

Ecomorphology of Carnivora challenges convergent evolution

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Abstract Convergent evolution is often reported in the mammalian order Carnivora. Their adaptations to particularly demanding feeding habits such as hypercarnivory and durophagy (consumption of tough food) appear to favour morphological similarities between distantly related species, especially in the skull. However, phylogenetic effect in phenotypic data might obscure such a pattern. We first validated the hypotheses that extant hypercarnivorous and durophagous large carnivores converge in mandibular shape and form (size and shape). Hypercarnivores generally exhibit smaller volumes of the multi-dimensional shape and form space than their sister taxa, but this pattern is significantly different from random expectation only when hunting behaviour categorisations are taken into account. Durophages share areas of the morphospace, but this seems to be due to factors of contingency. Carnivores that hunt in pack exhibit incomplete convergence while even stronger similarities occur in the mandible shape of solitary hunters due to the high functional demands in killing the prey. We identified a stronger phylogenetic signal in mandibular shape than in size. The

quantification of evolutionary rates of changes suggests that mandible shape of solitary hunters evolved slowly when compared with other carnivores. These results consistently indicate that the need for a strong bite force and robust mandible override sheer phylogenetic effect in solitary hunters.

Keywords Mandible shape · Durophagy · Hypercarnivory · Morphological disparity · Feeding adaptation · Constraint

Introduction

Members of the mammalian order Carnivora exhibit an extraordinary diversity of forms, both in extant and extinct clades (Ewer 1973; Goswami 2010). Although this diversity unfolds across a number of different ecomorphotypes, most carnivores are basically meat eaters that share a common digestive physiology (Clauss et al. 2010). As such, it is not surprising that patterns of iterative evolution are reported over and over in describing the evolution of carnivore skull and dental morphologies (Van Valkenburgh 1991, 1999, 2007). The main factor sought to explain carnivore skull shape differentiation is the adaptation to different feeding habits including hypercarnivory, herbivory and hard food consumption (Ewer 1973; Van Valkenburgh 1989, 1991; Holliday and Stepan 2004; Raia 2004; Goswami 2006; Figueirido et al. 2010, 2011, 2013). Meat and tough food consumption requires extreme modifications of the skull apparatus (cranium and the mandible) to withstand the high biomechanical loads imposed by catching and holding live prey and crushing and cracking hard food items such as bones. Through evolutionary time, these mechanisms favoured ecomorphological overlap between distantly related species (e.g. the giant and the lesser panda for feeding on bamboo, Figueirido et al. 2010) so that

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convergence (sensu Futuyma 2010) was repeatedly reported for the skull phenotype.

Detecting convergence presents many methodological issues especially when analysing multivariate data. Usually, skull phenotypic variation is simplified by multiple measurable traits so that the empirical morphospace they define can be explored with a multiple combination of vectors, which in turn allow testing for convergence. To avoid spurious interpretation of morphospace overlap, morphological disparity (volume of the multivariate morphospace) is tested for three different kinds of convergence as defined by Stayton (2006):

- i. Complete convergence implies overlap in the morphospace by the putatively convergent taxa;
- ii. Incomplete convergence applies when putatively convergent taxa occur in different areas of the morphospace (i.e. they do not overlap) but are closer to each other than their relatives are.
- iii. Parallel changes imply taxa showing parallel phenotypic changes away from their sister groups. In this case, convergent taxa do not share common areas of morphospace but share similar pathways of phenotypic transformation.

Our aim is to test for the presence of these three different kinds of convergence in mandibular size and shape (defined as morphospace, Bookstein 1989) of extant large carnivorans as due to previously identified adaptations (see Figueirido et al. 2011, 2013) that include: hypercarnivory (functionally defined as a diet including a high percentage of meat) or durophagy (a diet with substantial consumption of tough food, i.e. bamboo or bones). The mandible is favoured over the cranium here because this anatomical structure is entirely devoted to mastication and intimately linked with feeding habits in Carnivora (Raia 2004; Meloro 2011a; Meloro and O'Higgins 2011; Prevosti et al. 2012). Morphospace overlap between distantly related extant and fossil species has already been detected in the carnivoran mandible shape (Figueirido et al. 2011, 2013), yet no formal test for convergence was presented so far. We limited our data to large (body mass >7 kg, Van Valkenburgh 1985, 1988—except the lesser panda *Ailurus fulgens*, a tough food consumer, cf. Meloro 2011a) extant species because there are unequivocal data about their feeding habits, and also because they represent a set of highly interacting species with similar metabolic requirements that share mandibular shape traits (Meloro and O'Higgins 2011). By using geometric morphometrics (Adams 2014a; Adams et al. 2013), we analyse both size and shape of the mandible separately and in conjunction. Meloro and Raia (2010) found evidence for different rates of evolution in size and shape of the lower carnassial tooth (m1), and a similar pattern is expected for the mandible. If mandibular size and shape evolve at different rates, convergence might not be detected in the same way for size and shape space. Additionally, we included

explicitly phylogenetic relationships into our analyses, in order to control for the impact of shared ancestry in patterns of morphospace occupation and functional differences.

Materials and methods

We collected size and shape data for 307 mandibular specimens of 57 species of extant Carnivora from both suborders Caniformia and Feliformia (cf. sample in Meloro 2011a). Two-dimensional coordinates of 14 anatomical landmarks were digitised as described in Meloro (2011a, 2012; Meloro and O'Higgins 2011). The landmarks describe relative tooth positioning in the corpus (canine, premolar area, slicing vs crushing molar area) and the ramus mandibulae (see [Electronic supplementary material](#) for details). Size data were extracted using the natural log transformed centroid size from each landmark configuration (Bookstein 1989). Generalised Procrustes analysis was applied to extract shape coordinates (Rohlf and Slice 1990) on the overall sample and then averaged per species in order to generate an evolutionary morphospace that includes 57 data points (see Meloro et al. 2008). Averaged shape coordinates for the 57 carnivoran species were subjected to a principal component analysis (PCA). We identified two distinct morphospaces: the shape space defined by PC axes of all shape variables, and the form space defined by a PCA of all shape coordinates and the natural log transformed centroid size. The form space has been rarely employed (see O'Higgins and Jones 1998), and it is here explored to identify the impact of mandible size on patterns of morphospace occupation. The two distinct morphospaces defined by PC scores were first explored graphically to detect patterns of ecomorphological overlap between distantly related species (those belonging of different suborders) sharing similar feeding adaptations.

