Phylogenetically structured variance in felid bite force: the role of phylogeny in the evolution of biting performance

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Abstract

A key question in evolution is the degree to which morphofunctional complexes are constrained by phylogeny. We investigated the role of phylogeny in the evolution of biting performance, quantified as bite forces, using phylogenetic eigenvector regression. Results indicate that there are strong phylogenetic signals in both absolute and size-adjusted bite forces, although it is weaker in the latter. This indicates that elimination of size influences reduces the level of phylogenetic inertia and that the majority of the phylogenetic constraint is a result of size. Tracing the evolution of bite force through phylogeny by character optimization also supports this notion, in that relative bite force is randomly distributed across phylogeny whereas absolute bite force diverges according to clade. The nonphylogenetically structured variance in bite force could not be sufficiently explained by species-unique morphology or by ecology. This study demonstrates the difficulties in identifying causes of nonphylogenetically structured variance in morphofunctional character complexes.

Introduction

A key question in evolution is the extent to which adaptation and constraint affect morphology (Losos & Miles, 1994; Schluter, 2000; Gould, 2002). Can we regard an organism as a *tabula rasa* that has no significant history and that is subject to all the effects of natural selection in honing its morphology and adaptation, or are clades locked into particular anatomical pathways by their history? The concept of phylogenetic constraint has been discussed extensively by previous authors, but it is still one of the most difficult questions in evolution to summarize (Blomberg & Garland, 2002; McGhee, 2007); for example, McKitrick (1993) wrote 'in some sense, all evolutionary studies implicate phylogenetic constraint, and reviewing the topic is like trying to catch a greased pig' (p. 309). And because of this widespread discussion, phylogenetic constraint as an underlying influential

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factor that may limit evolutionary change is ill defined. However, its effect on evolution can be observed as phylogenetically conservative character evolution or as very strong phylogenetic signal in the data.

The role of phylogeny in morphofunctional character complexes is particularly important. Adaptations required in performing a certain function (or subsequent evolution of such morphofunctional characters in response to selection for that particular function) cannot occur independent of associated and necessary changes to underlying body plan (or bauplan). For instance, even if there was a particularly strong selective pressure to increase biting force and thus necessarily increase jaw adductor muscle mass, this cannot occur without associated changes to the cranial skeleton to allow for increased space. Such morphological changes can be restricted in how they can be achieved; for instance, expansion of cranial adductor space would be severely limited by the size of the brain case. There may or may not be some underlying constraint on how such adaptive changes can or cannot occur, something that may be inherited through evolutionary history, i.e. phylogeny. Identification of such phylogenetic signals in

morphofunctional data and quantifying the proportion of variance pertaining to phylogeny can indicate what scope there might be to seek other adaptive causes; these nonphylogenetically conservative features can be interpreted as evolving at a faster rate, perhaps as a result of strong selection. Comparative methods allow the phylogenetic signal in adaptations to be discerned and residual nonphylogenetic adaptation identified (Diniz-Filho *et al.*, 1998; Desdevises *et al.*, 2003).

In testing for phylogenetic signals in morphofunctional adaptations, we are particularly interested in focusing on biting performance, not only because biting is a simple and basic function that is important to food procuring, manipulation and processing in terrestrial tetrapods, but also because it is easy to define. Quantification of biting performance can be achieved through application of a simple biomechanical model, and the resulting single-value numerical indicator, bite force, can be used in further comparative analyses. Previous studies have shown that bite force is associated with not only food hardness (Van der Meij *et al.*, 2004) and food handling time (van der Meij & Bout, 2006), but also dietary categories (Wroe *et al.*, 2005; Christiansen & Wroe, 2007), and it is a widely used indicator of biting (or feeding) performance (Sinclair & Alexander, 1987; Thomason, 1991; Herrel *et al.*, 1998, 2002, 2005a,b; Binder & Van Valkenburgh, 2000; Erickson *et al.*, 2003, 2004; van der Meij & Bout, 2004; Christiansen & Adolfssen, 2005; Wroe *et al.*, 2005; Christiansen & Wroe, 2007). We are interested in the morphofunctional character complex that is summarized as bite force and any phylogenetic signal or indeed absence of it can be used to infer morphological changes associated with this particular biomechanical function.

We used Felidae for our case study because there is a recent phylogeny available to us that incorporates all extant species (Johnson *et al.*, 2006) and because the group underwent a relatively recent radiation (Johnson *et al.*, 2006). Therefore, we can test just how much phylogeny would affect simple biomechanical adaptations within members of a closely related and rapidly diversified group. Is there a strong phylogenetic signal as a result of rapid speciation in a short period of time, i.e. has the biting morphofunctional character complex remained relatively conserved across Felidae despite relatively rapid speciation? Or is there limited phylogenetic signal and could one then look for other explanations, including seeking independent evidence for adaptation in the evolution of biomechanical characters? Furthermore, Felidae includes some unique fossil taxa with interesting features, such as the cheetah-like convergence in the North American Cheetah-like Cat *Miracinonyx trumani*, the extremely large American Cave Lion *Panthera atrox* and the sabre-toothed cat *Smilodon fatalis*. Such unique fossil taxa help to augment ancestral states reconstructed from extant taxa and phylogeny.

Materials and methods

Body size index

Because most of the cranial specimens, from which measurements and bite force computations were made in this study, were either isolated or did not have any associated weight records, body size needs to be assigned accordingly. We used two measures of size: body mass and skull width. Body mass was used for species-level analyses, whereas skull width was used for specimen-level computation of size-adjusted functional indexes. Minimum and maximum body mass data were taken primarily from Nowak (2005). For *S. fatalis*, mass estimates from Christiansen & Harris (2005) were taken, whereas masses for *M. trumani* and *P. atrox* were estimated using the regression equation for lower molar length against body mass from Van Valkenburgh (1990). A mean body mass was computed for each species and used in further comparative analyses (Table 1).

Bite force

The most reliable source of bite force is actual physical measurements in live animals. However, in the case of felids, physical recordings of bite force, whether voluntary or involuntary, are not available from the literature. In addition, many felid species are endangered or rare in captivity and thus *in vivo* recordings are practically impossible to obtain. While some species are commonly kept in captivity, opportunities to measure bite force are rare as these animals can be sensitive if not dangerous. Specimens for myological sampling are also rare for similar reasons. Therefore, an alternative source of bite force measurements, a modified version of the dry skull method (Thomason, 1991) was used to estimate bite force in the 39 felid species from 178 dry skull specimens. The method outlined in Thomason (1991) was modified to better cope with certain complications (see Supporting Information for details). The unique advantage of the dry skull method is in its wide application to practically any mammalian species.

Using the dry skull method, the cross-sectional area (CSA) of each jaw-closing muscle can be approximated with ease and relative consistency (Fig. 1). CSA was measured from a series of digital photographs using the image analysis software ImageJ (Abramoff *et al.*, 2004; Rasband, W.S., ImageJ, US National Institute of Health, Bethesda, Maryland, USA. http://rsb.info.nih.gov/ij/, 1999–2009) to capture the range of possible muscle cross-sections (see Supporting Information). The median CSA value of each muscle group, i.e. temporal muscle group and masseter + pterygoid muscle complex, respectively, was computed and used for subsequent bite force calculation. The moment arm of each muscle group was measured not only from ventral and lateral view photographs of cranial specimens but also from actual mandibular specimens using a digital calliper, and a mean value of the moment arm

Table 1 Mean body mass (M_{Body}), median skull width (W_{sk}), mean dry skull bite force estimate (F_{DS}), corrected bite force (F_{Corr}), bite force predicted from skull width using an allometric equation (F_{Wsk}), size-adjusted bite force (BFQ) and number of specimens (n).

