Evolutionary Divergence and Convergence in Shape and Size Within African Antelope Proximal Phalanges

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Abstract Morphological convergence amongst species inhabiting similar environments but having different evolutionary histories is a concept central to evolutionary biology. Cases of divergent evolution, where there is morphological divergence between closely related species exploiting different environments, are less well studied. Here we show divergent evolution in the morphology of the proximal phalanges of several closely related African antelope species inhabiting different environments. This morphological divergence was consistently observed in both a neutral morphospace and an externally ordinated morphospace. Divergence, but not convergence, was also observed when size and shape were considered independently. Finally,

convergent evolution of the morphology of the proximal phalanges was observed, but only in the externally ordinated morphospace. Size shows less correlation with phylogeny than does shape. Therefore, we suggest that divergence in size will occur more readily when a species encounters new environmental conditions than divergence in shape. These findings are compatible with observations of rapid dwarfing on islands (Foster's rule).

Keywords Ecomorphology · Phalanges · Convergent evolution · Divergent evolution

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Introduction

Morphological convergence amongst species sharing a similar environment but different evolutionary histories is a well-reported phenomenon that has been observed in many lineages across time. It remains a key concept in evolutionary biology (e.g., Hertel 1994; Schluter 2000; Stayton 2005, 2006). However, an understanding of divergent evolution is critical for many theories examining the interaction between the environment and the evolution of phenotype. Adaptive radiation, for example, relies on the divergence of many species from a common ancestor once new environments are encountered (Schluter 2000). Documentation and, more importantly, empirical tests for morphological divergence are critical for modern evolutionary theory and provide a useful comparator for modern studies of genetic divergence (Gatesy et al. 1997; Hassanin and Ropiquet 2004).

Antelopes (Mammalia: Bovidae) are found throughout the Old World but are most common in African environments where they show the highest level of continent-wide diversity. In Africa, they inhabit a range of habitats from open grasslands to dense forest to wetland. They are abundant in fossil assemblages and have long been used as paleoenvironmental indicators (Gentry 1970; Vrba 1980).

Subsequently, postcranial elements commonly preserved in the fossil record have been the subject of ecomorphological analyses (Kappelman 1988, 1991; Plummer and Bishop 1994; Kappelman et al. 1997; DeGusta and Vrba 2003, 2005; Plummer et al. 2008; Bishop et al. 2011). These studies have revealed a correlation between habitat use and morphology of the antelope postcranial skeleton, as successful locomotor adaptation is intimately associated with organismal success (Plummer et al. 2008). Morphology will also be a consequence of the mechanical interaction between the postcranial skeleton and the substrate encountered (Scott 1985; Hamrick 2001; DeGusta and Vrba 2003). The morphology of the phalanges, in particular, should reflect these interactions as these elements are often in direct contact with the substrate being moved across (cursorial locomotion) or through (aquatic, arboreal, scansorial, and fossorial locomotion) (Kent and Miller 1997). Previous morphological analyses of mammalian phalanges have focused more on the distal phalanges (e.g., Macleod and Rose 1993; Hamrick 2001) as these are the most extreme elements of the limb and the most likely to be in contact with external environmental stimuli. There is also a rich body of literature examining primate and human phalanges in the context of hominin evolution (Deane and Begun 2008; Rolian et al. 2009; Almécija et al. 2009, 2010, 2012; Griffin and Richmond 2010; Kivell et al. 2011; Nelson et al. 2011; Congdon 2012).

Recently, the use of ecomorphological analyses of antelopes for habitat reconstructions has been questioned (Klein et al. 2010) on the basis that size, rather than shape, explains most of the morphological variation in antelope metapodials and astragali. Therefore, these authors argue, any discriminant analysis attempting to reconstruct habitats on the basis of elements that have not been independently linked to habitat or foraging strategy will make classification errors among like-sized genera. Body size is probably the most fundamental ecological trait, correlating with all aspects of an animal's biology including life history, physiology, behavior, and evolution (Peters 1983; Schmidt-Nielsen 1984; Damuth and MacFadden 1990), and understanding how body size changes in response to different environments has important implications for understanding macroevolutionary processes (Evans et al. 2012). In bovids, the physical structure of the environment, as well as diet, has been shown to correlate with size (Brashares et al. 2000; Bro-Jørgensen 2008). Closed habitats tend to favor smaller-bodied species because this trait facilitates movement through dense vegetation and allows for effective camouflage. Open habitats tend to favor larger-bodied species in part because of increased seasonality and the body-size restrictions related to a grazing lifestyle (Brashares et al. 2000; Bro-Jørgensen 2008). Although early ecomorphological analyses attempted to adjust morphological data for size, effectively trying to