Feeding adaptations were functionally identified according to previously suggested categorisations that were likewise applied to test for morphological convergence in the skull and the mandible of Carnivora (Van Valkenburgh 1991, 2007; Figueirido et al. 2011, 2013). Hypercarnivores include taxa adapted to high consumption (>75 %) of meat: large felids and hyenas (all except the aardwolf *Proteles*) and the fossa (among Feliformia), the polar bear, the wolverine (*Gulo gulo*) and the extant large canids (i.e. *Canis lupus*, *Cuon*, *Lycaon* and *Speothos*) among Caniformia. For consistency with previously identified convergence patterns, the hypercarnivores were subdivided also into pack hunters (all large wild canids and the spotted hyena, *Crocuta crocuta*) and solitary hunters (all felids, the fossa, the polar bear and the wolverine, see Figueirido et al. 2011). Together with hypercarnivory, we also wanted to test for convergence due to the consumption of tough food. In keeping with findings of Figueirido et al. (2013), we here define durophages as all the specialised bone

crackers and bamboo feeders including the spotted, the brown and the striped hyenas among the extant feliforms and the giant and lesser panda among the caniforms. Although bamboo and bones are different in composition, they have similar biomechanical properties (e.g. Young module), hence they both require high bite forces to be broken down during chewing (see Figueirido et al. 2013).

We formally tested for convergence due to hypercarnivory, pack or solitary hunting and durophagy by using morphological disparity test and analysis of covariance (ANCOVA) models, in keeping with Stayton's (2006) three types of convergence (see Fig. 3 in Stayton 2006, p. 828). The disparity test was employed to check for either complete or incomplete convergence. ANCOVA models using permutation tests (with and without phylogenetic correction, Adams and Collyer 2015) were applied to test for parallel changes in mandible shape between the putatively convergent feeding groups. The form data were not used for these analyses because the ANCOVA models are explicitly based on Procrustes distances.

The disparity test is based on the multidimensional convergence index (MCI) computed as the ratio of morphological disparity of the sister taxa to the morphological disparity of the potentially convergent species (those belonging to hypercarnivore/pack/solitary or durophagous dietary groups). $MCI > 1$ implies convergent species to occupy an area of the morphospace much smaller than that defined by their sister groups (Stayton 2006).

Significance in MCI was assessed by comparing the observed MCI vs randomly generated MCIs obtained by assigning the same number of species to convergent or non-convergent categories at random within clades (Stayton 2006). To test for the complete convergence, the random draw is generated extracting species from the overall sample, whereas for the test of incomplete convergence, the randomization is applied to the subsample of convergent plus their sister taxa only.

In order to identify sister taxa of species in different feeding groups, we generated a phylogenetic tree inclusive of the 57 large carnivoran species using the 10k tree project database (Arnold et al. 2010). The resulting topology and branch lengths (time of divergence in millions years) are based entirely on molecular datasets that were statistically treated using Bayesian phylogenetics (see [Electronic supplementary material](#)).

This phylogeny was also employed to test for the strength of phylogenetic signal in both mandible size and shape using the K statistic and its multivariate extension (Adams 2014a). K is a measure of phylogenetic signal that can vary between zero, 1 and more than 1. K equal to zero occurs when the structure of phenotypic data fit a star phylogeny (i.e. no phylogenetic signal), $0 < K < 1$ suggests that phylogenetic signal is present in the data with closely related species resembling each other less than expected by Brownian motion model,

and $K > 1$ support a strong phylogenetic structuring with close relatives being more similar than based on Brownian motion (Blomberg et al. 2003). Statistical significance of K was validated using randomization. Although K was designed for single continuous traits, Adams (2014a) recently developed a multivariate extension that we applied to mandibular size and shape data using the R package geomorph (Adams and Otárola-Castillo 2013). We also wrote an R script (available from the authors on request) to calculate MCI based on the disparity metric defined as averaged squared distances of multivariate data (Harmon et al. 2003) and to compare the observed MCI to the random MCIs computed in 2000 permutations.

ANCOVA models were applied to test for significant interaction in mandible shape data between the factors taxonomy (suborders Caniformia vs Feliformia) and diet (hypercarnivores/pack/solitary or durophages vs others). Adams and Collyer (2015) recently presented a way to apply this model to high-dimensional shape data by providing a permutation test for significance. Essentially, the ANCOVA model is based on procrustes distances rather than on PC scores of the shape space (Goodall 1991). The permutation test for significance (9999 runs) was applied before and after phylogenetic correction to take the phylogenetic covariance matrix explicitly into account as an error term (D -PGLS). Non-significant interaction between taxonomy and diet categories supports parallel evolution according to Stayton's (2006) definition.

The impact of size on mandibular shape was also tested using linear and phylogenetic generalised least square models to detect the degree of association between the two traits (again shape was quantified as procrustes distances with this model being equivalent to the multivariate regression of shape scores as dependent variables vs natural log centroid size as independent, cf. Meloro et al. 2008; Figueirido et al. 2010, 2013; Prevosti et al. 2012).

To provide an evolutionary interpretation of the dynamics of morphospace occupation by putatively convergent species, we, finally, computed phylogenetic evolutionary rates for shape data according to Adams (2014b). Phylogenetic evolutionary rates are described by the σ^2 statistic that quantifies the rate of accumulation of variance in a trait over time while accounting for the phylogenetic relationships among species. Adams (2014b) developed a multivariate extension of σ^2 that is relatively independent of sample size. To test for significant differences in the rates observed by two hypothetical groups, σ^2_{multiv} is computed independently for both of them and combined into a ratio. The observed ratio is compared with simulations that randomise the data on the phylogeny. If the observed ratio is greater than 1, it means that groups show distinct evolutionary rates (e.g. hypercarnivores evolve faster/slower than the other taxa). We applied this procedure to feeding groups and mandible data that showed evidence for convergence.

Results

Mandible shape

The first two principal components (PC1=39.96 % and PC2=27.34 % variance) of mandibular shape data clearly show a separation between Caniformia and Feliformia related to relative elongation and thickening of the corpus and the ratio of molar slicing vs crushing area (Fig. 1a). Along PC1, hypercarnivore taxa tend to occupy more positive scores while durophages caniforms and feliforms are clearly at opposite sides of PC2.