Taxa	M_{Body}	W_{sk}	F_{DS}	F_{Corr}	F_{Wsk}	BFQ	n
Acinonyx jubatus	47.0	123	486	732	669	110	3
Caracal aurata	11.0	86.0	227	381	341	112	2
Caracal caracal	13.0	83.0	195	335	316	106	2
Felis catus	4.00	62.0	94.0	177	187	94.0	16
Felis chaus	10.0	81.0	203	346	308	112	3
Felis margarita	2.00	70.0	104	195	230	85.0	2
Felis nigripes	2.00	65.0	95.0	180	203	88.0	2
Felis silvestris	6.00	66.0	104	195	211	93.0	7
Leopardus colocolo	3.00	70.0	129	233	238	97.0	7
Leopardus geoffroyi	4.00	59.0	88.0	169	170	99.0	2
Leopardus guigna	2.00	53.0	69.0	137	139	99.0	2
Leopardus pardalis	14.0	90.0	241	400	369	108	8
Leopardus tigrinus	2.00	59.0	84.0	163	171	95.0	3
Leopardus wiedii	3.00	68.0	120	220	222	98.0	2
Leptailurus serval	13.0	87.0	201	343	350	98.0	2
Lynx canadensis	11.0	89.0	204	348	360	97.0	4
Lynx lynx	23.0	103	307	493	482	106	3
Lynx pardinus	11.0	97.0	188	324	427	76.0	1
Lynx rufus	10.0	86.0	192	330	338	98.0	2
Miracinonyx trumani	27.0	132	450	686	757	91.0	1
Neofelis diardi	20.0	107	381	590	518	112	3
Neofelis nebulosa	20.0	112	402	621	559	111	5
Otocolobus manul	4.00	66.0	97.0	183	207	88.0	4
Panthera atrox	347	289	2370	2860	3250	88.0	1
Panthera leo	166	223	1440	1860	2010	91.0	18
Panthera onca	97.0	175	1040	1410	1290	109	3
Panthera pardus	59.0	142	625	907	870	105	12
Panthera tigris	186	217	1540	1970	1930	101	31
Panthera uncia	50.0	134	521	777	783	99.0	5
Pardofelis badia	4.00	65.0	114	210	203	104	2
Pardofelis marmorata	4.00	70.0	132	239	233	103	1
Pardofelis temminckii	12.0	92.0	278	453	383	118	2
Prionailurus bengalensis	5.00	68.0	136	245	224	108	3
Prionailurus planiceps	2.00	58.0	106	197	163	121	3
Prionailurus rubiginosus	1.00	50.0	69.0	138	125	111	2
Prionailurus viverrinus	11.0	92.0	299	484	383	126	2
Puma concolor	70.0	156	805	1130	1030	110	4
Puma yagouaroundi	7.00	64.0	122	224	195	119	2
Smilodon fatalis	220	178	774	1090	1320	83.0	1

measurements was used for further analyses (Table S1). Distance between the base of the canine and the jaw joint was taken as the biting moment arm (Table S1). Using a simple two-dimensional biomechanical model, bilateral dry skull bite force F_{DS} is calculated as:

$$F_{DS} = 2 \times \sigma \times \left[(CSA_T \times m_T) + (CSA_M \times m_M) \right] / m_{Bite}$$

where CSA_T and CSA_M are the median CSA of the temporal muscle and masseter + pterygoid muscle complex, respectively, m_T and m_M are the mean moment arms of the said muscles (effort arms), σ is the isometric muscle stress value at 0.3 N mm^{-2} (Thomason, 1991) and m_{Bite} is the biting moment arm (the load arm) taken at the base of the canine.

As dry skull bite force estimates are known to underestimate *in vivo* bite forces (Thomason, 1991), we corrected for this underestimation using Thomason's (1991) correction method. Thomason (1991) noted that in *Didelphis virginana* and *Rattus norvegicus*, bite forces estimated from myological data closely matched *in vivo* values. Therefore, under the assumption that myological bite force estimates provide reliable indication of *in vivo* bite forces and that this relationship persists in larger taxa, Thomason (1991) corrected his dry skull bite forces by using the fitted values for a regression equation of myological bite forces (B_p of Thomason, 1991) against dry skull bite forces (B_s of Thomason, 1991). However, because the application of this correction method to large carnivoran taxa involves extrapolation beyond the range observed and validated in *D. virginiana* (Thomason, 1991) and because there is no reason to believe that the same relationship still
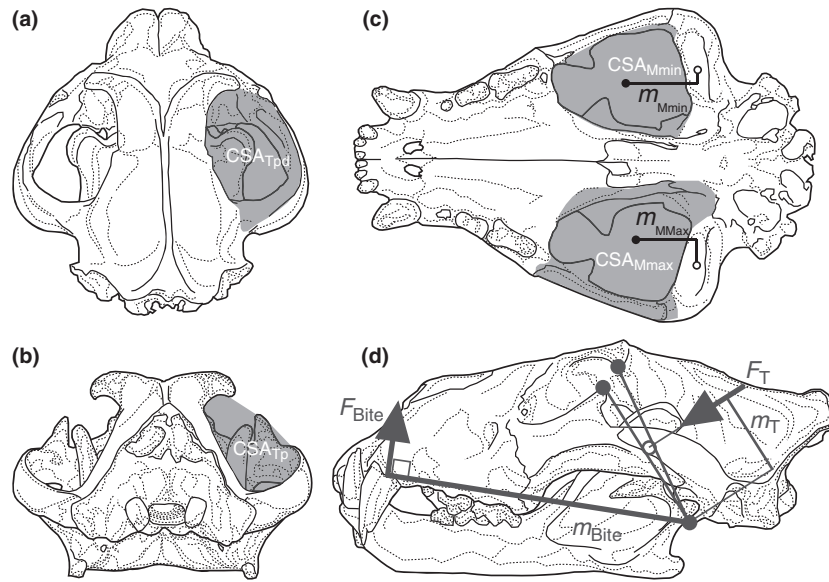
Fig. 1 Bite force was calculated in cat skulls following Thomason (1991) by taking photographs from different views. The skull of *Panthera leo* (BRSUG.28501) is shown with representative perspectives: (a), posterodorsal; (b), posterior; (c), ventral; and (d), lateral views. (a) and (b), CSA of the temporal muscle (CSA_T) can differ substantially depending on the angle of view, and thus a series of CSA_T were taken and an average was used. (c), similarly, the CSA of the masseter + pterygoid muscle complex (CSA_M) can differ depending on the bounding enforced, and thus a minimum and maximum CSA_M were taken. The perpendicular distance (thick solid line) between the centroid of the CSA_M (filled circle) and the centroid of the condyle (open circle) was taken as the moment arm of the muscle (m_M). (d), the moment arm for the temporal muscle m_T is taken as half the distance between the posterior edge of the mandibular fossa and the post-orbital process; distances from the jaw joint to the post-orbital processes (POP) of the frontal and jugal were taken and averaged to represent a mean condylo-POP length and then halved. The biting moment arm (m_{Bite}) is taken as the distance between the posterior extent of the mandibular fossa and the centre of the canine base. F_T and F_{Bite} are temporal muscle force and bite force, respectively.

holds in larger animals, subsequent authors had taken a tentative approach and had not adopted this correction method (Christiansen & Adolfssen, 2005; Wroe *et al.*, 2005; Christiansen & Wroe, 2007). Here, we demonstrate that our dry skull bite force estimates corrected using Thomason's (1991) correction method in the 36 extant felids have a bivariate distribution with respect to body mass that is not significantly different from that of *in vivo* bite forces in vertebrate predators of the same size range (1–200 kg; Fig. S1). Therefore, we determined that Thomason's (1991) correction method does indeed predict bite force values that are comparable to *in vivo* bite forces from animals of similar sizes, and we follow this approach to correct our dry skull bite force estimates in Felidae. The regression equation was derived from Thomason's (1991) bite forces at the canine biting position, and followed the form, $\log_{10} CB_P = 0.859 \times \log_{10} CB_s + 0.559$ ($R_8^2 : 1; p : < 2.2E - 16$), where CB_p and CB_s are Thomason's (1991) myological and dry skull bite force estimates, respectively. Replacing CB_s with our felid dry skull bite force estimates, F_{DS}, and taking the exponent would result in our corrected bite forces (F_{Corr}), or:

$$F_{Corr} = 10^{(0.859 \times \log_{10} F_{DS} + 0.559)}.$$

Bite force computation and correction were performed in R (R Core Development Team, 2009) at the specimen level (Table S2). Sample sizes for most species are small (Table 1) but where possible multiple specimens were used and an average taken.