remove size from shape (e.g., Kappelman 1988), this approach has been subsequently questioned and abandoned because of the intimate relationship between size and ecology (DeGusta and Vrba 2005; Kovarovic and Andrews 2007; Plummer et al. 2008; Bishop et al. 2011).

In order for any ecomorphological analysis to be valid, it must be demonstrated that the organisms under study exhibit both convergent and divergent evolution in response to new environmental conditions, and changes in size as well as shape are important aspects to consider. Although morphological convergence (and divergence) is assumed and qualitatively validated by ecomorphological studies (starting with Gentry (1970)), this has never been tested quantitatively, and particularly within a phylogenetic context. Here, therefore, we test the following two hypotheses: 1) that two closely related species in different environments are more different morphologically than two closely related species in the same environment (divergence); and 2) that two distantly related species in the same environment are more similar morphologically than two distantly related species in different environments (convergence). This is the first study where the influence of phylogeny (as opposed to taxonomy) is examined explicitly for analyses seeking to reconstruct habitats on the basis of bovid ecomorphological discriminant models. We focus on proximal phalanges because the morphology of these elements should reflect both a species' ambulation and the substrate because of their articulation with both the metapodials, and hence the limb, and the more distal phalanges, and hence the substrate.

Materials and Methods

We examined 343 proximal phalanges from 36 antelope species from: the American Museum of Natural History, New York, NY (AMNH), the National Museum of Natural History, Washington, DC (NMNH), and The Natural History Museum, London, UK (NHML). Adult, wild-shot specimens were measured in preference to zoo specimens, when available (zoo specimens, $n=10$). Each species was assigned to one of four broadly defined habitat preference categories used in previous ecomorphic studies (Scott 1985; Kappelman 1988, 1991; Kappelman et al. 1997) and the ethological literature (Dorst and Dandelot 1986; Kingdon 1997). These categories partition the environmental continuum from habitats generally lacking trees and bush to those with a continuous tree canopy. The habitat preference categories are: open (grassland, arid country, ecotones bordering open country), light cover (light bush, tall grass), heavy cover (heavy bush, woodland, densely vegetated swamp), and forest (Table 1).

We measured proximal phalanges following the measurement scheme detailed by Bishop et al. (2011) (Fig. 1, Table 2). We calculated a species mean for each measurement, and their

Table 1 Antelope species examined, common names, preferred habitats, and number of individuals examined (n)