Within the hypercarnivores, we also note a separation between pack hunters, which seem to occupy smaller region of PC1 vs PC2 morphospace, and solitary hunters.

A combination of PC2 vs PC3 (10.61 % of variance) reveals little separation among the feeding categories although durophages exhibit much higher scores in PC3 than all the other species. This axis describes changes in premolar row and relative height of the ramus (Fig. 1b).

MCI values based on all-shape PC scores are generally much larger than 1 when looking at hypercarnivores vs their sister taxa, while values smaller than 1 occur for durophages. The permutation tests yield significance in the hypercarnivorous solitary hunters, while pack hunters have significantly smaller volumes than their sister taxa only when randomization test is computed using a subsample that includes them and their sister taxa (Table 1). This supports complete convergence only in solitary hunters and incomplete convergence in pack hunters. Both the broad category of hypercarnivores (inclusive of all hunting types) and durophages exhibit no convergence. ANCOVA models show in some cases substantial changes in *P* values with and without phylogenetic correction (Table 2). In general, parallel shape changes between caniforms and feliforms occur among solitary hunters (in all cases, ANCOVA shows no interaction between this feeding categorization and suborder). For pack hunters, ANCOVA supports a non-significant impact of this factor on mandible shape data while in both hypercarnivore and durophages, the interaction term between diet and suborder remains significant after phylogenetic correction.

Mandible form

When size is associated to shape data, the empirical morphospace changes patterns of specimen distributions (Fig. 2). The first two PC axes (PC1=30.00 % of variance and PC2=20.15 %) identify a significant overlap between Caniformia and Feliformia that are better separated by a combination of PC1 vs PC3 (15.17 % of variance). The PC1 describes shape changes related to the shortening or enlargement of the premolar area with small canids and viverrids occupying positive scores. On PC2, major shape deformations occur in slicing vs crushing molar areas with hypercarnivores

Fig. 1 Scatter plot of the first vs second principal component (PC) (a) and the second vs the third PC (b) derived from a sample of 57 carnivoran mandible shape coordinates. Transformation grids visualise shape deformations relative to the mean at the positive and negative extremes of PC axes. Specimens are labelled according to suborder (circle, Caniformia; triangle Feliformia). The extreme feeding categories are coloured in black (solitary hypercarnivores), dark grey (pack hunters) or light grey (durophages); the area solitary hypercarnivores occupy is highlighted by a solid line and the area of pack hunters by a dashed line

occupying positive scores while the giant panda is at the extreme negative. The PC1 vs PC2 plot indicates strong clustering among solitary hunters while durophages are evenly distributed. This pattern changes when PC3 is also considered. PC3 is loaded on changes in the crushing area and the diastema/premolar area and the relative height of the ramus. In the PC2 vs PC3 morphospace, durophages occupy higher PC3 scores while the clustering of the other feeding categories is not evident (Fig. 2).

MCI values are generally smaller than 1, except for solitary and pack hunters and never statistically significant supporting no type of convergence (either complete or incomplete, Table 1).

Phylogenetic patterns

The phylogenetic signal was significant in both size and shape data. Size has a much lower *K* ($K=0.385$, $P<0.0001$) than shape ($K_{\text{multiv}}=0.631$, $P<0.001$) showing that closely related carnivorans might vary considerably in their mandibular size but not to the same extent in their shape. Goodall's ANOVA test showed a weak but significant impact of size on mandibular shape ($R^2=0.046$, $F=2.6739$, $P=0.032$), which was not significant when phylogeny was accounted for ($F=2.0823$, $P=0.221$).

The test for rate of evolution using convergent feeding categories as factor showed that the mandible shape of solitary hunters evolved at much slower rate ($\sigma=9.19e^{-6}$) than that of the rest of Carnivora ($\sigma=1.10e^{-5}$), and this pattern is significantly different from random (σ ratio=1.19, $P=0.017$). For pack hunters, the same test showed a non-significant pattern for the σ ratio that was not different from 1 (σ ratio=1.065, $P=0.601$). This means that pack hunter carnivorans did not evolve their mandible shape at a different rate than the rest of carnivorans. Comparing suborders also showed different rates of evolution in mandible shape, with a faster evolution in caniforms ($\sigma=1.29e^{-5}$) than feliforms ($\sigma=7.34e^{-6}$; σ ratio=1.752, $P=0.001$).

For mandibular size, the same test showed a significant difference in the rate of evolution only for solitary hunters, which again evolved size variation at a much slower rate ($\sigma=0.006$) than the other taxa ($\sigma=0.002$; σ ratio=2.358, $P=0.032$).

