Morphological evaluation of genetic evidence for a Pleistocene extirpation of eastern African impala

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Keywords:
antelope; entostyles; extirpations; founder populations; genetic population histories; Pleistocene climate changes

Dates:
Received: 20 June 2010
Accepted: 03 Oct. 2010
Published: 17 Nov. 2010

How to cite this article:
Reynolds SC. Morphological evaluation of genetic evidence for a Pleistocene extirpation of eastern African impala. S Afr J Sci. 2010;106(11/12), Art. #325, 7 pages. DOI: 10.4102/sajs.v106i11/12.325

This article is available at:
http://www.sajs.co.za

ABSTRACT

Palaeontology typically relies on fossil studies, in particular morphological differences, to reconstruct and interpret patterns of vertebrate evolution. However, genetic studies of population histories of extant species provide data about past population events (e.g. local extinctions, recolonisations) which are equally relevant to palaeontological questions. This study used morphological traits to evaluate a hypothesis based on genetic evidence that southern African impala (*Aepyceros melampus*) are the founder population for all other living African impala populations, after an eastern African extirpation event dating to around 200 000 years ago. Measurements of three horn metrics and the presence or absence of a particular dental trait were compared across four regional impala samples. Eastern African impala possess a unique combination of larger horns and a significantly higher occurrence of entostyles when compared to other impala populations. These traits are likely to have characterised a small group of founding impala which recolonised this region. This pattern appears consistent with the genetic evidence that a subset of the southern African impala gave rise to the eastern African populations. Other species with complex population histories, such as wildebeest, eland, topi and hartebeest may also therefore be expected to express variation in certain morphological traits in the fossil record because of similar patterns of recolonisations. The process of local extinction and subsequent repopulation over shorter timescales (10^2 – 10^3 years) may pass unnoticed in the fossil record, and lineages may appear uninterrupted. Instead, greater morphological variation within a species may be observed, which may be misinterpreted as reflecting a speciation event, or ecophenotypic variation. Combining data from genetic studies and palaeontology may provide further clues as to how faunal dispersals within Africa shaped the morphological variation in the fossil record, and how to best interpret such differences.

INTRODUCTION

Studies of fossil assemblages recovered at various sites, have, until very recently, been the only way to reconstruct and interpret patterns of species evolution and community response to large-scale climate shifts.[1,2] The Plio-Pleistocene African fauna, particularly species with extensive geographic ranges, provide important data regarding mammal responses, such as body size changes, to pronounced climate and seasonality shifts.[2] Several species co-occurring in southern and eastern Africa, show marked body size changes in eastern African conspecifics, relative to southern African counterparts. This pattern has been explained by dramatic climate changes over the last three million years, which may have been further exacerbated by different degrees of volcanism and tectonism in east Africa, which did not affect southern Africa to the same extent.[2]

Genetic studies of living populations provide a non-palaeontological perspective on recent population changes, local extinctions (known as extirpations) and recolonisations.[3,4,5] In particular, the genetic evidence across several mammal species suggests that dramatic late Pleistocene climate changes have profoundly altered their population structures. The amount of variation in microsatellite markers suggests a late Pleistocene extirpation (ca. 200 KYA) of several species in eastern Africa, including wildebeest (*Connochaetes taurinus*) and impala.[3,4,5] Other species exhibit more complex spatial patterns of refugia during the same temporal period; for example, phylogeographic studies of the hartebeest (*Alcelaphus buselaphus*), the topi (*Damaliscus lunatus*) and the eland (*Taurotragus oryx*), indicate that they survived during this period in small isolated areas of eastern and western Africa.[3,5]

Of all the African antelopes subjected to genetic studies to date, the impala stands out as an intriguing mix of evolutionary simplicity and complexity, offering a unique record of antelope evolution in Africa over several million years. Some authors consider this bovid tribe, the Aepycerotini, to be 'living fossils', little changed over the course of their evolution.[6] With apparently almost no speciation during the Plio-Pleistocene, they contrast markedly with other, more speciose tribes, such as the Alcelaphini or the Antilopini.

The earliest specimens assigned to the *Aepyceros* genus are known from Lothagam, northern Kenya, from deposits which are dated to between 7 MYA and 4 MYA.[7] Impala also are abundant in the Shungura, Member B (Ethiopia) deposits, which date to 2.95 ± 0.05 MYA.[1,8] The Shungura species (*Aepyceros shungurae*) had a smaller overall body size and somewhat different horncore morphology.[2] The genus has also been identified from one early deposit in southern Africa, Makapansgat, palaeomagnetically dated to approximately three million years old; but these specimens have not been assigned to a species.[9,10]

Impala are widespread in modern times, inhabiting savannah environments over a discontinuous distribution throughout sub-Saharan Africa.[11,12] Only males possess ridged, thin, S-shaped horns. In present-day South Africa, impala are confined to game reserves and, as reintroduced populations, to the eastern coast of South Africa (Figure 1). Because this species has been well studied, from behavioural, genetic, palaeontological and morphological perspectives, it provides a useful model to assess whether or not variation in morphological traits can be reconciled with current genetic evidence of population history.