Size-independent biting function index

As bite force is highly correlated with body size (Meers, 2002; van der Meij & Bout, 2004; Huber *et al.*, 2005; Wroe *et al.*, 2005), its unique morphofunctional aspect needs to be isolated from the overwhelming effect of size. Following Wroe *et al.* (2005) and Christiansen & Wroe (2007), we calculated bite force quotients (BFQ) as the standardized residuals of bite force with respect to body size. First, we computed expected values of bite force at a given size using skull width (W_{Sk}) as the size indicator. This F_{Wsk} is computed as the exponent of the fitted values of a least squares regression model of $\log_{10} F_{Corr}$ against $\log_{10} W_{Sk}$, $\log_{10} F_{Corr} = 1.86 \times \log_{10} W_{sk} - 1.06$ ($R_{176}^2 : 0.984; P : < 2.2E - 16$) (Fig. S2), or $F_{Wsk} = 10^{(1.86 \times \log_{10} W_{sk} - 1.06)}$. Skull width was selected as an

appropriate indicator of size as it was determined in a preliminary analysis to be the best predictor of bite force and because it is the only indicator of size available for each specimen (Sakamoto, 2008). Second, the ratio between the corrected dry skull bite force estimates (F_{Corr}) and the bite force values predicted from skull width (F_{Wsk}) was taken and standardized, so that an expected bite force value for a given skull width would equal 100. Thus, BFQ is calculated as:

$$BFQ = F_{Corr}/F_{Wsk} \times 100.$$

where F_{Wsk} is the bite force predicted from skull width or the exponent of the fitted values of the regression between $\log_{10}F_{Corr}$ and $\log_{10}W_{sk}$.

BFQ were computed at the specimen level (Table S2) from which species-mean values were computed (Table 1) and used for further analyses.

Phylogeny

The phylogeny of Johnson *et al.* (2006) formed the basis of the felid tree topology used in this study (Fig. 2). This topology was selected over others such as Salles (1992), Pecon-Slattery *et al.* (2004) or the supertree of Bininda-Emonds *et al.* (1999) because it is fully resolved, and above all, it is the most recent and the only study encompassing all extant valid felid species. Two taxa in the original tree of Johnson *et al.* (2006), *Felis bieti* and *F. lybica*, were excluded because of a lack of specimens in our sample. Three extinct species, *M. trumani*, *P. atrox* and *S. fatalis*, were inserted into the relevant positions according to recent studies (Burger *et al.*, 2004; Barnett *et al.*, 2005, 2009). The tree was first constructed manually in MacClade and run through the APE library

Fig. 2 Dated phylogeny of Felidae with basal node age of 16.9 Ma. The phylogenetic hypothesis of the Felidae used for analyses of evolution in biting function is based on that of Johnson *et al.* (2006) with fossil species inserted into the relevant positions according to recent phylogenetic analyses. Branch durations are estimated using node age estimates (Johnson *et al.*, 2006) and first occurrence fossil dates. The extinct taxa, *Smilodon fatalis*, *Miracinonyx trumani* and *Panthera atrox*, are shown at their youngest geological age at 0.0117 Ma (or 11 700 years ago), which is nearly indistinguishable from the extant taxa relative to the depth of the current tree (16.9 Ma at the basal node). The basal node is dated here according to fossil dates of *Pseudaelurus*. Numbers at the nodes represent the node numbers assigned in R and correspond to the node numbers in Tables S9–S12.
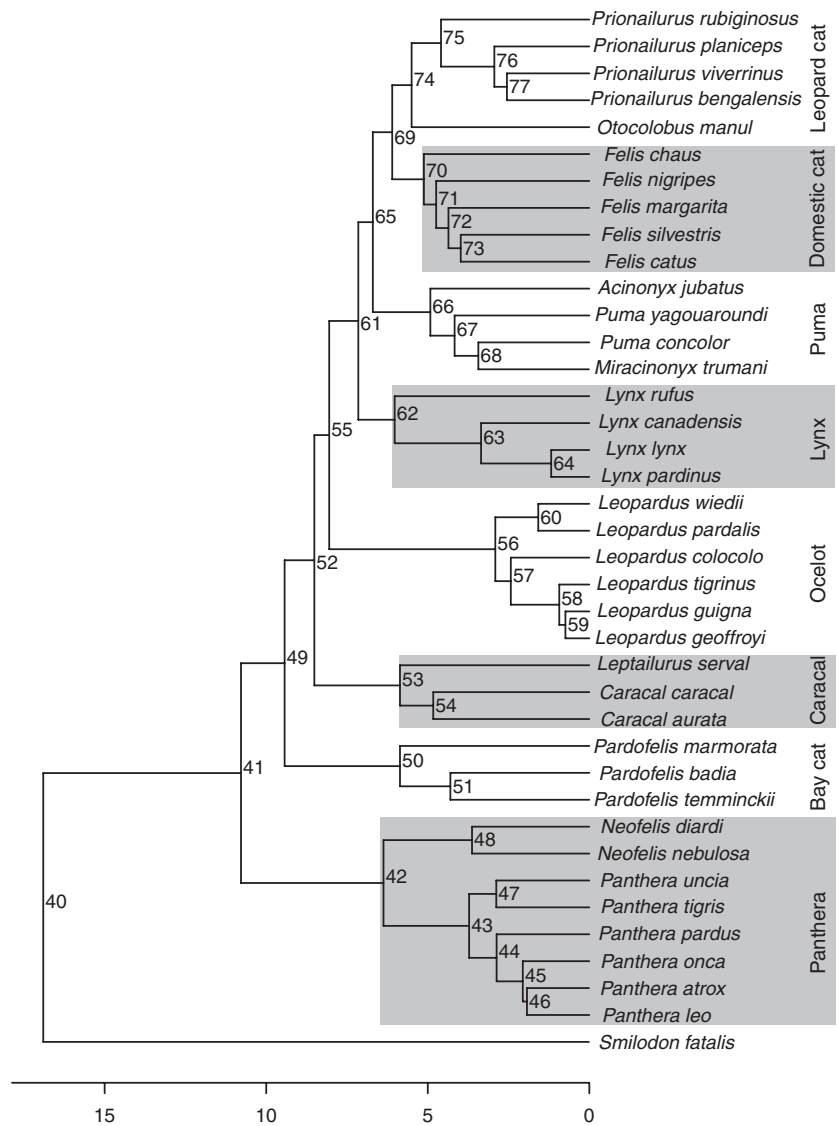
(Paradis *et al.*, 2004) in R to date the tree and estimate initial branch durations. In dating the tree we first took each terminal age [in millions of years (Ma)] as the first appearance implied by the dates of Johnson *et al.* (2006) or the earliest occurrence of that taxon in the fossil record collected from the Paleobiology Database(http://paleodb.org; April 2009) (Table S3), whichever was older, and each node age as the date from Johnson *et al.* (2006) or the earliest fossil occurrence of all the descendants of that node, again, whichever was oldest. Where a taxon only had either a molecular age or a fossil age then this was used. After this initial dating, some zero duration branches existed (an inevitable consequence in the standard dating method used by palaeontologists), and at this point, the method of Brusatte *et al.* (2008) was applied such that zero duration branches were lengthened by sharing time equally with a directly ancestral branch of positive duration. This is a modification of the approach of Ruta *et al.* (2006) where sharing is proportional to character changes, which was not possible or desirable in the present context (R code for implementing both methods is available from http://www.graemetlloyd.com/). Terminal branches were extended to fit the last occurrence dates for each terminal taxon, i.e. 0 Ma for extant and 0.0117 Ma (end of the Pleistocene) for extinct taxa. For the basal node age, two dates were assigned: the oldest occurrence of *Pseudaelurus*, the supposed ancestral stock of both felines and machairodontines, at 16.9 Ma; and the earliest fossil occurrence of any member of the Machairodontinae, at 23.03 Ma. Both dates were retrieved from the Paleobiology Database (2009).

Phylogenetic eigenvector regression

To test for phylogenetic signals in phenotypic variables, the phylogenetic eigenvector regression (PVR) of Diniz-Filho *et al.* (1998) was conducted. First, pairwise phylogenetic distances were extracted from branch duration information (millions of years) in R. The phylogenetic distance matrix was further subjected to a principal coordinate (PCo) analysis to reduce the complex topological information down to a few manageable columns of PCo axes (Tables S4 and S5, respectively, for the 16.9 and 23.03 Ma trees) that still maximally represented phylogenetic relationships. Following Diniz-Filho *et al.* (1998), a broken-stick model (Frontier, 1976; Jackson, 1993) was used to select the appropriate numbers of axes to use in further analyses. In this way, the first nine PCo axes were kept for both the 16.9 and 23.03 Ma trees, explaining 85% and 87% of the total variance, respectively. Bite force and BFQ were each subjected to a multiple regression analysis as the response variable and the PCo axes as the predictor variables. The R^2 values are read as the proportion of variance in the morphofunctional variable explained by phylogeny. A step function available in R, which is based on Akaike's Information Criterion (AIC; Akaike, 1973), was implemented to select the predictor variables with significant input to the models. We also permuted *P* values using Permute! version 3.4 alpha 9 (Casgrain, 2009) over 999 permutations.