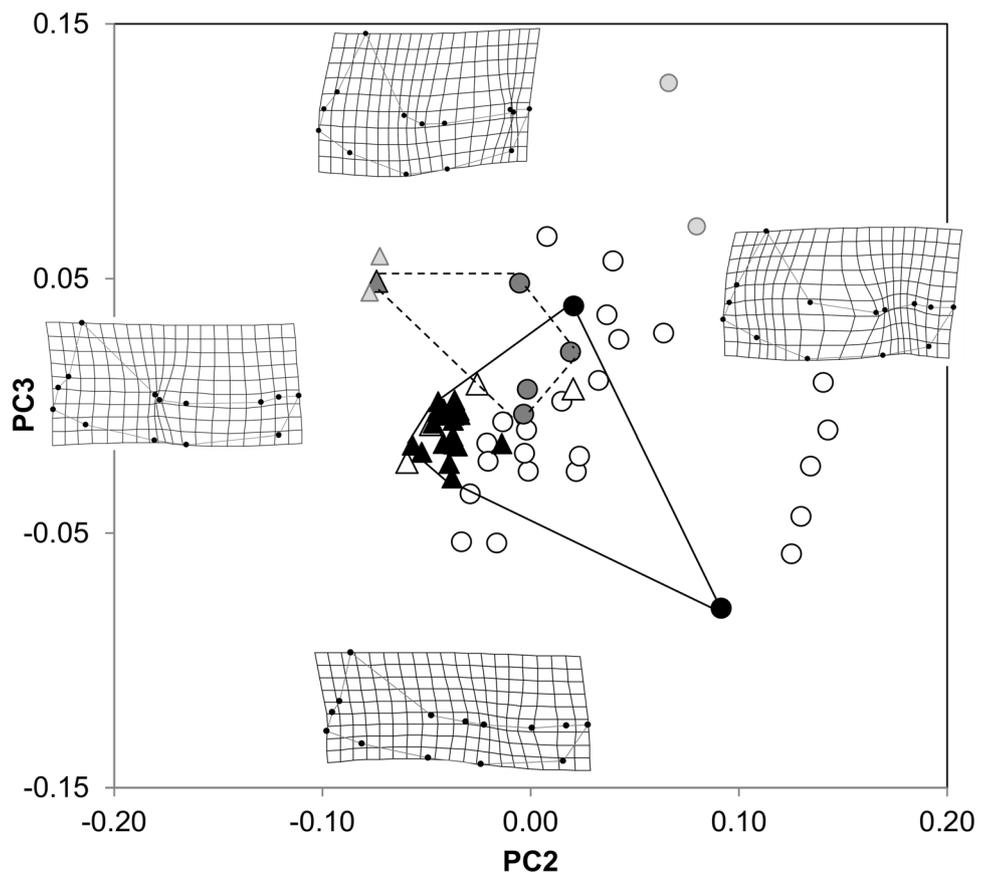
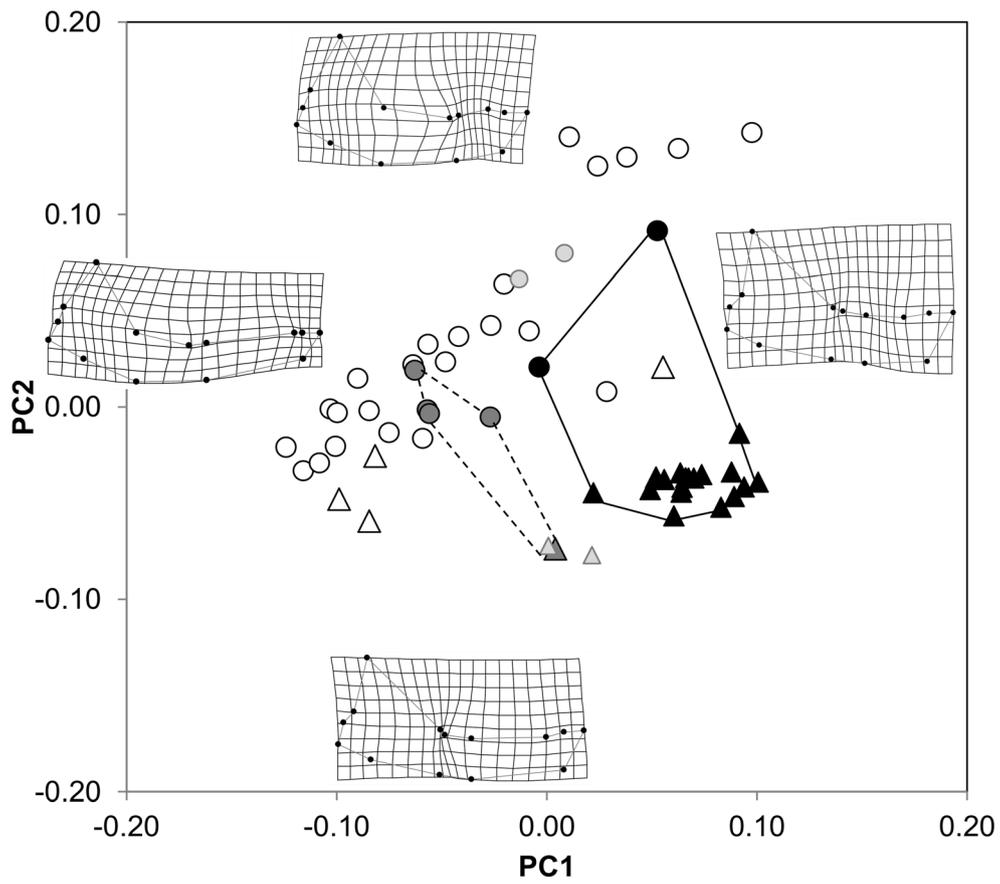


Table 1 Morphological disparity values (MD), multidimensional convergence index (MCI) and probability values after 9999 data permutation accounting for different hypotheses of convergence based on species-averaged sample ($N=57$) of mandible shape

	Shape				Form			
	MD	MCI	Complete P	Incomplete P	MD	MCI	Complete P	Incomplete P
Hypercarnivores ($N=28$)	0.013				53.491			
Sister taxa ($N=16$)	0.0161	1.241	0.615	0.221	51.433	0.961	0.937	0.706
Pack ($N=5$)	0.0088				46.415			
Sister taxa ($N=5$)	0.0162	1.828	0.084	0.008*	59.673	1.286	0.076	0.116
Solitary ($N=21$)	0.0086				53.441			
Sister taxa ($N=9$)	0.0267	3.105	<0.0001*	<0.0001*	55.098	1.031	0.491	0.541
Durophages ($N=5$)	0.0243				66.679			
Sister taxa ($N=14$)	0.0221	0.912	0.657	0.657	66.213	0.993	0.698	0.809

* $P<0.005$, level of significance

Discussion

Detecting phenotypic convergence in multivariate traits such as those generated by geometric morphometrics is challenging. Multiple landmark coordinates can be reduced by factorial analyses such as principal component as to generate an empirical morphospace, but graphical representation of PCA might be misleading due to its high dependence on sample size and distribution (i.e. altering number of cases might alter the structure of the covariance matrix hence the PC scores, Adams et al. 2011). This can result in a wrong interpretation of functional convergence.

Using morphological disparity, we identified convergence to occur in the mandible shape of solitary hunter carnivorans. Incomplete convergence was also identified in pack hunters, yet non-behavioural ecological characterisations based on feeding style, such as hypercarnivory or durophagy, are not associated with convergence in the mandible of large carnivorans. This result suggests that sharing phenotypic

morphospace might not be a solid indication of convergence when compared with methods based on morphospace volume and direct comparison with random models (Stayton 2006). In all of our analyses, hypercarnivorous taxa exhibit smaller morphological disparity than other species or their sister groups (Table 1), thus strongly confirming previous assertions on carnivoran ecomorphology (Holliday and Stepan 2004), although this pattern is not statistically distinct from randomness. In Holliday and Stepan's study, discrete skull characters were analysed in a sample including both fossil and extant Carnivora. We analysed only mandibular size and shape, and only extant species for which a more field observation-oriented definition of hypercarnivory applies (i.e. diet including >75 % of meat).