ID	Tribe	Species	Common name	Preferred Habitat	n
1	Tragelaphini	<i>Tragelaphus imberbis</i>	Lesser Kudu	Heavy Cover	5
2	Tragelaphini	<i>Tragelaphus strepsiceros</i>	Greater Kudu	Heavy Cover	11
3	Tragelaphini	<i>Tragelaphus scriptus</i>	Bushbuck	Forest	27
4	Tragelaphini	<i>Tragelaphus euryceros</i>	Bongo	Heavy Cover	18
5	Tragelaphini	<i>Tragelaphus speki</i>	Sitatunga	Heavy Cover	28
6	Aepycerotini	<i>Aepyceros melampus</i>	Impala	Light Cover	19
7	Neotragini	<i>Madoqua kirkii</i>	Kirk's Dik-dik	Heavy Cover	14
8	Antilopini	<i>Antidorcas marsupialis</i>	Springbok	Open	12
9	Neotragini	<i>Ourebia ourebi</i>	Oribi	Light Cover	11
10	Antilopini	<i>Gazella granti</i>	Grant's Gazelle	Open	13
11	Antilopini	<i>Gazella thomsoni</i>	Thomson's Gazelle	Open	15
12	Reduncini	<i>Redunca fulvorufula</i>	Mountain Reedbuck	Light Cover	5
13	Reduncini	<i>Redunca arundinum</i>	Southern Reedbuck	Light Cover	2
14	Reduncini	<i>Redunca redunca</i>	Bohor Reedbuck	Light Cover	6
15	Reduncini	<i>Kobus megaceros</i>	Nile Lechwe	Heavy Cover	1
16	Reduncini	<i>Kobus ellipsiprymnus</i>	Waterbuck	Heavy Cover	4
17	Reduncini	<i>Kobus kob</i>	Kob	Light Cover	3
18	Cephalophini	<i>Cephalophus monticola</i>	Blue Duiker	Forest	11
19	Cephalophini	<i>Cephalophus leucogaster</i>	White-bellied Duiker	Forest	5
20	Cephalophini	<i>Cephalophus niger</i>	Black Duiker	Forest	1
21	Cephalophini	<i>Cephalophus weynsi</i>	Weyne's Duiker	Forest	4
22	Cephalophini	<i>Cephalophus natalensis</i>	Red Forest Duiker	Forest	2
23	Cephalophini	<i>Cephalophus nigrifrons</i>	Black-fronted Duiker	Forest	3
24	Cephalophini	<i>Sylvicapra grimmia</i>	Common Duiker	Light Cover	9
25	Cephalophini	<i>Cephalophus dorsalis</i>	Bay Duiker	Forest	4
26	Cephalophini	<i>Cephalophus silvicultor</i>	Yellow-backed Duiker	Forest	8
27	Alcelaphini	<i>Connochaetes gnou</i>	Black Wildebeest	Open	5
28	Alcelaphini	<i>Connochaetes taurinus</i>	Blue Wildebeest	Open	3
29	Alcelaphini	<i>Alcelaphus buselaphus</i>	Hartebeest	Open	23
30	Alcelaphini	<i>Damaliscus lunatus</i>	Tsessebe	Open	16
31	Alcelaphini	<i>Beatragus hunteri</i>	Hirola	Open	4
32	Alcelaphini	<i>Damaliscus pygargus</i>	Bontebok	Open	13
33	Hippotragini	<i>Addax nasomaculatus</i>	Addax	Open	4
34	Hippotragini	<i>Oryx gazella</i>	Gemsbok	Open	12
35	Hippotragini	<i>Hippotragus equinus</i>	Roan Antelope	Open	6
36	Hippotragini	<i>Hippotragus niger</i>	Sable Antelope	Open	16

$\log_{10}$ values were used in both Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA). These values are listed in the online [supplementary information](#). Both sex and forelimb and hind limb phalanx data were pooled in the analyses, as two-way NPMANOVAs showed no significant interactions between sexes and habitat groups ($p=0.137$) or limb position and habitat groups ($p=0.8582$).

To investigate the role of phylogeny in ecomorphological analyses, a phylogenetic tree was constructed from genetic data based on sequences of the mitochondrial gene cytochrome b from Genbank. Accession numbers are listed in the online

[supplementary information](#). The sequences were aligned using MUSCLE (Edgar 2004) under default parameters, resulting in a matrix of 55 taxa and 1144 characters. The character matrix was analyzed in a parsimony framework using TNT v. 1.1 (Goloboff et al. 2008) utilizing the New Technology Search, ratchet, and drift options (100 random addition sequences), with the pronghorn (*Antilocapra americana*) as the outgroup. The data were also run on TNT (maximum parsimony) and RaxML (maximum likelihood) frameworks; the resulting topologies were nearly identical, therefore the parsimony analysis was used with a strict consensus tree created from the most parsimonious trees. The patristic distance

Fig. 1 Measurement scheme used on antelope proximal phalanges. Abbreviations as follows: *MAXLEN* maximum length; *MINLEN* minimum length; *PMAXHT* maximum height of proximal end; *PARTHT* proximal articular height; *PMAXBR* maximum breadth of proximal end; *PARTBR* proximal articular breadth; *MAP* midshaft

anteroposterior diameter; *MML* midshaft mediolateral diameter; *DMAXAP* maximum anteroposterior dimension of the distal end; *DMINAP* minimum anteroposterior dimension of the distal end; *DMAXML* maximum mediolateral dimension of the distal end; *DMINML* minimum mediolateral dimension of the distal end

(the number of apomorphic step changes separating two taxa in the cladogram) between each species pair was calculated on the basis of this tree.