Morphometric eigenvector regression

Following the logic of PVR, to test for correlations between bite force/BFQ and overall skull morphology, bite force and BFQ were subjected to multiple linear regression analyses against principal components (PC) of a simple morphometric analysis, here onwards referred to as morphometric eigenvector regression (MVR). First, six morphometric variables (simple linear measurements) were taken from each specimen, and species-mean values were computed (Table S6). Then, to quantify overall morphology, these six variables were subjected to a principal components analysis (PCA) (Table S7), where the first PC (PC1) axis explained 99% of the total variance in the morphometric data. However, because this axis is also highly associated with size, to capture other subtleties in morphological variation, the remaining PC axes (except the last, PC6) were retained for further analyses. Multiple linear regression analyses were conducted where either bite force or BFQ are the response variables and the five PC axes are the predictor variables. A step function was implemented to select the predictor variables with significant input to the models. As with PVR, significance was tested by permutational means using Permute.

Phylogenetically structured phenotypic variance in felid bite force

The earlier mentioned procedures for PVR and MVR can illustrate how much of the variance in bite force/BFQ can be explained by phylogeny and morphology, respectively, but some unknown proportion of either variance may actually overlap, forming a proportion of variance explained by a combination of phenotype (morphology and/or size) and phylogeny, the phylogenetically structured phenotypic variance (Fig. 3) following the naming sense of Diniz-Filho *et al.* (1998). On the other hand, the remainder of the variance may be partitioned into components that can be explained solely by either phenotype or phylogeny and a component that is unexplained by the current model. To quantify the proportion of variance in bite force and BFQ explained by phenotypic variables (size and morphology), by phylogeny and the phylogenetically structured phenotypic variance, we performed variance partitioning using PVR following the protocols of Desdevises *et al.* (2003) and Kriloff *et al.* (2008). First, a simple (or multiple) linear model was fitted on bite force or BFQ against phenotypic variable(s), the R^2 value of which explains the total

proportion of variance explained by phenotype (partition a + b). Second, bite force/BFQ was subjected to a multiple linear regression against the first nine PCo axes representing phylogeny (as outlined earlier). The multiple R^2 of this corresponds to the total amount of variance explained by phylogeny (partition b + c). Third, a multiple regression on bite force/BFQ against phenotype + phylogeny was conducted, the R^2 of which representing the overall proportion of variance explained by phenotype and phylogeny combined (partition a + b + c). Fourth, to obtain the individual values of the partitions a, b and c, R^2 values from the separate regression analyses were used: $a = R^2_{a+b+c} - R^2_{b+c}$; $b = R^2_{a+b} - a$; and $c = R^2_{b+c} - b$. Fifth, the residual variation (or the unexplained portion of the total variation) was computed as: $d = 1 - R^2_{a+b+c}$, or $d = 1 - (a + b + c)$.

For bite force, three models were initially constructed: Model 1, bite force against phenotype (body mass + morphometrics) and phylogeny; Model 2, bite force against phenotype (morphometrics) and phylogeny; and Model 3, bite force against phenotype (body mass) and phylogeny.

For BFQ, only one model was considered, i.e. partitioning variance in BFQ between morphometrics and phylogeny because BFQ is size independent already, so body mass need not be included in the model.

Regardless of any elimination of predictor variables because of the step-wise elimination procedures outlined for the PVR and MVR methods previously, all predictor variables were included in the variance partitioning models because insignificant variables in one model may actually have some significance in the presence of variables from the other model, i.e. phenotypic or phylogenetic models, respectively.

Environmentally structured variance in bite force

Bite force has been tested repeatedly against feeding ecology (Herrel *et al.*, 2005a; Wroe *et al.*, 2005; Christiansen & Wroe, 2007), and here we also follow that trend. Previous studies have used dietary categories, whether as feeding specializations (Christiansen & Wroe, 2007) or prey size categories (Meachen-Samuels & Van Valkenburgh, 2009). However, as our methods work better using continuous variables rather than categorical variables, we compiled a list of known prey for 35 of the 39 cat species in our dataset from various sources (see Supporting Information). Diet is unknown for *Pardofelis badia* (no such study exists) and the three extinct taxa, *M. trumani*, *P. atrox* and *S. fatalis*, so these taxa were excluded from further analyses. For each of the remaining cat species, we assigned a minimum and maximum prey size using body mass data taken primarily from Nowak (1999) but also from other sources (Table S7). For prey taxa that had ambiguous assignments such as 'pig' or 'rat', we assigned a range of body mass for genera under the common name that

is most commonly sympatric with the cat species of interest. For each cat species, the midpoint value between the minimum and maximum prey body mass values was computed (Table S7) as the mean prey mass. We used midpoints instead of preferred prey size because although many cats are known to have a specific prey preference despite their wide range in documented prey size, such information is unknown in most cat species, and we preferred to conform to a standard protocol. Further, there is not enough information to compute the mode of the distribution in prey size either.

To investigate whether variance in bite force can be sufficiently explained by prey size alone, a least squares regression analysis was first conducted on $\log_{10} F_{Corr}$ against $\log_{10}$ prey size using R. Further, to test whether prey size can still significantly explain variance in bite force in the presence of other explanatory variables, i.e. cat size, morphology and phylogeny, we conducted a multiple linear regression analysis on bite force against prey size, cat size, morphology (PC axes) and phylogeny (PCo axes), and an AIC-based procedure of variable selection was performed using the step function in R. Morphology is quantified as principal components as before but using the morphometric dataset for the reduced taxonomic sample. Phylogeny is quantified as the principal coordinates extracted from a phylogenetic distance matrix computed from a pruned tree reflecting the reduced taxonomic sample (Table S8). Using the broken-stick model, we kept the first eight PCo axes, which explained roughly 82% of the total variance, for further analyses.

To investigate whether variance in BFQ can be sufficiently explained by prey size in relation to cat size, we computed measures of size-independent relative prey size much in the same way as computing BFQ. First, a least squares linear model was fitted to $\log_{10}$ midpoint prey size with respect to $\log_{10}$ cat body mass (Fig. S3): $\log_{10}$ prey size $= 1.47 \times \log_{10}$ cat size $- 0.724$ (R^2_{33}: 0.692; p: 5.92E-10). The residuals of this regression were then used to compute relative prey size as:

$$\text{Relative prey size} = \frac{\text{Prey size}}{\text{Prey size predicted for cat size}} \times 100$$

where prey size predicted for cat size is the exponent of the fitted value of the regression. Relative prey size is shown in Table S7.

A least squares regression model was fitted to BFQ against relative prey size to determine the proportion of variance in BFQ explained by relative prey size. To test for any nonlinear correlation between the two variables, a Spearman rank correlation test was conducted. Absolute prey size was also tested for any significant correlation with BFQ using a least squares linear model and Spearman rank correlation test.

Bite force evolution in Felidae as assessed by character optimization

To visually trace the evolution of bite force and BFQ, we optimized these characters onto the tree by computing nodal values throughout phylogeny. We employed the maximum likelihood (ML) method of ancestor reconstruction (Schluter *et al.*, 1997), which is the equivalent of the weighted squared-change parsimony method of Maddison (1991) (Webster & Purvis, 2002). We preferred ML over phylogenetically independent contrasts (Felsenstein, 1985) because the former is an algorithm specifically designed to estimate ancestral values whereas the latter only computes these values as intermediate steps in making biological observations statistically independent (Felsenstein, 1985). Further, preliminary analyses showed that differences between ML and independent contrasts ancestor values are small and the overall magnitude of the values and relative relationships are comparable; there are no significant differences between the ancestor values estimated by the two methods. Regardless of which method is employed, accurate branch durations are important (Laurin, 2004; Laurin *et al.*, 2009). This is because both methods assume a Brownian motion model of evolution, where rates of character change per unit branch should follow a normal distribution across the phylogeny (Garland *et al.*, 1992; Laurin, 2004; Laurin *et al.*, 2009). However, if evolution of our character does not follow the Brownian motion model or if our branch durations are somehow systematically biased, then this would yield undesirable statistical artefacts where there would be a lack of bivariate normality of contrasts and a lack of homoscedasticity of the residuals (Diaz-Uriarte & Garland, 1996). To test whether our branch durations meet the assumptions [or adequately standardize the contrasts of bite force and BFQ (Garland *et al.*, 1992)], we used the four diagnostic tests available in the PDAP module (Midford *et al.*, 2005) of MESQUITE (Maddison & Maddison, 2009). While contrasts of BFQ were adequately standardized, the same was not true for bite force; although it passed three of the four tests, there was a significant (albeit weak) positive correlation between the absolute value of the standardized contrasts and their estimated value of the base node. This implied that the larger nodal values were associated with unusually high contrasts, i.e. taxa with higher character values systematically evolve faster (the opposite situation to the example given in Laurin *et al.* (2009)). Varying branch durations using the Stratigraphic Tools package (Josse *et al.*, 2006) in MESQUITE as in Laurin (2004) or Laurin *et al.* (2009) with stratigraphic constraints taken from the International Stratigraphic Chart (2008) did not sufficiently improve the situation, so the whole tree was subjected to Grafen's ρ transformation (Grafen, 1989), which allowed for adequate standardization of the contrasts. Branch durations were only transformed for estimating ancestor values in bite force.