Our results only partially support a previous investigation by Figueirido et al. (2011) where hypercarnivores strongly overlapped in mandibular morphospace due to shared traits: shortening of the molar crushing area, elongation of the slicing area, enlarged canine area, relatively shorter corpus and

Table 2 Summary statistics for different ANCOVA models with shape as dependent variable

	Non-phylogenetic					PGLS				
	SS	MS	R^2	F	P value	SS	MS	R^2	F	P value
Hypercarnivory	0.150	0.150	0.229	18.685	0.001*	0.001	0.001	0.067	3.898	0.356
Hypercarnivory×suborder	0.023	0.023	0.035	2.875	0.108	0.000	0.000	0.009	0.523	0.019*
Pack	0.014	0.014	0.022	1.627	0.301	0.001	0.001	0.071	4.179	0.161
Pack×suborder	0.006	0.006	0.010	0.731	0.773	0.000	0.000	0.021	1.214	0.189
Solitary	0.174	0.174	0.266	22.632	0.001	0.002	0.002	0.104	6.269	0.228
Solitary×suborder	0.008	0.008	0.012	0.995	0.640	0.000	0.000	0.009	0.526	0.333
Durophagy	0.038	0.038	0.057	4.766	0.015*	0.001	0.001	0.035	1.944	0.001*
Durophagy×suborder	0.026	0.026	0.039	3.241	0.057	0.000	0.000	0.012	0.683	0.001*

Dependent variables are listed in the first column together with their interaction terms. PGLS represent the models including phylogenetic covariance matrix into account

* $P<0.005$, level of significance

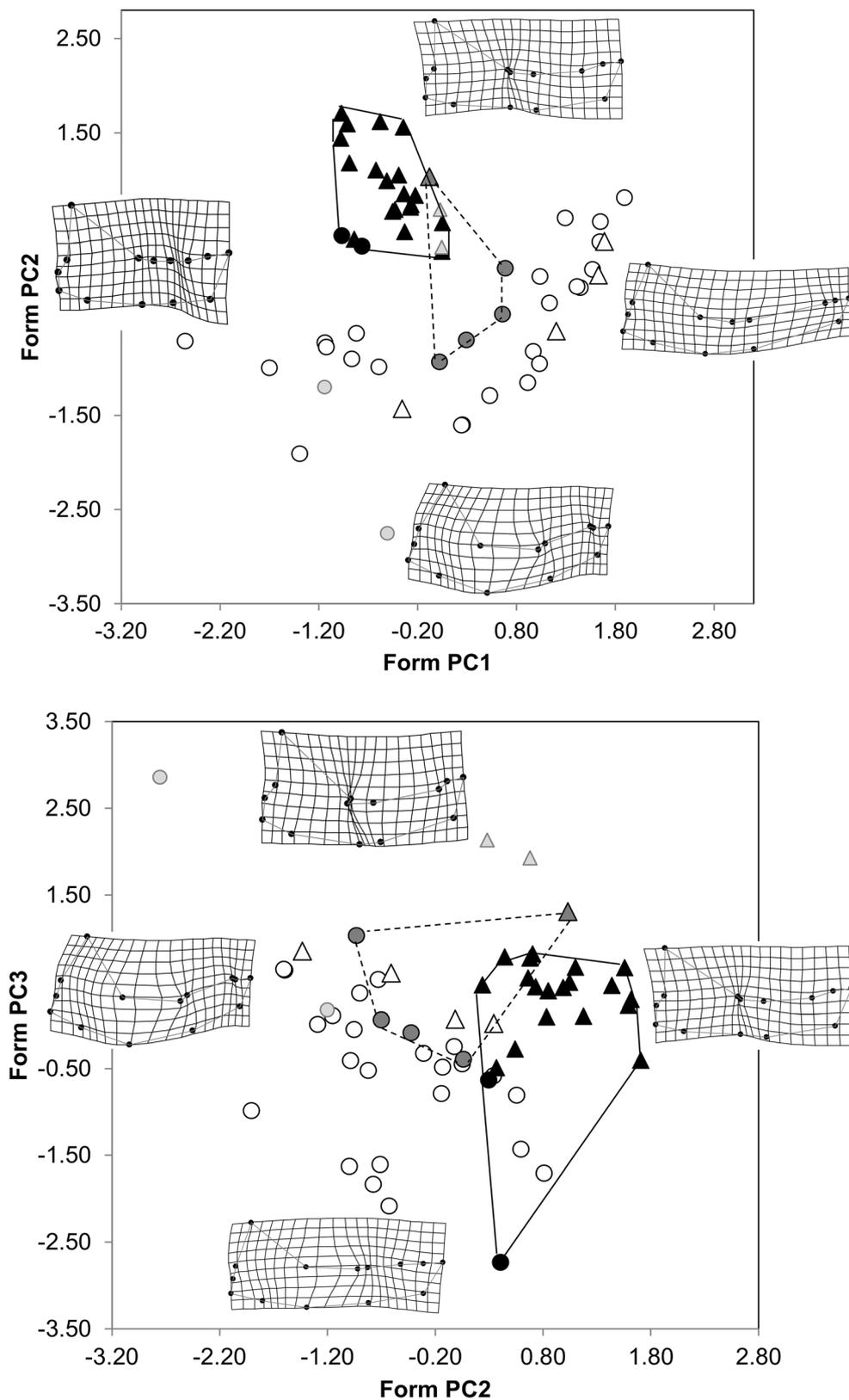


Fig. 2 Scatter plot of the first vs second component (PC) (a) and the first vs the third PC (b) derived from a sample of 57 carnivoran mandibular form (size+shape) coordinates. Transformation grids visualise form deformations relative to the mean at the positive and negative extremes of PC axes. Specimens are labelled according to suborder (*circle*, Caniformia; *triangle*,