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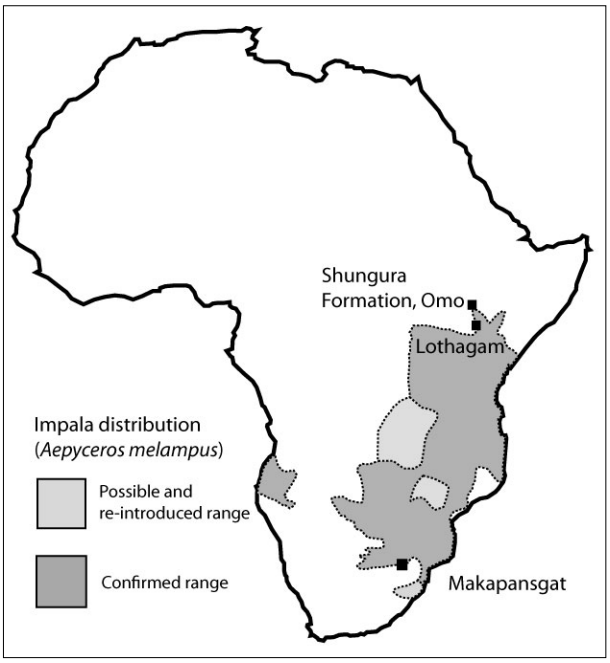

FIGURE 1
Present-day distribution of impala (*Aepyceros melampus*).[7] Dark shaded areas show the confirmed range of this species, and lighter shaded areas the regions where impala have been reintroduced. The earliest appearances of the genus are at the fossil localities of Lothagam (northern Kenya), where deposits date to 4 MYA– 7 MYA[7] and the contemporaneous Shungura Formation (Ethiopia) and Makapansgat (South Africa), which date to ca. 3 MYA[8,9]

Modern geographic variation in impala

Most authors recognise between three and four subspecies of impala, (1) the long-horned impala from East Africa (*Aepyceros melampus suara*), (2) the common impala from the southern African regions (*A. m. melampus*), (3) the subspecies *A. m. johnstoni*, from a geographically intermediate region – Zamibia, Malawi and Zimbabwe and (4) the rare black-faced subspecies (*A. m. petersi*).[13] The latter subspecies is known only from a small region of northern Namibia and south-western Angola (Figure 1) and is clearly distinguishable from other impala subspecies based on both pelage and mitochondrial DNA differences.[14] Within the continuous distribution of common impala from eastern and southern African regions, the two subspecies (*A. m. suara* and *A. m. melampus*) are recognised primarily by horn size differences.[7] Male eastern African impala horns are reported to be significantly longer than southern African conspecifics.[15,16] Apart from horn size differences, detailed cranial and dental studies could find almost no size differences between southern African and eastern African impala samples.[2]

Several previous studies have examined geographic variation of impala, using both craniodental and horn morphology,[2,15,16] other studies have examined how pelage differences relate to previously recognised subspecies. Horn dimensions differ between impala populations (subspecies) and this 'diversity may have genetic, evolutionary, ecological or biogeographic importance'[16].

Lorenzen and colleagues used their study of genetic diversity and spatial population structuring to deduce that southern Africa is the founder population for all modern impala.[4] The genetic perspective appears at odds with the current fossil evidence, where impala are abundant in many fossil sites in eastern Africa, but are sparsely represented in the southern African sites. In this study, I therefore used horn metrics and the presence or absence of simple folds and basal entostyles in the upper molar teeth of impala samples across their geographic range to test for regional differences, and to establish whether there are morphological traits which could support the genetic hypothesis.