We computed ancestor values and their 95% confidence intervals using the APE library (Paradis *et al.*, 2004) in R. Ancestor estimates along with their respective 95% CI were plotted against time (in millions of years). The ancestor values estimated for bite force do not have the same relationship with time as BFQ does because of the transformed branch durations, but we plotted them against time as well, just for visual purposes. We did not perform any statistical analyses on ancestor estimates with regard to time or any other means of detecting trends, because our dataset is predominantly composed of extant taxa, there is no appropriate method to do this. A good approach would have been to follow Laurin (2004) and regress independent contrasts of bite force/BFQ against independent contrasts of taxon duration since clade origin, but because the majority of our taxa are extant, this is not possible. We did not test for correlation between ancestor values with respective clade rank (or node age) as in Carrano (2000), nor did we assess ancestor-descendant changes for directionality as in Butler & Goswami (2008). The latter was avoided because ancestor values in the ML method are estimated to minimise overall change (sum of squared changes) across phylogeny and as such, these changes tend to converge towards a distribution with a mean of 0, i.e. by default, we would expect to see no directionality.

Results

Phylogenetic eigenvector regression

PVR results indicate that roughly 80% of the variance in bite force can be explained by the 16.9 Ma phylogeny (multiple R^2_{29}: 0.793; adjusted R^2_{29}: 0.728; *p*: 9.515E-08; AIC_{11}: −12.7). Of the nine PCo axes, only PCo1, PCo3, PCo7 and PCo9 were significant as explanatory variables (Table 2), but a step function in R retained a further two axes, PCo5 and PCo6 to the model explaining 77% of the total variance (multiple R^2_{32}: 0.772; adjusted R^2_{32}: 0.730; *p*: 4.935E-09; AIC_8: −15.1). Adjusted R^2 values and AIC values indicate that elimination of nonsignificant variables does not greatly improve the model. Results for the 23.03 Ma phylogeny were nearly identical with very minor differences.

In BFQ, the 16.9 Ma phylogeny explains roughly 47% of the total variance (multiple R^2_{29}: 0.465; adjusted R^2_{29}: 0.299; *p*: 0.0169; AIC_{11}: −129), but only PCo8 has any significant input to the model (Table 2). A step function eliminated four axes and retained PCo2, PCo4, PCo6, PCo8 and PCo9 in the model. The reduced model explained roughly 44% of the variance in BFQ (multiple R^2_{33}: 0.443; adjusted R^2_{33}: 0.358; *p*: 0.0019; AIC_7: −136). Using the 23.03 Ma tree resulted in nearly identical results except after elimination of the same four predictor variables using the step function (multiple R^2_{33}: 0.416; adjusted R^2_{33}: 0.328; *p*: 0.00236; AIC_7: −134).

Table 2 Significance of predictor variable input to PVR models for absolute and relative bite forces. *P*-values with significance are reported for the full set of predictor variables (phylogenetic eigenvectors) and after a step-wise elimination procedure. Bold indicates the step at which a variable was eliminated through the step-wise elimination procedure in R. *P*-values are computed through 999 permutations in Permute.

Variable	Absolute bite force		Relative bite force	
	Full	Step-wise	Full	Step-wise
PCo1	0.00200**	0.00200**	0.852	**2**
PCo2	0.308	**2**	0.0800	0.0740
PCo3	0.0160*	0.0140*	0.822	**1**
PCo4	0.350	**1**	0.118	0.0960
PCo5	0.182	0.184	0.852	**3**
PCo6	0.0780	0.0820	0.0840	0.0760
PCo7	0.0100*	0.00800**	0.282	**4**
PCo8	0.378	**3**	0.00200**	0.00200**
PCo9	0.0120*	0.0140*	0.100	0.0900

Significant at: ***, 0.001; **, 0.01; *, 0.05.

Morphometric eigenvector regression

MVR results show that 99% of the total variance in bite force can be explained by morphology as represented by PC axes (multiple R^2_{33}: 0.988; adjusted R^2_{33}: 0.986; *p*: < 2.2E-16; AIC_8: −133). However, only PC1, PC2 and PC5 are significant variables (Table 3). PC1 is most likely size-associated morphology whereas PC2 is primarily the contrast between the length and width measurements, i.e. how foreshortened or elongated the skull is. PC5 primarily reflects the contrast between the width of the braincase and the width of the skull, and thus perhaps associated with the width of the cranial adductor chamber, i.e. the size of the adductor muscles. A step function did not eliminate any variables.

In BFQ, morphology explained only 38% of the total variance (multiple R^2_{33}: 0.38; adjusted R^2_{33}: 0.286; *p*: 0.00567; AIC_8: −132) and only PC2 having any significant input to the model (Table 3). A step function reduced the

Table 3 Significance of predictor variable input to MVR models for absolute and relative bite forces. *P*-values with significance are reported for the full set of predictor variables (phylogenetic eigenvectors) and after a step-wise elimination procedure. Bold indicates the step at which a variable was eliminated through the step-wise elimination procedure in R. *P*-values are computed through 999 permutations in Permute.

Variable	Absolute bite force		Relative bite force	
	Full	Step-wise	Full	Step-wise
PC1	0.00200**	–	0.376	**4**
PC2	0.0140*	–	0.00200**	0.00200**
PC3	0.140	–	0.412	**3**
PC4	0.0780	–	0.694	**2**
PC5	0.00400**	–	0.702	**1**

Significant at: ***, 0.001; **, 0.01; *, 0.05.

predictor variable to only include PC2 (multiple R^2_{37}: 0.346; adjusted R^2_{37}: 0.329; *p*: 8.183E-05; AIC_3: −137).

Phylogenetically structured phenotypic variance

Comparison of the three models using AIC reveals that Model 1 (AIC_{17}: −151) was the best of the three but only slightly better than Model 2 (AIC_{16}: −143), whereas Model 3 was considerably worse than the other two (AIC_{12}: −84.1). Therefore, only Models 1 and 2 were kept for variance partitioning.

Partitioning of bite force variance between phenotype and phylogeny reveals that much of the variance can be explained by phylogenetically structured phenotype. In Model 1, the proportions of variance explained by phenotype (partition a, size and morphology) was 20.3% whereas that explained by phylogeny (partition c) was negligible at 0.65%. On the other hand, the phylogenetically structured phenotypic variance (partition b, phenotype and phylogeny) composed the largest partition at 78.6%. Finally, the proportion of variance unexplained by the model (partition d) was almost nonexistent (0.44%). In comparison, with the exclusion of size from the phenotype (Model 2), partitioning of variance does not change much from that of Model 1: a (morphological variance), 20.2%; b (phylogenetically structured morphological variance), 78.7%; c (phylogenetic variance), 0.61%; and d (unexplained), 0.57%.

In BFQ, the partitioning of variance between phenotype (morphology) and phylogeny are as follows: a, 24%; b, 14%; c, 32.5%; and d, 29.5% (Fig. 3).

Environmentally structured variance in bite force

Prey size can explain roughly 70% of the total variance in bite force (R^2_{33}: 0.688; *p*: 7.55E-10) on its own. However, with the addition of other variables, cat body size, morphology and phylogeny, prey size loses any significance to the overall model (Table 4). In fact, a stepwise elimination procedure would ultimately eliminate this variable altogether. An AIC-based comparison reveals that in the presence of other predictor variables, the addition of prey size has negligible impact on the AIC value (without prey size, AIC_{16}: −141.2; with prey size, AIC_{17}: −141.4).

BFQ on the other hand has no linear correlation with either relative prey size (R^2_{33}: 0.0324; *p*: 0.301) or absolute prey size (R^2_{33}: 0.0547; *p*: 0.176). Spearman rank correlation tests also reveal no correlations between BFQ and relative prey size (ρ_{33}: 0.238; *p*: 0.169) or absolute prey size (ρ_{33}: 0.210; *p*: 0.227).