Feliformia). The extreme feeding categories are coloured in *black* (solitary hypercarnivores), *dark grey* (pack hunters) or *light grey* (durophages); the area solitary hypercarnivores occupy is highlighted by a *solid line* and the area of pack hunters by a *dashed line*

posteriorly projected coronoid (cf. Fig. 1a). These traits represent functional adaptation to a range of biomechanical loadings imposed by the necessity of dealing with a food that is not easy to catch—live prey (Meloro et al. 2011), and they consistently occur in solitary hunters. This group is the only one showing complete convergence. Our interpretation is that mandibular design must be optimised in order to cope with the high functional demands of subduing and killing a prey alone, especially since applying killing bites imposes high biomechanical loading on the canines (cf. Prevosti et al. 2012). This killing behaviour occurs consistently in all extant large felids and is present also in the fossa and in two large caniforms: the wolverine and the polar bear. For the polar bear, the idea that solitary hunting imposes a functional constraint on skull morphology was advanced by Slater et al. (2010), who identified a rapid phenotypic change in this species relative to other omnivorous bears. Field studies on the wolverine support the notion that these species individuals are capable of killing prey as large as caribou and moose, which greatly outweigh them (Ewer 1973; Lofroth et al. 2007).

The evolution of adaptations to killing prey as lonely hunters appears to occur in parallel in caniforms and feliforms, as evidenced by ANCOVA statistical models (Table 2). On the other side, the incomplete convergence detected for pack hunters is possibly the result of little variation among clades since only large canids and one feliform (the spotted hyena) exhibit this behaviour. With incomplete convergence in these taxa, one might argue that pack hunting can be less biomechanically demanding than solitary hunting and is hence less functionally constrained. Cooperative behaviour might sometimes occur also in felids (e.g. the lion, Schaller 1972) although their killing technique is clearly distinct from that of wild canids and hyenas (Biknevicius and Van Valkenburgh 1996). While prey killing is operated by a single individual even in a pride of lionesses, the usual killing technique in pack hunters such as hunting dogs, spotted hyenas and wolves involves repeated bites to the gonads and the bellies delivered by different individuals (Kruuk 1972; Mech 1980; Creel and Creel 2002).

A broader taxonomic overview including also small carnivorans might possibly alter the pattern of convergence detected in relation to social hunting behaviour. Yet, it is clear that rapid, repeated bites aimed to kill prey by bleeding as in typical pack hunters are not as demanding as single prolonged bites aiming to kill prey by suffocation as in the lion. Still, small carnivores rarely focus on prey larger than themselves when in groups (see Friscia et al. 2007).

We note that previous attempts to detect convergence in Carnivora generally restricted the sample to large taxa inclusive of fossil species (cf. Werdelin 1989; Figueirido et al. 2011, 2013). Since ecological categorisation of fossil species is generally inferred by ecomorphology, extinct taxa should be considered with caution because this might generate circular

arguments in testing convergence unless other methods ('morphology-free') are employed to categorise them (e.g. geochemical dietary proxies or tooth microwear). This is particularly relevant given the huge importance of killing behaviour on convergence the present study suggests.

High biomechanical demands apparently favour convergence in the carnivoran mandible. If this was the case for solitary hunters, why was there no convergence detected in durophagous species? Our analyses always support, for this category, a similar or even larger morphological disparity than sister or remaining clades in mandible shape. In keeping with recent findings of Figueirido et al. (2013), we also detected shared morphospace along the first and the third PC axes (Fig. 1b), which describes a suite of shared traits in the mandible for durophagous species: enlargement of the coronoid region and premolar area, higher curvature at the posterior area of the molars, shortening of diastema and canine (Fig. 1b). There are different possible explanations to support the lack of convergence in durophages:

1. Durophagy does not impose a common 'environmental' selective regime (sensu Futuyma 2010); although tough food such as bamboo and bones might share similar material properties, they are chewed differently and constitute different fraction of a carnivoran diet (bamboo constitute more than 99 % of pandas diet in the wild (Johnson et al. 1988; Wei et al. 1999) while bones account for some 20 % of the spotted hyena diet (Van Valkenburgh 1996));
2. Durophagy is not an obligatory feeding behaviour; pandas experience hour-long cyclic loading on their cheek teeth during mastication because they are selective bamboo feeders, whereas hyenas (and any other bone-chewing carnivorans) have to withstand high loadings that occur rather episodically. Additionally, hyenas generally kill relatively large prey that fights for their life once grappled (Kruuk 1972; Schaller 1972) and hence require also traits of the hypercarnivory morphospace;
3. If durophagy is defined as bone crushing, it might be more widespread across Carnivora than usually assumed. All carnivorans require a supply of calcium in their diet, which they generally obtain from the bones of their prey (Allen et al. 1996), without any distinction to consumers of both large and small prey. Even small cats, the alleged 'non-bone consumers' par excellence, consume bones when eating mice or other small prey as a whole (for a detailed discussion, see Meloro 2011a, pp. 430–431; Meloro 2012).

The non-convergence pattern identified for mandibular form space (Fig. 2) supports size changes between species as a primary mechanism of niche differentiation in Carnivora (Carbone et al. 1999, 2007). Meloro and Raia (2010) already identified this pattern by looking at lower carnassial length,

and our findings confirm a trend observed also in fossil species. PGLS does not support interspecific allometry in the dataset employed, but this appears to be a trend that might change depending on taxa included (cf. Meloro et al. 2008; Figueirido et al. 2011; Meloro and O'Higgins 2011; Prevosti et al. 2012). As a generalisation confirmed also by previous studies, interspecific allometry explains only a small portion of mandibular shape changes in Carnivora, suggesting that shape change might be related to different ecological factors.