MATERIALS AND METHODS

A total of 276 modern impala skulls of both sexes from four African regions were examined (Table 1). Both adult and sub-adult (third molars erupting) specimens were included. Only wild-caught specimens with known country provenance were included. The impala were divided into four groups corresponding to geographic localities, in order to characterise the degree of potential variation from each region. Group 1 (*A. m. melampus)* represents the subspecies from the Republic of South Africa, while Group 2 (*A. m. johnstoni*) represents those from Zambia, Malawi and Zimbabwe. Group 3 (*A. m. suara*) represents Uganda, Kenya and Tanzania and includes the subspecies *A. m. rendilis*, which is considered a synonym for *A. m. suara*.[16] The final group, Group 4 (*A. m. petersi*), contains the rare black-faced impala from Angola, Namibia and Botswana. Specimens were studied at the National Museums of Kenya (Nairobi), the Natural History Museum (London), the Powell-Cotton Museum (Kent), the University Museum of Zoology (Cambridge), the University of the Witwatersrand (Johannesburg) and the Northern Flagship Institute (Pretoria).

Traits examined

Three horn metrics were measured on 164 male impala skulls: total horn length, greatest width between horns and width at tips; these metrics were recorded on unbroken horn sheaths, using a standard tape measure, to the nearest 3 mm (Figure 2). Means for all groups were tested using the Mann-Whitney U test.[17] The presence or absence of (1) simple folds and (2) entostyles on teeth of both sexes also was recorded (Figure 3). Where both traits occurred, these were scored once only as 'entostyles present'. Sexes from each regional subsample were first compared separately, and then as pooled data. The Chi-square (x^2) goodness-of-fit test was used to compare data to a null distribution of equal numbers of simple folds and entostyles across impala groups.[18]

RESULTS

The large horn dimensions of the eastern African impala, *A. m. suara*, are considered by several authors to be a diagnostic feature of this subspecies.[12] However, while the eastern African subspecies does possess the largest means of all three horn metrics examined (Table 2), the total length of the horns of east African impala (Group 3) were not significantly different to that of the South African (Group 1) impala ($p > 0.05$, Mann-Whitney U test; Tables 2 and 3, Figure 4). The only metric which differed significantly between these two groups was the greatest width between horns, with the eastern African impala having a significantly greater mean width between horns ($p < 0.05$, Mann-Whitney U test; Tables 2 and 3, Figure 5). The overlap between Group 1 (*A. m. melampus*) and Group 3 (*A. m. suara*) therefore renders this trait less useful as an indicator of a particular subspecies in every case (Figures 4 and 5). However, for the

TABLE 1
The number of specimens of four separate *Aepyceros melampus* subspecies examined in this study

Subspecies	Countries of origin	Female	Male
Group 1 (*A. m. melampus*)	South Africa	68	94
Group 2 (*A. m. johnstoni*)	Zambia, Zimbabwe, Malawi	26	24
Group 3 (*A. m. suara/ rendilis*)	Uganda, Kenya, Tanzania	7	24
Group 4 (*A. m. petersi*)	Angola, Namibia, Botswana	11	22
Total		**112**	**164**

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Group 2 impala (from Malawi, Zimbabwe and Zambia), all horn metrics were found to be significantly smaller than in their counterparts from either eastern or southern Africa (Tables 2 and 3) – a pattern which strongly differentiates this subspecies (*A. m. johnstoni*) from all other subspecies (Figures 4 and 5). The horn metrics of Group 4, the black-faced impala, were most similar to the southern African impala, with all three horn metrics not being significantly different (Table 3).

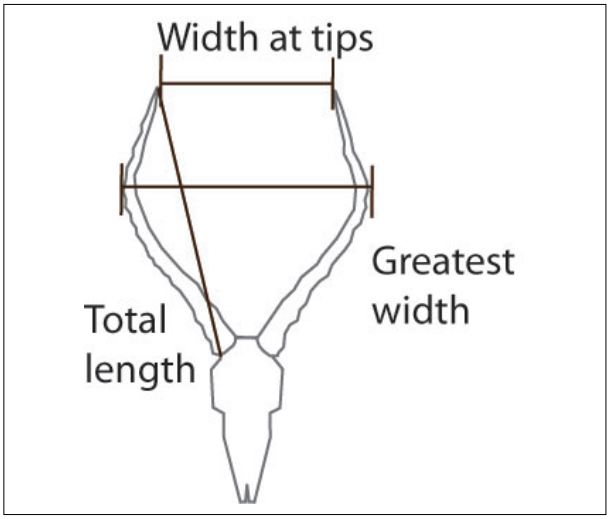

FIGURE 2
Three measurements taken on undamaged male impala horns. These measurements – greatest width between horns, total length and width at tips – are comparable with measurements used in other studies of modern impala subspecies variation[16]

For the dental traits, teeth of both sexes had simple folds and entostyles, although these traits were not equally distributed between males and females (Table 4). Increased sample sizes may clarify whether or not there are differences between sexes, and why such traits may differ. Entostyles were most commonly observed in the eastern African impala, occurring in 51.6% of specimens. In contrast, only 16.7% of the South African impala (Group 1) had entostyles (Table 4). The genetically differentiated black-faced impala, *A. m. petersi* (Group 4), had the second highest occurrence of entostyles at 36.4% (12 of a total of 33 specimens). The occurrence of entostyles in Group 2 (*A. m. johnstoni*) was intermediate between the southern and eastern African groups, occurring in 26% of specimens (Table 4). Simple folds were most common in Groups 2 and 3 (occurring in 16.0% and 12.9% of specimens, respectively; Table 4). They occurred least often in the South African group, with only 5 of 130 specimens (3.1%) having simple folds.