Bite force evolution in Felidae as assessed by character optimization

The evolution of absolute bite force (represented by F_{Corr}) and relative bite force (BFQ) was optimized onto

Table 4 Significance of predictor variable input to the multiple linear model of absolute bite force against cat body size ($\log_{10}M_{Body}$), absolute prey size ($\log_{10}M_{Prey}$), morphology and phylogeny. *P*-values with significance are reported for the full set of predictor variables and after a step-wise elimination procedure. Bold indicates the step at which a variable was eliminated through the step-wise elimination procedure in R. *P*-values are computed through 999 permutations in Permute.

Variable	Full	Step-wise
$\log_{10}M_{Body}$	0.270	0.064
$\log_{10}M_{Prey}$	0.268	**7**
PC1	0.00200**	0.00200**
PC2	0.0280*	0.006**
PC3	0.294	**4**
PC4	0.460	**3**
PC5	0.00400**	0.00200**
PCo1	0.376	**6**
PCo2	0.292	0.644
PCo3	0.696	**5**
PCo4	0.104	0.138
PCo5	0.104	0.112
PCo6	0.848	**2**
PCo7	0.0660	0.032*
PCo8	0.988	**1**

Significant at: ***, 0.001; **, 0.01; *, 0.05.

phylogeny using ML ancestor reconstructions on log-transformed values (Figs 4 and 5; Tables S9 and S10). Absolute bite force for the common ancestor of Felidae was estimated at 534 N with lower and upper 95% confidence intervals of 379 and 752 N, respectively. Bite force for the common ancestor of all crown-group felines (Felinae) was estimated to be 461 N (95% confidence intervals: 371 ∼ 572 N). However, 95% confidence intervals for these two ancestor estimates overlap extensively and there is no significant change. There is a clear divergence in bite force at the split between the *Panthera + Neofelis* clade (or the *Panthera* lineage sensu Johnson *et al.* (2006)) and all other crown felines; the common ancestor of *Panthera* and *Neofelis* is estimated to have had a significantly higher bite force at 709 N (95% CI: 564 ∼ 890 N) compared to the common ancestor of all

other crown-group felines with an estimated bite force value at 393 N (95% CI: 325 ∼ 475 N).

Within the *Panthera + Neofelis* clade, there is a definitive increase towards the [(*P. atrox*, *P. leo*), *P. onca*] subclade when compared to the common ancestor of the *Panthera + Neofelis* clade. All ancestor values are significantly higher than those within the other crown feline clades, with the exception of ancestor estimates in the Puma clade (or Puma lineage sensu Johnson *et al.* (2006)).

The Puma clade exhibits increased ancestor values compared to those of the other non-*Panthera + Neofelis* feline clades, but overlaps in 95% confidence intervals show no significant differences among these ancestor values. Terminal values of this clade (with the exception of *Puma yagouaroundi*) on the other hand all fall outside of the range of its sister clades, i.e. Domestic Cat and Leopard Cat clades, but fall within the range of the *Panthera* and *Neofelis* taxa (Fig. 4). Ancestor estimates within all other clades are not significantly different from each other, and terminal values typically occupy a vast range in bite force value. *Smilodon fatalis* overlaps with values of the *Panthera + Neofelis* clade.

Relative bite force, BFQ, on the other hand is reconstructed at the base of Felidae to be lower than average at 96.2, but associated error is large (95% confidence intervals: 84.5 ∼ 110) encompassing the average value of 100. BFQ at the common ancestor of crown felines is estimated at 102 (95% CI: 94.2 ∼ 110) and not significantly different from that of Felidae. All ancestor estimates within Felinae have excessive overlapping in 95% confidence intervals and show a typical funnel-shaped pattern of increasing variance associated with increasing number of nodes up the tree. *Smilodon fatalis* is outside the range of all feline taxa with the exception of *Lynx pardinus* that has the lowest BFQ.

Discussion

In this study, we have explored the relative roles of phylogenetic and nonphylogenetic structures in bite force, a functional character of cats that summarizes a
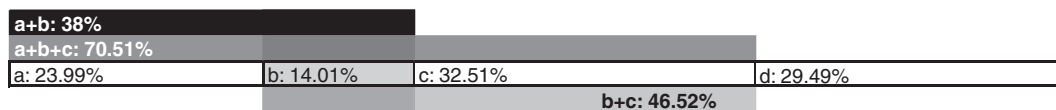

a+b: 38%			
a+b+c: 70.51%			
a: 23.99%	b: 14.01%	c: 32.51%	d: 29.49%
	b+c: 46.52%		

Fig. 3 Variance partitioning using PVR. Variance in absolute bite force or relative bite force (BFQ) can be partitioned between phenotypically and phylogenetically structured portions. Partition a is the proportion of variance explained by phenotype alone, whereas partition c is the proportion of variance explained by phylogeny alone. On the other hand, partition b represents the phylogenetically structured phenotypic variance (shaded). Lastly, partition d is the proportion of variance unexplained by the current model. The example here is the partitioning of variance in BFQ, with the total variance represented as the whole length of the open bar further compartmentalized into the above four partitions. The black bar represents the total amount of variance in BFQ explained by morphology (partitions a + b; R^2 from MVR results). The light grey bar represents the total amount of variance explained by phylogeny (partitions b + c; R^2 from PVR). The dark grey bar represents the total amount of BFQ variance explained by both morphology and phylogeny (partitions a + b + c) or the R^2 value of a multiple linear regression of BFQ against morphology and phylogeny combined.

Fig. 4 Bite force optimized onto phylogeny (16.9 Ma tree) using the maximum likelihood ancestor estimation method. Bite force was optimized onto the 16.9 Ma tree with Grafen's ρ (Grafen, 1989) transformed branch durations using the ML method in the ape library of R. The ancestor estimates and 95% confidence intervals are plotted against time. Grafen's ρ transformation (Grafen, 1989) results in disassociation of branch durations with time, but nonetheless ancestor values are plotted against time for visual purposes only. Error bars indicate upper and lower 95% confidence intervals of each ancestor estimate and represent uncertainties associated with the estimation. Note that 95% confidence intervals get wider deeper down the tree. Node symbols reflect clade (lineage) membership according to Johnson *et al.* (2006) (see Fig. 2).
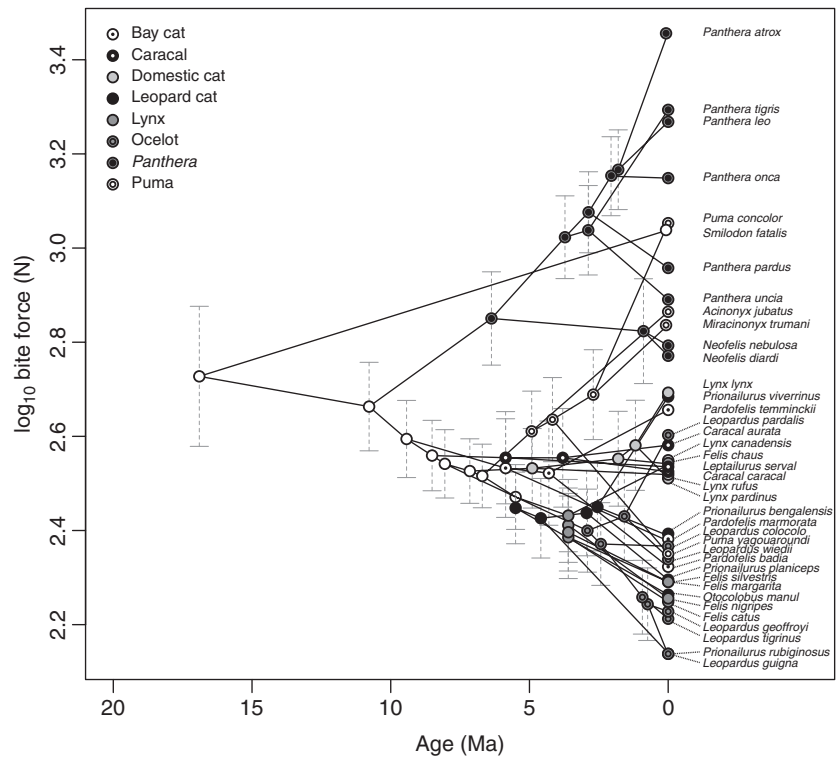