A neutral morphospace was constructed by subjecting the morphological variables to a Principal Component Analysis (PCA). This is a commonly employed algorithm in morphological studies aimed at extracting the most informative components of a multivariate dataset. A size-proxy variable was determined by comparing the first Principal Component (PC1) score of each species with its average species body mass (from Smith et al. 2004). We interpret PC2, which is independent of PC1, as a shape-proxy variable. Hereafter,

Table 2 List of measurements taken on the proximal phalanges and abbreviations used throughout the text

Abbreviation	Measurement
MAXLEN	Maximum length
MINLEN	Minimum length
PMAXHT	Maximum height of proximal end
PARTHT	Proximal articular height
PMAXBR	Maximum breadth of proximal end
PARTBR	Proximal articular breadth
MAP	Midshaft anteroposterior diameter
MML	Midshaft mediolateral diameter
DMAXAP	Maximum anteroposterior dimension of the distal end
DMINAP	Minimum anteroposterior dimension of the distal end
DMAXML	Maximum mediolateral dimension of the distal end
DMINML	Minimum mediolateral dimension of the distal end

we refer to these proxies as ‘size’ and ‘shape,’ respectively, cognizant that they are not a perfect representation of the true size or shape factors. We constructed a morphospace ordinated under the set of external conditions defined by habitat type using a Discriminant Function Analysis (DFA), with open, light cover, heavy cover, and forest as classifying variables. This type of analysis is commonly employed by paleoecologists attempting to reconstruct the likely habitat allocation of fossil skeletal elements (Plummer and Bishop 1994; Kovarovic et al. 2011). It maximizes the differences among species occurring in different habitat types and allows the assignment of unknown elements to a probable habitat type. On the basis of the two morphospaces, we were able to determine the morphological distance separating every species pair by calculating the Euclidean distance in the plot of the first two components for each space. This enabled us to construct two morphological distance matrices, one neutral (PCA) and the other ordinated externally (DFA).

Comparison between the patristic distance and the morphological distance matrices (PCA, DFA, size, and shape) was accomplished by Mantel tests using 9999 random permutations. Topology of the phylogenetic tree was mapped onto the two morphospaces using the methods described in O’Keefe (2002) and Stayton (2005). In this method each point representing a species in the morphospace corresponds to a terminal node on the phylogenetic tree. Centroids are calculated for each clade comprised of sister species or sister groups. Points are then plotted in the morphospace for each of these centroids, and branches connecting the nodes to the

sister groups are drawn. These points correspond to internal nodes, and will always lie on the line connecting two groups or species, with the exception of an unresolved node in the cladogram, which is drawn as a polygon in the morphospace with the centroid and associated branch extending from its centre. This algorithm is then repeated successively for deeper nodes up to each basal node; however, in this study the basal node is not shown in order to maximize clarity of the resulting figure. This method in essence shows the phylogenetic relationships (represented by lines connecting closely related species or nodes) graphically in the morphospace. Two species or sister groups closely related but morphologically disparate are represented by a longer line, whereas two species or sister groups closely related and morphologically similar are represented by a shorter line. The middle node from each species or sister group pair in the resulting figure corresponds to an internal node of the cladogram, and represents the group centroid of these pairs in morphospace.

In this study, we define divergent evolution as the morphological divergence of two closely related species when these species exploit different environments. In a quantitative framework, this equates to a statistically significant difference in morphology between two closely related species inhabiting different environments relative to the difference in morphology between two closely related species inhabiting the same environment. We determined the cut off for closely related species by calculating the average patristic distance among all congeneric species and adding two standard deviations. Closely related species pairs were those whose patristic distance was less than this value, otherwise species were considered distantly related. Each species pair was then assigned to one of four categories: closely related same environment, closely related different environment, distantly related same environment, and distantly related different environment. Divergence and convergence were analysed by non-parametric Mann-Whitney U-tests.