DISCUSSION

Variation in both metric and non-metric traits is often observed in geographically widespread species. One of the most common forms of geographic variation is body size clines, with larger-bodied conspecifics found in South Africa and smaller-bodied individuals in eastern Africa. This pattern has been interpreted as a selection for larger body sizes in more seasonal southern African environments[2,15] and has been identified for several

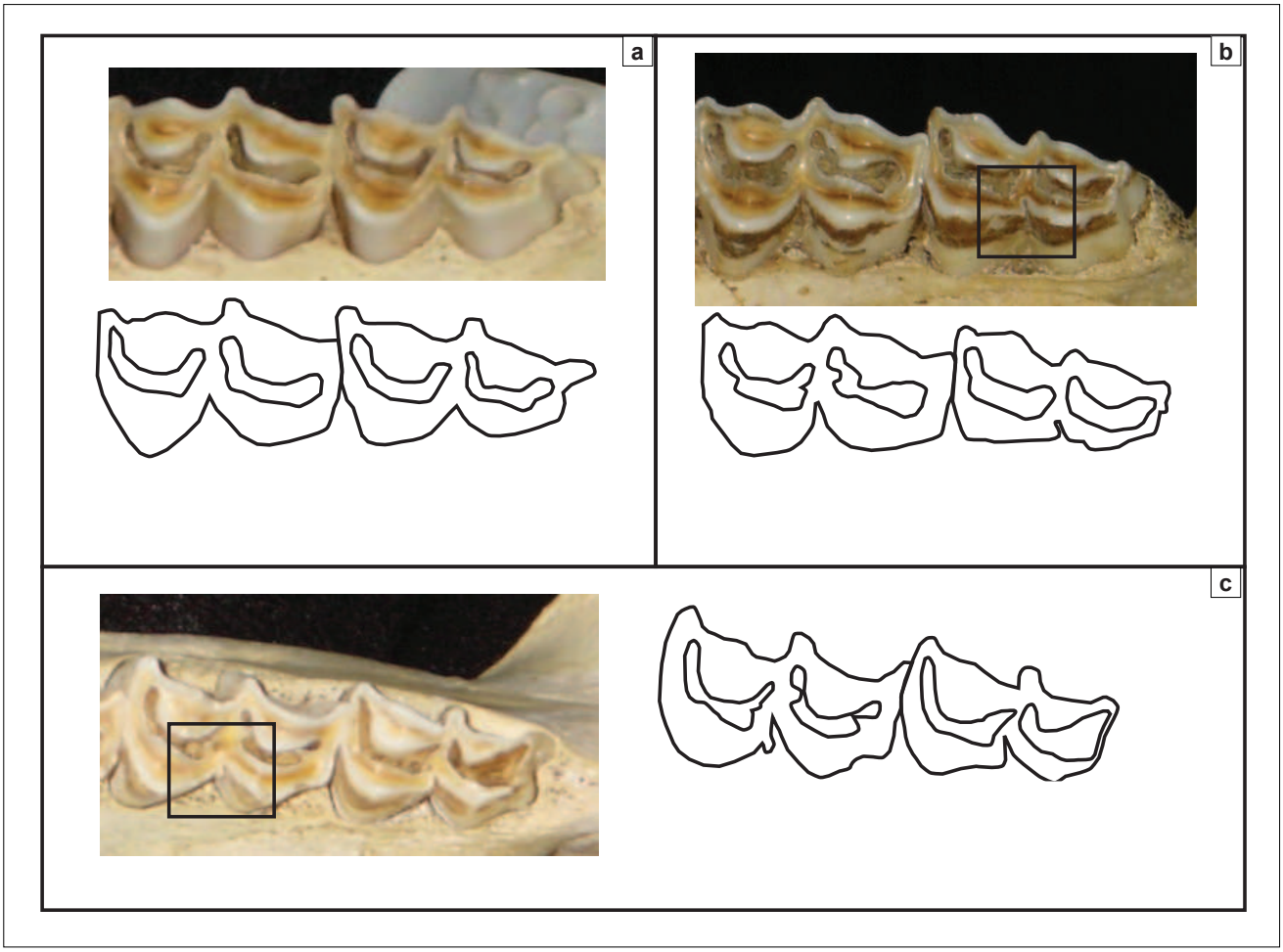

FIGURE 3
Upper molar dental traits recorded in all adult impala individuals: a) a complete absence of simple folds or entostyles, b) the presence of simple enamel folds and c) the presence of entostyles of the second upper molar