Fig. 5 BFQ optimized onto phylogeny (16.9 Ma tree) using the maximum likelihood ancestor estimation method. BFQ was optimized onto the 16.9 Ma tree using the ML method in the ape library of R. The ancestor estimates and 95% confidence intervals are plotted against time. Error bars indicate upper and lower 95% confidence intervals of each ancestor estimate and represent uncertainties associated with the estimation. Note that 95% confidence intervals get wider deeper down the tree. Node symbols reflect clade membership according to Johnson *et al.* (2006) (see Fig. 2).
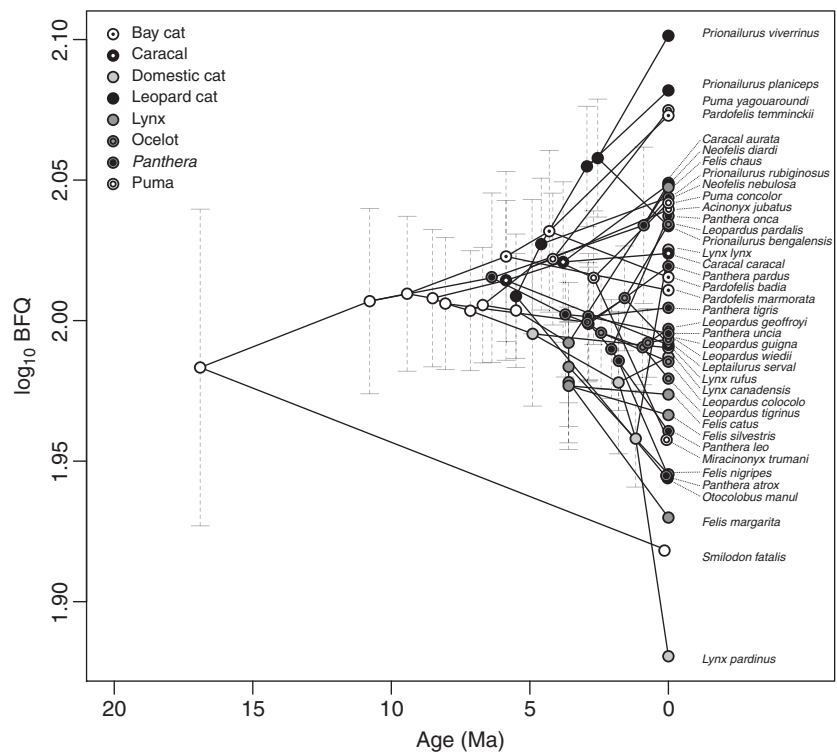
great number of anatomical attributes. Importantly, we show that the results can depend on how the functional complex is measured: relative bite force is randomly distributed across phylogeny whereas absolute bite force diverges according to clade. This shows that body size is critically important here, in that different subclades

exhibit different mean body sizes and that the phylogenetic constraint working on body size also ultimately works on bite force.

Phylogenetic constraint in biting performance

An important but not clearly defined nor well-understood feature of evolution is phylogenetic constraint. Evolution of a certain trait can be considered phylogenetically constrained when a certain trait state is shared across a broad sample of closely related taxa, e.g. a synapomorphy, and when that trait state seemingly persists throughout the group's evolutionary history with little or no change. On the other hand, one could also argue that constraint is observed as the rate of change that closely mirrors phylogeny; change is proportional to branch durations, and differences among terminal values can be explained by phylogenetic distance. In this case, we would be observing changes in character states from the ancestral condition (e.g. increase in bite force), but these changes could be slow and more similar in closely related taxa than between distantly related taxa, i.e. mirroring phylogeny. Phylogenetically conserved traits could be constrained or they could exhibit inertia. The former is a situation where changes from one state to another are difficult (e.g. developmentally/genetically constrained; tetrapods typically do not sprout a third pair of limbs), while a possible cause for the latter is simply a retention of characters because there are limited selective pressures for change. The underlying factor influencing evolutionary change, i.e. constraint or inertia, perhaps can never be identified, but the effect of phylogenetic conservatism can be observed.

In morphofunctional character complexes, in our case exemplified by simple biomechanical models, phylogenetic conservatism, if any, can be attributed to constraints such as the difficulties of moving one muscle complex from attachment point A to another attachment point B perhaps further along the jaw towards the rostrum. A muscle attachment point B would exert higher mechanical advantage, thus enabling more efficient biting (assuming that the distance to the biting point remains the same), but a muscle attachment simply cannot jump from point A to point B, but occurs as a slow migration (or a gradual shift in the population mean of the attachment location) over many generations. Similarly, the length of the tooth row and consequently the palatal/facial lengths cannot change readily without numerous anatomical changes in associated craniofacial skeletal elements. One cannot simply increase or decrease biting moment arms without affecting other vital organs, such as the volume of the sinus or field of vision, which would potentially have some serious consequences.

The dominance of phylogenetically structured phenotypic variance in absolute bite force indicates a strong phylogenetic signal and thus inertia in phenotypic changes associated with this biomechanical variable. However,

because our morphometric variables still incorporate size information (PC1 is greatly associated with size and explains most of the morphological variance), and as Diniz-Filho & Nabout (2009) report that 65% to 67% of the variance in felid body mass is explained by phylogeny, the inertia or constraint put upon the evolution of absolute bite force is perhaps mostly because of whatever evolutionary constraint that works on body size evolution. Indeed, skeletal muscle mass has been observed to represent a constant proportion of total body mass (Calder, 1984; Schmidt-Nielsen, 1984), and muscle force has been also argued to be constrained by size increase at a theoretical scaling factor of $2/3$ [or $M^{0.67}$ where M is body mass (Calder, 1984; Schmidt-Nielsen, 1984; Swartz & Biewener, 1992)]. Because the greatest source of variation in bite force is attributed to muscle force and fundamentally muscle cross-sectional areas (the absolute values of this parameter greatly exceed that of mechanical advantage by up to five orders of magnitude), bite force would also theoretically be constrained by size increase with a certain scaling factor closely following that proposed for muscle force [e.g. $M^{0.66}$ (Huber *et al.*, 2005)].

The residual variance in bite force after size is taken into account, i.e. relative bite force or BFQ shows reduced phylogenetic signal, and the remaining variance is attributed to morphology (albeit only a quarter) and unknown sources. Thus, once size is of no concern, then the evolution of the morphofunctional character complex associated with biting performance is relatively free of phylogenetic constraint and character values for terminal taxa are not so much governed by their phylogenetic positions; character optimization also shows a rather random distribution of BFQ values across phylogeny. This opens up possibilities that taxon-specific variation as a result of individual evolutionary history is causal to the nonphylogenetic variance in BFQ. However, whatever this taxon-specific variation may be attributed to, morphology as currently quantified in this study was insufficient as the primary source of variance because the morphometric partition of BFQ variance is relatively small.

Other potential sources, absolute and relative prey sizes, fail even more miserably; BFQ variance is not explained by these variables at all. This illustrates difficulties in pinning down a single or multiple causal factors for morphofunctional variance from its possibly complex and even convoluted interaction with the environment. For instance, relative prey sizes are largest (and extremely large at that) for the middle-sized cats, in particular *Lynx lynx*, *L. rufus* and *Neofelis* (albeit some ambiguity regarding maximum prey size for *Neofelis*). While both species of *Neofelis* have marginally high BFQ values (112 and 111, respectively, for *N. diardi* and *N. nebulosa*), the two *Lynx* species do not; in fact they have typical bite forces for cats of their sizes. The ability to take down such enormous prey thus does not seem to be determined by the ability or inability to bite much harder than other cats of similar sizes. This is counter to

what we would expect intuitively. For instance, for *L. rufus* to kill prey that can be up to 10 times larger than itself in body mass, this cat must be able to penetrate the ever thicker and tougher skin that necessarily increases with larger prey size and still be able to bite down deep enough to inflict a fatal bite, whether to suffocate the prey or to cause haemorrhaging (Sunquist & Sunquist, 2002). A higher biting force would make this task easier for a much smaller predator. On the other hand, both *Lynx* and *Neofelis* species are known to have a wide taxonomic range of prey items, and although much less is known for *Neofelis*, at least in *Lynx*, large prey hunting seems to be out of necessity rather than by preference, i.e. during the winter. So, perhaps predator-prey dynamics is not as simple as being 'the ability to kill relatively large prey is determined by a 'better' biomechanical performance', but more like, 'the ability to kill large prey is determined by food availability and how desperate the predator would be'. In more specific terms, morphofunctional character complexes and biomechanical performance may not be the limiting factors to prey procurement but the desperation for food may in fact be forcing a predator to perform to the maximum or even beyond the capabilities predicted by biomechanical modelling. However, the uncertainties and limited information regarding prey sizes may be obscuring some otherwise present signal. Furthermore, discrepancies between our results and those of previous studies regarding correlation between biting performance and ecology (Wroe *et al.*, 2005; Christiansen & Wroe, 2007) could very well be attributed to the wider taxonomic coverage of the latter studies (i.e. Carnivora as opposed to just Felidae) and possibly even a wider range of bite force relative to body size; i.e. the range of felid relative bite force may be more confined when compared to that in Carnivora as a whole. After all, more broad-scale studies encompassing widely varying skull morphologies across Carnivora will necessarily capture a greater range of phenotypic variance that may very well better match that of ecology. However, unlike these previous studies we use continuous values for the ecological variable (in the sense of prey size), and this may in fact be the reason for the discrepancy.