Results

Phylogenetic Results

The strict consensus tree constructed from the six most parsimonious trees had 3247 steps (Fig. 2). This tree formed the basis from which the patristic distances were calculated. The topology of the phylogenetic tree mapped onto the morphospaces is shown in Fig. 3.

Functional Results

Summary statistics are listed in Tables 3–5. PC1 was strongly correlated with the log of average body mass ($r=0.9627$,

Fig. 2 Strict consensus tree of the 36 species of antelope examined

$p<0.001$). Overall, forest-adapted antelopes were smaller than other species, as evinced by their lower PC1 values. Their lower PC2 values equate to relatively shorter phalanges (MAXLEN, MINLEN) combined with larger medio-lateral dimensions at the distal end (DMAXML, DMINML) and wider midshaft mediolateral diameters (MML). Overall, this equates to squatter, broader phalanges at the distal end, where the proximal phalanges articulate with the middle phalanges. Heavy cover species also had generally lower PC2 values. However, they were generally larger in size. Light cover species were the second smallest (low PC1), but with high values of PC2, suggesting narrower phalanges at the distal end. Open adapted species all had high PC1 values, indicating large body sizes, but as a group they were not distinguishable along PC2.

Forest dwelling species were distinguished by low values of the first canonical variate associated with the DFA (DF1). This variate is positively affected by proximal articular breadth (PARTBR) and negatively by the maximum height (MAXHT). Hence, forest species are distinguished by taller, narrower proximal ends of the phalanges, where they articulate with the metapodials. Conversely, open adapted species were distinguished by high DF1 values, corresponding to shorter, wider proximal ends. Heavy cover species were distinguishable from other antelopes in having high DF2 values.

Fig. 3 Mapping the phylogenetic topology onto the different morphospaces examined. The ecologically weighted morphospace, **a** and **b** is represented by the first two components of a Discriminant Function Analysis using four habitat classification categories: forest (dark green), heavy cover (green), light cover (blue), and open (red). The unbiased morphospace, **c** and **d** is represented by the first two components of a Principal Component Analysis. For each of the habitat categories, squares represent the group centroid, outlines (shown in **(a)** and **(c)**) represent convex hulls. Phylogenetic relationships are shown only to tribal level with the exception of Aepycerotini and Neotragini, which is grouped with the Antilopini. Numbers refer to

species as follows: 1 *Tragelaphus imberbis*, 2 *T. strepsiceros*, 3 *T. scriptus*, 4 *T. euryceros*, 5 *T. spekii*, 6 *Aepyceros melampus*, 7 *Madoqua kirkii*, 8 *Antidorcas marsupialis*, 9 *Ourebia ourebi*, 10 *Gazella granti*, 11 *G. thomsoni*, 12 *Redunca fulvorufula*, 13 *R. arundinum*, 14 *R. redunca*, 15 *Kobus megaceros*, 16 *K. ellipsiprymnus*, 17 *K. kob*, 18 *Cephalophus monticola*, 19 *C. leucogaster*, 20 *C. niger*, 21 *C. weynsi*, 22 *C. natalensis*, 23 *C. nigrifrons*, 24 *Sylvicapra grimmia*, 25 *C. dorsalis*, 26 *C. silvicultor*, 27 *Connochaetes gnou*, 28 *C. taurinus*, 29 *Alcelaphus buselaphus*, 30 *Damaliscus lunatus*, 31 *Beatragus hunter*, 32 *D. pygargus*, 33 *Addax nasomaculatus*, 34 *Oryx gazella*, 35 *Hippotragus equinus*, 36 *H. niger*

This corresponds to a combination of narrower proximal articular breadth (PARTBR) and wider proximal breadth (PMAXBR). In other words, in these species the proximal end of the phalanx bulges out from the articular surface.

Size and Shape

In comparisons between morphological distances and phylogenetic distance, shape showed the highest correlation

($r=0.3096$, $p<0.0001$) whereas size showed the lowest ($r=0.163$, $p=0.0067$). Both the PCA and DFA morphospaces were significantly correlated with patristic distance (PCA: $r=0.1641$, $p=0.0066$; DFA: $r=0.3029$, $p<0.001$).