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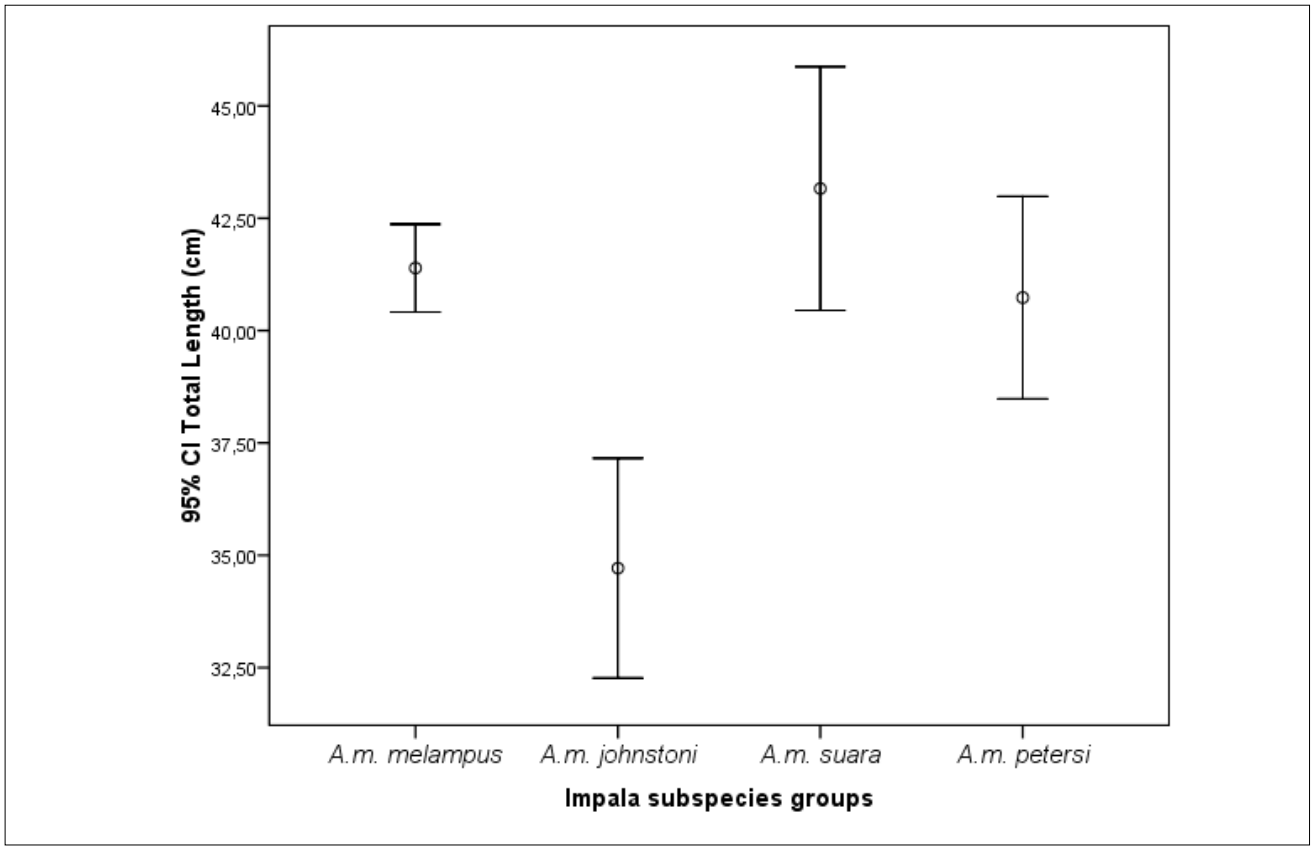

FIGURE 4
Means and 95% confidence intervals of total horn length of the different subspecies of impala. Impala from Malawi (*A. m. johnstoni*) have the shortest horns, while the eastern African subspecies (*A. m. suara*) have the longest horns