The tests for phylogenetic signal and evolutionary rate provide stronger support for a lack of convergence in form space and size-shape differences. We confirmed mandible size to be less conservative within carnivorans than its shape. This pattern contrasts with recent findings on primates (Meloro et al. 2015) that show exactly the opposite trend, thus suggesting that mammals might exhibit distinct patterns of evolutionary allometry in relation to feeding adaptations. With size being more evolutionary malleable in Carnivora, it allows rapid diversification to be tightly linked to environmental adaptations (Davies et al. 2007; Meiri et al. 2009). Evolutionary rates indicate morphological stasis in solitary hunters, whose size and shape evolved consistently slower than other taxa. Once an optimal design to deal with such high functional demands is in place, it changes little within clades: that is the case especially of felids, which represent the highest percentage of solitary carnivoran hunters. In relation to this, it is also not surprising to find feliforms generally evolving at much lower rate in mandibular shape than caniforms. The early establishment of some particular morphologies was also identified in previous studies on the mandible, and it supports the tight link between feeding adaptation and mandible shape in groups with limited dietary variation (e.g. felids, Meloro and O'Higgins 2011; Prevosti et al. 2012; perissodactyls, Raia et al. 2010). This does not preclude convergence to occur on a broader taxonomic scale but confirms the importance of attaining certain optimal designs.

Conclusions

The interpretation of morphological disparity allowed us to identify convergence in the mandible shape of solitary large carnivorans. This metric represents a feasible way to deal with multivariate shape data and already provided support to patterns of macroevolutionary diversification within carnivorans and other meat-eating mammalian groups (Holliday and Stepan 2004; Meloro 2011b; Prevosti et al. 2012; Bennett and Goswami 2013; Echarri and Prevosti 2015). We emphasise the strong functional demands on the mandibular design required to kill prey alone for terrestrial carnivorans, while consumption of bamboo and bones is not statistically associated with convergence among distantly related taxa (the pandas and hyenas).

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Conflict of interest The authors declare no conflict of interests in this publication and confirm that it is an original work that did not involve humans or animals. In addition, all the authors confirm that they were actively involved in the realisation of this study.

References

- Adams, D. C. (2014a). A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Systematic Biology*, *63*, 685–697.
- Adams, D. C. (2014b). Quantifying and comparing phylogenetic evolutionary rates for shape and other high-dimensional phenotypic data. *Systematic Biology*, *63*, 166–177.
- Adams, D. C., & Collyer, M. L. (2015). Permutation tests for phylogenetic comparative analyses of high-dimensional shape data: what you shuffle matters. *Evolution*, *69*, 823–829.
- Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*, 393–399.
- Adams, D. C., Cardini, A., Monteiro, L. R., O'Higgins, P., & Rohlf, F. J. (2011). Morphometrics and phylogenetics: principal components of shape from cranial modules are neither appropriate nor effective cladistic characters. *Journal of Human Evolution*, *60*, 240–243.
- Adams, D. C., Rohlf, D. C., & Slice, D. E. (2013). A field comes of age: geometric morphometrics in the 21st century. *Hystrix, the Italian Journal of Mammalogy*, *24*, 7–14.
- Allen, M. E., Oftedal, O. T., & Baer, D. J. (1996). The feeding and nutrition of carnivores. In D. G. Kleiman, M. E. Allen, K. V. Thompson, & S. Lumpkin (Eds.), *Wild mammals in captivity. Principles and Techniques* (pp. 139–147). Chicago: University of Chicago Press.
- Arnold, C., Matthews, L. J., & Nunn, C. L. (2010). The 10k trees Website: a new online resource for primate phylogeny. *Evolutionary Anthropology*, *19*, 114–118.
- Bennett, C. V., & Goswami, A. (2013). Statistical support for the hypothesis of developmental constraint in marsupial skull evolution. *BMC Biology*, *11*(1), 52.
- Biknevicius, A. R., & Van Valkenburgh, B. (1996). Design for killing: craniodental adaptations of mammalian predators. In J. L. Gittleman (Ed.), *Carnivore behavior, ecology, and evolution, Vol. 2* (pp. 393–428). Ithaca, New York: Cornell University Press.
- Blomberg, S. P., Garland, T., Jr., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, *57*, 717–745.
- Bookstein, F.L. (1989). Size and shape: a comment on semantics. *Systematic Zoology*, *38*, 173–180.
- Carbone, C., Georgina, M. M., Roberts, S. C., & Macdonald, D. W. (1999). Energetic constraints on the diet of terrestrial carnivores. *Nature*, *402*, 286–288.
- Carbone, C., Teacher, A., & Rowcliffe, J. M. (2007). The cost of carnivory. *PLoS Biology*, *5*, e22.
- Clauss, M., Kleffner, H., & Kienzle, E. (2010). Carnivorous mammals: nutrient digestibility and energy evaluation. *Zoo Biology*, *29*, 687–704.
- Creel, S., & Creel, N.M. (2002). *The African wild dog: behavior, ecology, and conservation*. Princeton University Press.