Another potential source of variation in relative bite force that is not explored here is the unknown effect of size-associated or allometric shape change. Even after standardization of size, as employed in geometric morphometrics, allometric shape change can be observed in the skulls of various tetrapods including felids (Christiansen, 2008; Slater & Van Valkenburgh, 2008). While the mechanisms of allometric shape change (i.e. the what and the how) are well documented, its causes (i.e. the why) are currently not understood. Whatever the cause, the presence of allometric shape change in felid crania indicates that size-independent measures of biting performance, i.e. BFQ, may be influenced in part by this unknown allometric factor, despite the elimination of

size influence. Thus, some of the unexplained variance in BFQ could potentially be attributed to such unknown and poorly understood underlying biological factors; our measurement-based morphometrics is insufficient to explain much of the variance in BFQ, and future research should facilitate landmark-based morphometrics to test their impact on the residual variance in BFQ. Overall, it is extremely difficult to identify and pinpoint the causal factor(s) of the nonphylogenetically structured variance in biomechanical performance, i.e. BFQ.

Adaptations and convergence

Regardless of the strength of the phylogenetic signal in phenotypic traits, character evolution can be a result of adaptations; traits undergo natural selection and evolve as adaptations to environmental pressures, whether rapidly without much constraint (as in adaptive radiation) or slowly with some form of constraint as discussed earlier. As biting performance is directly linked with feeding function and thus more likely contributes to survival (i.e. biting performances suited for certain niches increases fitness), it is reasonable to assume that this biomechanical trait would be under selection and thus can be regarded as an adaptation. However, as noted previously, our analyses indicate that there are no correlations between ecology/environment (absolute and relative prey size) and biting performance (both absolute and relative bite force), indicating rather strongly that biting performance is not an adaptation as a response to prey size. This does not, however, preclude the possibilities that biting performance is an adaptation to some sort of selection, it merely cannot be explained by prey size. Perhaps biting performance is an adaptation towards other aspects of feeding ecology or behaviour, for instance, hunting techniques, average time in killing prey or biting/chewing cycles. Frequent and repetitive use of a certain biting action over another would presumably work as a selection pressure for adaptations in morphofunctional complexes that are well suited for that performance. Assuming that biting performance quantified as BFQ is an adaptation for a specific feeding behaviour, we can see that such adaptations in Felidae are relatively randomly distributed across phylogeny (ancestor reconstructions converge towards an average value; Fig. 5) and cluster into vague groupings (Fig. 5) regardless of taxonomic affinities, and thus we see convergences in BFQ values. For instance, there is a tight clustering of ten cats with relatively high BFQ values that includes seven of the eight major feline lineages (sensu Johnson *et al.*, 2006). Because such clusters are formed regardless of phylogeny, they can represent convergences into different morphofunctional niches with regard to biting performance. A notable convergence in biting performance is that between the Lion *Panthera leo* and the extinct North American Cheetah-like Cat *M. trumani*. Interestingly, *M. trumani* is

disparate in BFQ from its presumed present-day ecological analogue, Cheetah *Acinonyx jubatus*, and instead morphofunctionally convergent with Lion. Another interesting convergence is that among the extinct American Cave Lion *P. atrox*, black-footed Cat *Felis nigripes* and Manul *Otocolobus manul*. In either case, the extinct taxa are convergent with taxa with lower-than-average BFQ. On the other hand, the extinct sabre-toothed cat *S. fatalis* falls outside the BFQ radiation of the crown-group felines (with the exception of *L. pardinus*) and occupies a unique morphofunctional niche, consistent not only with its disparate craniofacial morphology compared to modern felids but also with results of previous studies (Wroe *et al.*, 2005; McHenry *et al.*, 2007). This implies that the use of jaw adduction in generating biting force was quite different in *S. fatalis* compared to crown felines, and as suggested elsewhere (Akersten, 1985; McHenry *et al.*, 2007), that *S. fatalis* probably did not rely heavily on jaw muscle-driven bite force in driving their hypertrophied canines into prey but perhaps on significant contributions from cervical muscle activity.

Detecting phylogenetic signals and testing evolutionary hypotheses

Using a phylogenetic framework has become common practice in studying comparative data and evolutionary biology (Harvey & Pagel, 1991). It is important to note that biological observations on any level are not statistically independent because of shared history (Felsenstein, 1985) and comparative methods help to account for the unknown amount of phylogenetic influence inherent in comparative data (Harvey & Pagel, 1991). Testing certain evolutionary hypotheses such as those pertaining to body size (e.g. Bergmann's rule or Cope's rule) also must be conducted within a phylogenetic framework (e.g. as in Laurin (2004) for testing Cope's rule); a notable increase in body size associated with latitude or time may in fact just be attributed to some underlying phylogenetic relationship, i.e. geographical or temporal distribution may be highly correlated with phylogeny that may in turn also be correlated with body size. The distribution of character traits and its evolution within a taxonomic group of animals can also be studied under a phylogenetic framework, such as by optimization of character evolution on a phylogeny. This can test, for instance, whether a widely distributed phenotypic character (e.g. high bite force) is derived or primitive within that group and further allows one to trace the evolutionary history of a character through phylogeny. However, it is imperative that the presence of a phylogenetic signal be tested in the comparative data a priori. If there are no phylogenetic signals in the data, then there is no reason to believe that phylogenetic influence would affect the outcome of comparative or evolutionary analyses but worse yet may even introduce some undesired errors instead, e.g. the unreliability of ancestor reconstructions where there are no phylogenetic signals detected for the character trait in question (Laurin, 2004). The various methods for detecting phylogenetic signals are reviewed in detail elsewhere (Diniz-Filho *et al.*, 1998; Laurin, 2004), and their accuracy in detecting phylogenetic signal is beyond the scope of this study, but it is worthy of note here that regardless of the reliability of certain methods over others (e.g. regression approaches over Mantel test) and differences in the results, it is vitally important to be able to use such tests to quantify the proportion of variance in the phenotypic data that can be attributed to phylogeny. Then and only then, we can fully appreciate the variation in the data that can be attributed to other factors such as species-unique phenotypic traits or ecology.

Acknowledgments

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Supporting information

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

Appendix S1 Supporting figures.

Appendix S2 Institutional abbreviations.

Appendix S3 Dry skull method.

Appendix S4 Literature cited in supporting information.

Table S1 Craniofacial and biomechanical measurements per specimen. Measurements are in mm except for cross-sectional area (CSA) which are in mm^2.

Table S2 Dry skull bite force estimates (F_{DS}), corrected bite forces (F_{Corr}), bite force predicted from skull width (F_{Wsk}), and relative bite force BFQ for each specimen.

Table S3 Age range of terminal taxa in millions of years (Ma).

Table S4 PCo scores extracted from a phylogenetic distance matrix of the 16.9 Ma felid tree.

Table S5 PCo scores extracted from a phylogenetic distance matrix of the 23.03 Ma felid tree.

Table S6 Craniofacial measurements (mm) and principal components scores per species.

Table S7 Minimum and maximum prey size, midpoint prey size, and relative prey size for each species of cat. Unless otherwise specified, prey item and prey size data are compiled from Nowak (2005) and Nowak (1991) respectively. Missing data: NA.

Table S8 PCo scores extracted from a phylogenetic distance matrix of the reduced-taxa 16.9 Ma felid tree.

Table S9 Log10 bite force ML ancestor reconstruction and 95% confidence intervals using the 16.9 Ma tree. Node numbers correspond to those on Figure S2.

Table S10 Log10 BFQ ML ancestor estimates and 95% confidence intervals using the 16.9 Ma tree. Node numbers correspond to those on Figure S2.

Table S11 Log10 bite force ML ancestor reconstruction and 95% confidence intervals using the 23.03 Ma tree. Node numbers correspond to those on Figure S2.

Table S12 Log10 BFQ ML ancestor reconstruction and 95% confidence intervals using the 23.03 Ma tree. Node numbers correspond to those on Figure S2.

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