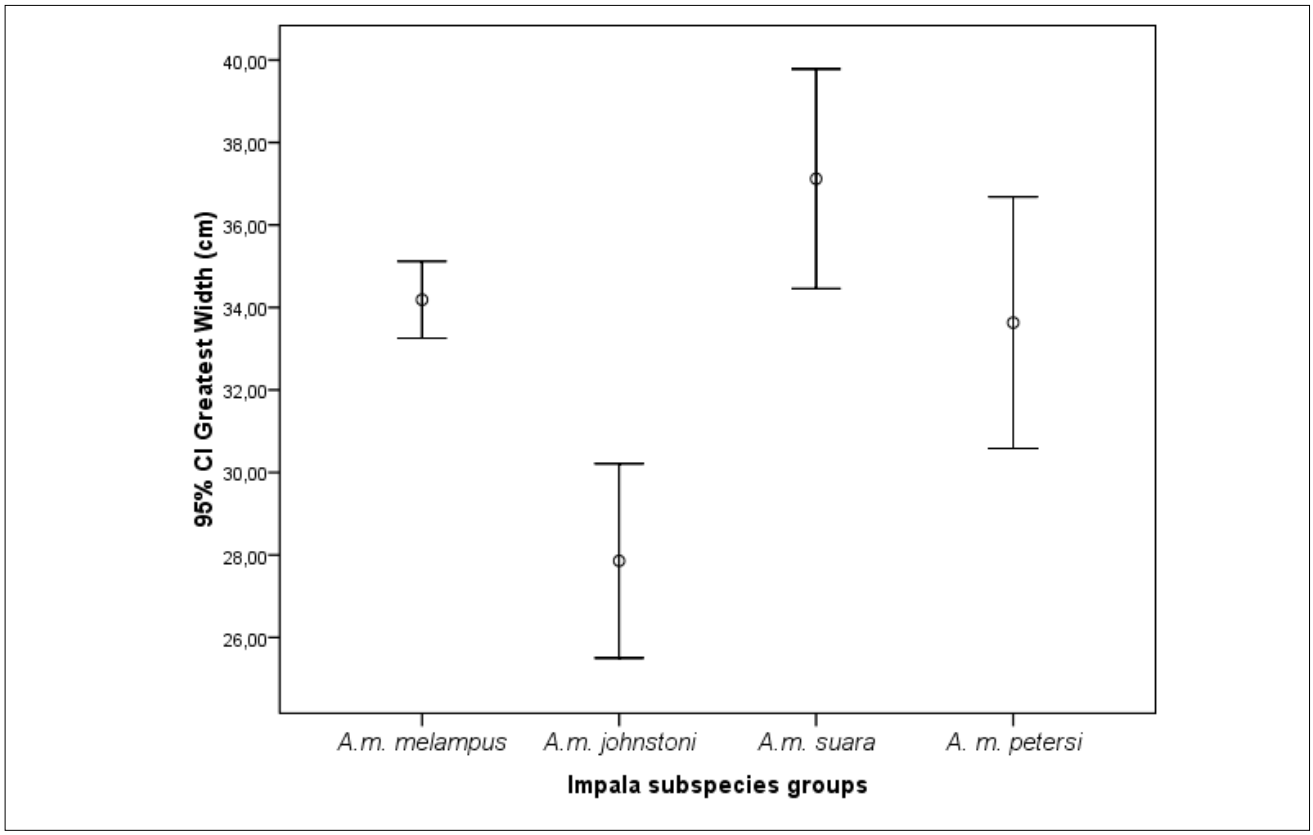

FIGURE 5
Means and 95% confidence intervals of the greatest width between the horns of the different subspecies of impala. Impala from Malawi (*A. m. johnstoni*) have the narrowest width between horns, and the eastern African subspecies (*A. m. suara*) have the largest, followed closely by those of the genetically differentiated black-faced impala from Namibia (*A. m. petersi*)

species including spotted hyaena (*Crocuta crocuta*) and plains zebra (*Equus burchelli*). While the geographic variation in these species is easier to understand in terms of seasonality differences, impala show a pattern consisting of (1) a lack of body size differences between regions, (2) slight horn size differences (except for the greatest width between horns) and (3) a greater number of entostyles in the eastern African impala. These data do suggest that the populations of impala may have undergone different population histories relative to the spotted hyaena and plains zebra. The local extirpation and recolonisation pattern has not been observed for the other two species and may account for the complex regional patterns visible in impala.