The topology of the phylogenetic tree mapped onto the morphospaces is shown in Fig. 3. Although the neutral morphospace (PCA) shows some degree of separation among habitat groups (Fig. 3c), the externally ordinated morphospace (DFA) shows the best separation among

Table 3 Summary statistics of Discriminant Function Analysis and Principal Component Analysis

	Eigenvalue	% variance	Wilk's Lambda	Chi-square	df	Sig.
PCA1	0.359806	97.432	—	—	—	—
PCA2	0.00649092	1.7577	—	—	—	—
DF1	6.421	79.9	0.042	88.605	30	<0.001
DF2	1.095	13.6	0.313	32.485	18	0.019

Table 4 Component scores (PC) for each antelope species examined calculated in the Principal Component Analysis

Species	PC1	PC2
<i>Tragelaphus imberbis</i>	0.26277	0.031737
<i>Tragelaphus strepsiceros</i>	0.76717	1.74×10^{-6}
<i>Tragelaphus scriptus</i>	-0.15292	-0.01062
<i>Tragelaphus euryceros</i>	0.7454	-0.12836
<i>Tragelaphus spekii</i>	0.29868	0.014623
<i>Aepyceros melampus</i>	0.10433	0.12632
<i>Madoqua kirkii</i>	-1.146	0.064116
<i>Antidorcas marsupialis</i>	-0.16588	0.1532
<i>Ourebia ourebi</i>	-0.51802	0.12577
<i>Gazella granti</i>	0.11363	0.14063
<i>Gazella thomsoni</i>	-0.37145	0.1357
<i>Redunca fulvorufula</i>	-0.23848	0.062926
<i>Redunca arundinum</i>	-0.01285	0.087957
<i>Redunca redunca</i>	-0.02987	0.10742
<i>Kobus megaceros</i>	0.305	-0.01038
<i>Kobus ellipsiprymnus</i>	0.72159	-0.06221
<i>Kobus kob</i>	0.20994	0.005699
<i>Cephalophus monticola</i>	-1.4122	-0.08541
<i>Cephalophus leucogaster</i>	-0.69739	-0.0697
<i>Cephalophus niger</i>	-0.79516	-0.08504
<i>Cephalophus weynsi</i>	-0.61757	-0.10281
<i>Cephalophus natalensis</i>	-0.93792	-0.10096
<i>Cephalophus nigrifrons</i>	-0.59642	-0.03192
<i>Sylvicapra grimmia</i>	-0.66779	0.008647
<i>Cephalophus dorsalis</i>	-0.61783	-0.11892
<i>Cephalophus silvicultor</i>	0.056969	-0.05248
<i>Connochaetes gnou</i>	0.47871	-0.06691
<i>Connochaetes taurinus</i>	0.68792	-0.06878
<i>Alcelaphus buselaphus</i>	0.57088	0.026385
<i>Damaliscus lunatus</i>	0.54839	0.033348
<i>Damaliscus hunteri</i>	0.37175	0.04186
<i>Damaliscus dorcas</i>	0.1919	0.035858
<i>Addax nasomaculatus</i>	0.37741	-0.07116
<i>Oryx gazella</i>	0.66554	-0.07127
<i>Hippotragus equinus</i>	0.805	-0.025
<i>Hippotragus niger</i>	0.69478	-0.04026

habitat categories (Fig. 3a). Morphological divergence is clearly visible on the DFA morphospace (Fig. 3b). In particular, the separation of the cephalophine *Sylvicapra grimmia* (24) away from the forest group centroid (the dominant habitat classification for the Cephalophini) towards the light cover group centroid, the reduncines *Kobus megaceros* (15) and *K. ellipsiprymnus* (16) away from the light cover group centroid (the dominant habitat classification for the Reduncini) to the heavy cover group centroid, and the neotragine *Madoqua kirkii* (7) away from the light cover and open

group centroids (the dominant habitat classification of the Antilopinae) to the heavy cover group centroid. With the exception of *M. kirkii*, similar patterns can be seen in the neutral morphospace, but this is a result of differences in size (PC1) as well as shape (PC2) (Fig. 3d).