- Davies, J. T., Meiri, S., Barraclough, T. G., & Gittleman, J. L. (2007). Species co-existence and character divergence across carnivores. *Ecology Letters*, *10*, 146–152.
- Echarri, S., & Prevosti, F. J. (2015). Differences in mandibular disparity between extant and extinct species of metatherian and placental carnivore clades. *Lethaia*, *48*, 196–204.
- Ewer, R. F. (1973). *The carnivores*. New York: Cornell University Press.
- Figueirido, B., Serrano-Alarcón, F. J., Slater, G. J., & Palmqvist, P. (2010). Shape at the cross-roads: homoplasy and history in the evolution of the carnivoran skull towards herbivory. *Journal of Evolutionary Biology*, *23*, 2579–2594.
- Figueirido, B., MacLeod, N., Krieger, J., De Renzi, M., Pérez-Claros, J. A., & Palmqvist, P. (2011). Constraint and adaptation in the evolution of carnivoran skull shape. *Paleobiology*, *37*, 490–518.
- Figueirido, B., Tseng, Z. J., & Martin-Serra, A. (2013). Skull shape evolution in durophagous carnivorans. *Evolution*, *67*, 1975–1993.
- Frischia, A. R., Van Valkenburgh, B., & Biknevicius, A. R. (2007). An ecomorphological analysis of extant small carnivorans. *Journal of Zoology (London)*, *272*, 82–100.
- Futuyma, D. J. (2010). Evolutionary constraint and ecological consequences. *Evolution*, *64*, 1865–1884.
- Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society. Series B (Methodological)*, *285*–339.
- Goswami, A. (2006). Morphological integration in the carnivoran skull. *Evolution*, *60*, 122–136.
- Goswami, A. (2010). Introduction to carnivoran evolution. In A. Goswami & A. Frischia (Eds.), *Carnivoran evolution: new views on phylogeny, form, and function* (pp. 1–24). Cambridge: Cambridge University Press.
- Harmon, L. J., Schulte, J. A., Losos, J. B., & Larson, A. (2003). Tempo and mode of evolutionary radiation in iguanian lizards. *Science*, *301*, 961–964.
- Holliday, J. A., & Stepan, S. J. (2004). Evolution of hypercarnivory: the effect of specialization on morphological and taxonomic diversity. *Paleobiology*, *30*, 108–128.
- Johnson, K. G., Schaller, G. B., & Jinchu, H. (1988). Comparative behavior of red and giant pandas in the Wolong Reserve, China. *Journal of Mammalogy*, *69*, 552–564.
- Kruuk, H. (1972). *The spotted hyena (a study of predation and social behaviour)*. Chicago and London: The University of Chicago Press.
- Lofroth, E. C., Krebs, J. A., Harrower, W. L., & Lewis, D. (2007). Food habits of wolverine *Gulo gulo* in montane ecosystems of British Columbia, Canada. *Wildlife Biology*, *13*, 31–37.
- Mech, D. (1980). *The wolf. The ecology and behaviour of an endangered species*. Minneapolis, London: University of Minnesota Press.
- Meiri, S., Guy, D., Dayan, T., & Simberloff, D. (2009). Global change and carnivore body size: data are stasis. *Global Ecology and Biogeography*, *18*, 240–247.
- Meloro, C. (2011a). Feeding habits of Plio–Pleistocene large carnivores as revealed by their mandibular geometry. *Journal of Vertebrate Paleontology*, *31*, 428–446.
- Meloro, C. (2011b). Morphological disparity in Plio–Pleistocene large carnivore guilds from Italian peninsula. *Acta Palaeontologica Polonica*, *56*, 33–44.
- Meloro, C. (2012). Mandibular shape correlates of tooth fracture in extant Carnivora: implications to inferring feeding behaviour of Pleistocene predators. *Biological Journal of the Linnean Society*, *106*, 70–80.
- Meloro, C., & O'Higgins, P. (2011). Ecological adaptations of mandibular form in fissiped Carnivora. *Journal of Mammalian Evolution*, *18*, 185–200.
- Meloro, C., & Raia, P. (2010). Cats and dogs down the tree: the tempo and mode of evolution in the lower carnassial of fossil and living Carnivora. *Evolutionary Biology*, *37*, 177–186.
- Meloro, C., Raia, P., Piras, P., Barbera, C., & O'Higgins, P. (2008). The shape of the mandibular corpus in large fissiped carnivores: allometry, function and phylogeny. *Zoological Journal of the Linnean Society*, *154*, 832–845.
- Meloro, C., Raia, P., Carotenuto, F., & Cobb, S. (2011). Phylogenetic signal, function and integration in the subunits of the carnivoran mandible. *Evolutionary Biology*, *38*, 465–475.
- Meloro, C., Cáceres, N.C., Carotenuto, F., Sponchiado, J., Melo, G.L., Passaro, F., & Raia P. (2015). Chewing on the trees: constraints and adaptation in the evolution of the 1 primate mandible. *Evolution* (in press).
- O'Higgins, P., & Jones, N. (1998). Facial growth in *Cercocebus torquatus*: an application of three dimensional geometric morphometric techniques to the study of morphological variation. *Journal of Anatomy*, *193*, 251–272.
- Prevosti, F. J., Turazzini, G. F., Ercoli, M. D., & Hingst-Zaher, E. (2012). Mandible shape in marsupial and placental carnivorous mammals: a morphological comparative study using geometric morphometrics. *Zoological Journal of the Linnean Society*, *164*, 836–855.
- Raia, P. (2004). Morphological correlates of tough food consumption in carnivores. *Italian Journal of Zoology*, *71*, 45–50.
- Raia, P., Carotenuto, F., Meloro, C., Piras, P., & Pushkina, D. (2010). The shape of contention: adaptation, history, and contingency in ungulate mandibles. *Evolution*, *64*, 1489–1503.
- Rohlf, F. J., & Slice, D. E. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, *39*, 40–59.
- Schaller, G. B. (1972). *The Serengeti lion (a study of predator–prey relations)*. Chicago and London: The University of Chicago Press.
- Slater, G. J., Figueirido, B., Louis, L., Yang, P., & Van Valkenburgh, B. (2010). Biomechanical consequences of rapid evolution in the polar bear lineage. *PLoS One*, *5*(11), e13870.
- Stayton, C. T. (2006). Testing hypotheses of convergence with multivariate data: morphological and functional convergence among herbivorous lizards. *Evolution*, *60*, 824–841.
- Van Valkenburgh, B. (1985). Locomotor diversity between past and present guilds of large predatory mammals. *Paleobiology*, *11*, 406–428.
- Van Valkenburgh, B. (1988). Trophic diversity in past and present guilds of large predatory mammals. *Paleobiology*, *14*, 155–173.
- Van Valkenburgh, B. (1989). Carnivore dental adaptations and diet: a study of trophic diversity within guilds. In J. L. Gittleman (Ed.), *Carnivore behavior, ecology, and evolution, volume 1* (pp. 410–436). Ithaca: Cornell University Press.
- Van Valkenburgh, B. (1991). Iterative evolution of hypercarnivory in canids (Mammalia: Carnivora): evolutionary interactions among sympatric predators. *Paleobiology*, *17*, 340–362.
- Van Valkenburgh, B. (1996). Feeding behaviour in free-ranging, large African carnivores. *Journal of Mammalogy*, *77*, 240–254.
- Van Valkenburgh, B. (1999). Major patterns in the history of carnivorous mammals. *Annual Review Earth Planetary Science*, *27*, 463–493.
- Van Valkenburgh, B. (2007). Déjà vu: the evolution of feeding morphologies in the Carnivora. *Integrative Comparative Biology*, *47*, 147–163.
- Wei, F., Feng, Z., Wang, Z., Zhou, A., & Hu, J. (1999). Use of the nutrients in bamboo by the red panda (*Ailurus fulgens*). *Journal of Zoology (London)*, *248*, 535–541.
- Werdelin, L. (1989). Constraint and adaptation in the bone-cracking canid *Osteoborus* (Mammalia: Canidae). *Paleobiology*, *15*, 387–401.