Overall horn metrics for the southern African impala (Group 1) and the eastern African impala (Group 3) are similar, while the dental non-metric traits strongly differentiate these groups (Table 4). The entostyle evidence is consistent with a founder population scenario, where impala recolonising the eastern African region possessed high numbers of entostyles and larger horn dimensions. All three horn metrics of the black-faced impala do not differ significantly from the southern African impala (Tables 2 and 3). However, the black-faced impala show a higher incidence of simple folds and entostyles than are found in southern African impala (Table 4). This finding appears to confirm studies which suggest that *A. m. petersi* is a separate,

TABLE 2
Comparison of the mean horn metrics for four impala subspecies

Group	Greatest width between horns (cm)	Width at tips (cm)	Total length (cm)
1: South Africa (n = 69)	34.19	24.34	41.39
2: Zambia, Zimbabwe, Malawi (n = 21)	27.86	17.00	34.71
3: Uganda, Kenya, Tanzania (n = 23)	37.12*	25.52*	43.16*
4: Angola, Namibia, Botswana (n = 15)	33.63	21.63	40.73

*, Highest values.

TABLE 3
Results of the pairwise comparisons of three horn metrics between four impala subspecies

		p-values		
		Group 2	Group 3	Group 4 (n = 15)
Greatest width between horns (cm)	Group 1 (n = 69)	0.000*	0.012*	0.797
	Group 2 (n = 21)	-	0.000*	0.003*
	Group 3 (n = 25)	0.000*	-	0.050*
Width at tips (cm)	Group 1 (n = 67)	0.003*	0.436	0.666
	Group 2 (n = 21)	-	0.006*	0.103
	Group 3 (n = 24)	0.006*	-	0.520
Total length (cm)	Group 1 (n = 69)	0.000*	0.118	0.669
	Group 2 (n = 24)	-	0.000*	0.001*
	Group 3 (n = 25)	0.000*	-	0.100

*, Significant values.

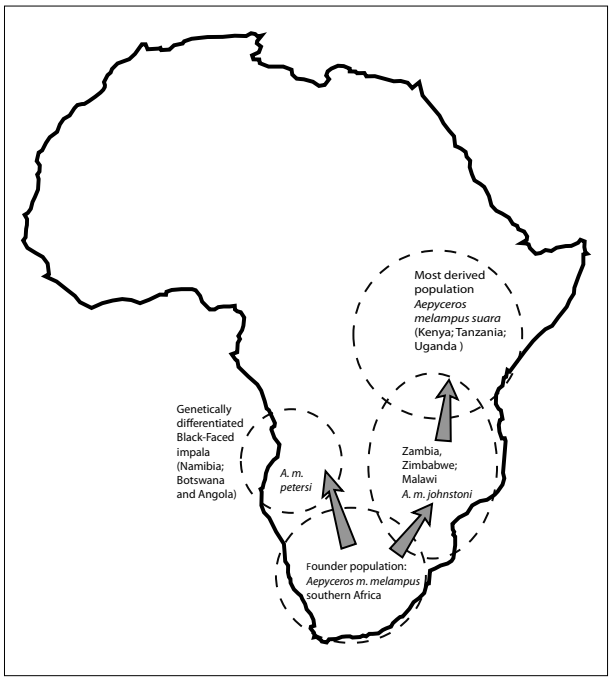

FIGURE 6
Schematic representation of how the recolonisation from the founder population is hypothesised to have progressed: two separate groups dispersed to the northwest and to the northeast. The northeast branch culminates in the most derived population of eastern Africa, with large horn sizes and a high prevalence of entostyles. This model cannot presently explain why the impala from Malawi and Zimbabwe and Zambia differ equally from eastern African and southern African populations. The dispersal to the northwest leads to the genetically isolated population of the black-faced impala

TABLE 4
Incidence of upper molar traits, by sex and by geographical group

	Upper molar traits		
Group	Entostyles present	Simple folds present	Absent
Group 1			
Females (n = 68)	8.8% (6)	2.9% (2)	88.2% (60)*
Males (n = 94)	22.3% (21)	3.2% (3)	74.5% (70)*
Combined (n = 162)	16.7% (27)	3.1% (5)	80.2% (130)*
Group 2			
Females (n = 26)	15.4% (4)	7.7% (2)	76.9% (20)*
Males (n = 24)	37.5% (9)*	25.0% (6)	37.5% (9)*
Combined (n = 50)	26.0% (13)	16.0% (8)	58.0% (29)*
Group 3			
Females (n = 7)	57.1% (4)*	28.6% (2)	14.3% (1)
Males (n = 24)	50.0% (12)*	8.3% (2)	41.7 (10)
Combined (n = 31)	51.6% (16)*	12.9% (4)	35.5% (11)
Group 4			
Females (n = 11)	18.2% (2)	27.3% (3)	54.5% (6)*
Males (n = 22)	45.5% (10)	4.5% (1)	50.0% (11)*
Combined (n = 33)	36.4% (12)	12.1 (4)	51.5% (17)*

Note: Percentages of group total and numbers of specimens are given. Numbers of specimens are indicated in brackets after the percentages.
*, Highest totals.