In the neutral morphospace (Fig. 4a), two closely related species inhabiting the same environment are more similar than two closely related species inhabiting different environments (divergence; $p < 0.001$). Two distantly related species inhabiting the same environment are not more similar than

Table 5 Canonical scores (DF) for each antelope species examined calculated in the Discriminant Function Analysis

Species	DF1	DF2
<i>Tragelaphus imberbis</i>	-0.111736331	1.53632483
<i>Tragelaphus strepsiceros</i>	0.401857205	2.783031448
<i>Tragelaphus scriptus</i>	-1.137581335	2.29258763
<i>Tragelaphus euryceros</i>	-1.453974396	2.561167179
<i>Tragelaphus spekii</i>	-0.313062022	2.314558043
<i>Aepyceros melampus</i>	1.083497203	0.005758353
<i>Madoqua kirkii</i>	-0.445372338	0.839242648
<i>Antidorcas marsupialis</i>	2.768485301	-0.394452383
<i>Ourebia ourebi</i>	0.41063056	-1.255179589
<i>Gazella granti</i>	2.381220916	-1.569363689
<i>Gazella thomsoni</i>	1.622377304	-1.604874941
<i>Redunca fulvorufula</i>	1.736887166	-0.848111244
<i>Redunca arundinum</i>	2.037730562	-3.738083719
<i>Redunca redunca</i>	0.503604188	-1.464884844
<i>Kobus megaceros</i>	0.54075455	1.49640981
<i>Kobus ellipsiprymnus</i>	0.648430724	1.636134282
<i>Kobus kob</i>	0.087556896	-0.296468594
<i>Cephalophus monticola</i>	-4.693046641	-1.268701309
<i>Cephalophus leucogaster</i>	-4.561965982	-0.705991924
<i>Cephalophus niger</i>	-3.583573693	-0.837535916
<i>Cephalophus weynsi</i>	-3.9993855	-0.125564024
<i>Cephalophus natalensis</i>	-3.907700342	-0.951842837
<i>Cephalophus nigrifrons</i>	-4.519370378	0.390561507
<i>Sylvicapra grimmia</i>	-2.310611383	-0.29729561
<i>Cephalophus dorsalis</i>	-4.617705788	-0.731512887
<i>Cephalophus silvicultor</i>	-3.006854285	-0.980379811
<i>Connochaetes gnou</i>	0.879407681	1.203541667
<i>Connochaetes taurinus</i>	2.844528946	-0.90742172
<i>Alcelaphus buselaphus</i>	2.314255165	1.037370915
<i>Damaliscus lunatus</i>	3.177217145	-0.473643418
<i>Beatragus hunteri</i>	1.577723773	-0.162720871
<i>Damaliscus dorcas</i>	2.702371713	0.18421258
<i>Addax nasomaculatus</i>	3.472015863	0.563202615
<i>Oryx gazella</i>	2.145551042	0.65853956
<i>Hippotragus equinus</i>	2.89607888	-0.127785946
<i>Hippotragus niger</i>	2.429757631	-0.760827791

two distantly related species in different environments (convergence; $p=0.13$; Fig. 4a). This pattern is equivalent to the test for size and shape (Fig. 4b, d; $p<0.001$ and $p=0.1391$; and $p=0.003$ and $p=0.07$, respectively). In the externally ordinated ecospace (Fig. 4c), both convergence and divergence are observed (both $p<0.001$).

Discussion

Congruent with previous studies (Brashares et al. 2000; Bro-Jørgensen 2008), we found forest adapted species had smaller proximal phalanges than other antelopes. However, we found that the antelopes preferring heavy cover were of similar size to light cover species, at least in our sample. This trend cannot be explained completely by diet, as many

of the Tragelaphini examined in this habitat category are browsers or mixed feeders (Gagnon and Chew 2000). Size varies not only as a result of diet but also other ecological traits such as competition and predation, and it is likely that these variables are also influencing this trend. Both forest and heavy cover species had relatively broad distal ends of the proximal phalanges. In horses, broader phalanges have been suggested to be an adaptation to soft substrates (van Aspen 2010), and this explanation might be possible for antelopes as well. More likely though, this shape would give greater support to phalanges that manoeuvre in more cluttered habitats, and therefore experience greater loads from all directions compared to open habitat species.

