring 1974), thus stretching of this muscle may not substantially hinder bite force production during most feeding situations in these bats.

Consistent with the expected trade-off between maximizing mechanical advantage vs. gape, species with high mechanical advantages in their feeding apparatus experience some of the largest reductions in bite force at wide gapes. Increases in mechanical advantage can be achieved, in part, via the evolution of a shorter rostrum (out-lever), which positions the bite point closer to the jaw adductor attachment sites. In this study, short-faced skull morphologies were found to be associated with the reduction in bite force, albeit non-significantly. Two of the species with the highest mechanical advantages (durophagous frugivores *Centurio senex* and *Sphaeronycteris toxophyllum*) have the shortest rostra and some of the highest temporalis stretch factors (Fig. 1a, Table 2). Conversely, nectarivorous species have a long rostrum and muscle attachment locations that result in low muscle stretching and relatively wider gape (Fig. 1a) at the expense of the capacity to generate high bite forces. These results indicate that morphological determinants of muscle function (e.g., origin and insertion sites) may also play a role in gape-bite force relationships. The common vampire bat (*Desmodus rotundus*) presents an exception to these trends; although it has a large reduction in bite force and high temporalis stretch factor, it does not have a high mechanical advantage when compared to other species. *Desmodus rotundus* has a relatively weak bite force that is further decreased by gape changes, as would be expected by its reliance on a liquid food source that is accessed by piercing the prey's skin with its incisors (Greenhall 1972).

Altogether, the results from morphological and biomechanical comparisons indicate that frugivorous and insectivorous phyllostomids are specialized for producing high bite forces at low to intermediate gapes (also see Dumont *et al.* 2009). Although data on bat feeding kinematics are virtually nonexistent, it is known that frugivorous phyllostomids thoroughly chew fruit pulp to extract nutritious contents (Bonaccorso & Gush 1987; Dumont 1999), and prolonged chewing of insect prey by insectivores results in greater food breakdown (Santana, Strait & Dumont 2011b). Therefore, bite force production at low gapes may be of particular importance for the feeding performance of these bats. Carnivorous and omnivorous phyllostomids are similar to carnivorans in their low temporalis and high masseter stretch factors (Herring & Herring 1974; Table 2), and this potentially allows them to produce bite forces at the wider gapes that are required for capturing and consuming large and mechanically challenging vertebrate prey. Although these species do not have a relatively high skull mechanical advantage, they are larger animals, which is another evolutionary strategy for increasing maximum bite force (Anderson, McBrayer & Herrel 2008).

The interplay between bite force and gape at proximate and evolutionary scales is of particular importance to ecomorphology because these factors may jointly determine the physical spectrum of food items animals can consume (e.g., Herrel *et al.* 2001; Aguirre *et al.* 2003; Meyers & Irschick 2015). Taxonomic and physical (hardness) dietary categories, however, failed to explain the functional trends described here. Dietary hardness characterizations have been effective in explaining major evolutionary trends in bite force and mechanical advantage in phyllostomids (Santana, Dumont & Davis 2010; Dumont *et al.* 2014), but the results presented here evidence that other, more complex metrics of diet may be necessary for explaining composite performance metrics, such as the effect of gape on bite force. Future studies that measure actual prey item sizes, hardness and, in particular, their variation within and across species would allow for these analyses. Importantly, bats seem to exhibit strong intraspecific variation in bite force at different gapes, and the extent in which they do so across their natural diets may be a significant factor explaining dietary diversity. This study presents a framework for generating concrete predictions on the dietary differences among bats based on quantifiable aspects of their feeding ecology.

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Data accessibility

Data deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.gg526, (Santana 2015).

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Supporting Information

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

Fig. S1. Bite force meter setup.
Table S1. Data on dietary hardness and cranial shape.