Light cover species and most open habitat species had relatively narrower and longer distal ends, suggesting locomotion over harder/drier substrates in less structurally

Fig. 4 Box plot of the Euclidean distances between each species pair for (a) neutral morphospace, (b) size space, (c) externally ordinated morphospace, and (d) shape space. Species classified according to level of relatedness based on average patristic distance between congeneric species, and environments represented by the habitat classification categories of forest, heavy cover, light cover, and open. For each

group, the 25–75 percent quartiles are drawn using the box, the median is shown with a horizontal bar in the box, the whiskers accompanying the box show minimum and maximum values, and outliers are shown as points. Asterisks indicate significant differences ($p \leq 0.05$) between the groups

complex habitats. Elongation of distal limb elements is also an adaptation for more cursorial species that typically inhabit more open habitats (Hildebrand and Goslow 2001; Pough et al. 2008). In mammals exhibiting an unguligrade posture, such as antelopes, the weight of the body is supported entirely by the distal phalanges, with the proximal and medial phalanges effectively contributing to the stride-length of the animal (Pough et al. 2008). Muscles in the distal part of the limb are reduced in favor of using tendons to transmit muscular forces in order to reduce the weight of this part of the limb (Hildebrand and Goslow 2001). This effectively reduces limb inertia, increasing speed and long-distance travel, but at the cost of decreased manoeuvrability. Therefore, we would expect antelopes found in more open habitats to have narrower, more gracile phalanges compared to more forest adapted species, as these species are more likely to rely on speed and endurance over open terrain for predator evasion than manoeuvrability through dense vegetation. Finally, open habitat species were larger on average (higher PC1 scores) than antelopes in the other habitat categories. This is possibly a consequence of diet as most are grazers, although increased size may also contribute to increased speed in open habitats. In contrast, small compact bodies appear to be adaptive in dense habitats for greater manoeuvrability (Bro-Jørgensen 2008).

Our results demonstrate morphological divergence between closely related antelope species inhabiting different environments, regardless of phylogeny (Fig. 4a, c). Divergence was observed in both size and shape (Fig. 4b, d), suggesting that any size-related changes occurring as a result of habitat differentiation would be accompanied by changes in shape. Convergent evolution, however, was only observed in the externally ordinated ecospace (DFA).

The mantel tests indicated that shape is more closely correlated with phylogeny than is size, which shows the lowest correlation. Our results suggest size may be more labile to a change in environmental conditions than shape as it is not as phylogenetically constrained. If flexibility in size over shape is real, the first phenotypic changes observable once a species is exposed to different environmental conditions should be size related. This conclusion is borne out by observations of island dwarfing (Foster's rule [Foster 1964]), where changes in size have been observed as occurring as quickly as within 150 years (Yom-Tov et al. 1999).

Our results have important implications for paleoecologists. First, we show that convergence and divergence were observed in an externally ordinated morphospace, supporting ecomorphological analyses in this space. Therefore, if convergent and divergent evolution of a skeletal element under investigation can be demonstrated in this space, such as it has here, then this means this element satisfies the assumptions of ecomorphological analyses aimed at discriminating habitats. Although phylogeny plays a role in

both the size and shape of any skeletal element, we show that size is less correlated with phylogeny than shape. However, neither of the neutral morphospaces (shape nor size) fulfilled the convergence/divergence assumptions of ecomorphological models, and hence may not be suitable for building discriminant models for habitat prediction. We also show that an externally ordinated morphospace may more accurately reflect the phylogenetic relationships of the species under examination, as well as their functional similarities, compared to a neutral morphospace.

Finally, our study provides a quantitative method for testing divergence amongst species within a phylogenetic context. Analyses and tests for divergence impact many fields of evolutionary biology and are just as important as those for convergence. Our results suggest that size may be relaxed more quickly than shape in the context of new environmental conditions. African antelopes are continental species, and it would be interesting to see if the pattern of divergence we describe is followed in island species, where the ecological pressures for a change in size are considered much greater (e.g., Sondaar 1991; Yom-Tov et al. 1999; Millien and Damuth 2004).

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