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distinct subspecies, most closely related to the common, southern African impala populations.[14]

Possible dispersal routes of impala

High genetic diversity indicates that impala dispersed northwards from a founder population in southern Africa.[4] Low dispersal distances presumably aided the genetic differentiation, especially in the black-faced impala.[19] With no significant differences in horn metrics between Group 1 and Group 4 impala and only slight differences in upper molar traits, it is plausible that the founder population gave rise directly to the black-faced form (Figure 6). However, other authors have proposed that the subspecies is a relict population of an earlier westward range expansion, before populations became geographically separated as a result of Pleistocene climate shifts.[4]

The *A. m. johnstoni* subspecies from Malawi, Zambia and Zimbabwe is a unique group: it has similar dental traits to the black-faced impala subspecies, but also has the smallest horn metrics of any impala subspecies examined here. The sample, albeit small, shows possible sexual dimorphism in dental traits (Table 4). Whether these traits were characteristic of the impala which colonised this region, or whether they represent ecophenotypic adaptation to the region is unclear, and should be the focus of future studies. The observed high genetic diversity of this subspecies has been ascribed to its intermediate position in the gene flow between populations.[4]

Implications for understanding the fossil record

While the presence of entostyles have been inferred to be a primitive trait,[20] within the context of this study, it appears that characteristics such as horn size and entostyles, may increase and decrease through time in response to the extirpation and recolonisations of certain subspecies. As a result, even though the geographic range is seemingly continuous, the localised effects of other variables, such as disease outbreaks, inbreeding effects, or climate changes, may act over smaller spatial and temporal scales. In essence, regional populations may function as separate evolutionary entities, influencing how morphological variation appears in the fossil record.

The recent population history of impala clearly illustrates how regional populations (or subspecies) do not share the same evolutionary trajectory, with one population surviving for ca. 200 KYA in the south, while another failed to survive in eastern Africa. Given that the *Aepyceros* lineage stretches back to between 4 MYA and 7 MYA, at Lothagam, in northern Kenya,[7] it is reasonable to assume that this scenario of population extirpations and recolonisations would have been repeated in different places within the temporal and geographical range of this genus. For instance, it is possible, given the dearth of Pliocene impala material in southern Africa, that the Makapansgat impala population may have been extirpated and later replaced by a different population of southward-dispersing impala, possibly originating in eastern Africa. In order to test this type of scenario, it may be more informative to consider entostyles and other distinguishing morphological characteristics as population markers, rather than traits which are uniformly 'primitive' or 'derived'. The presence and absence of such traits could indicate possible genetic relationships between regional groups through time, even beyond the timescales for which genetic population studies can provide information.

CONCLUSIONS

Two traits which differ among the four impala populations have been identified. The *A.m. suara* subspecies from eastern Africa have the largest horn metrics measured in this study, as well as the highest occurrence of entostyles (51.6%). While it is not possible to know exactly why the distribution of entostyles and specific horn sizes differ among regional populations, the recent genetic studies provide a framework within which such variation can be interpreted. In the light of the genetic evidence,[4,5] differentiation of horn and upper molar traits can be interpreted as a result of the dispersal of impala populations from a founder population elsewhere. The high occurrence of entostyles in the eastern African region suggests that the founder population that recolonised this region possessed high frequencies of this trait. While entostyle loss is considered a derived trait[20] here the entostyles in the eastern African impala do not represent a 'primitive' population; instead the trait appears to dominate this subspecies because it was more prevalent in the population which dispersed to eastern Africa.

In the fossil record, such processes of local extinction and repopulation of areas over shorter timescales (in this case, approximately 200 KYA) may be virtually palaeontologically invisible, apart from cases similar to this one, where population genetics is able to contribute an alternative perspective on species changes in the past. Instead, fossil lineages may appear continuous, but with possible variability in certain traits, which could be interpreted as speciation, or ecophenotypic adaptation to different environments. This pattern may be even more relevant for extinct species, where palaeontological and palaeoclimatic evidence alone must be used to develop scenarios of adaptation, endemism and extinction. Extant species with similarly complex population histories would also be expected to show some morphological variation as a result of population changes after the Pleistocene climate shifts. Species such as the wildebeest, eland, topi and hartebeest, may possess somewhat different morphological traits in the fossil record. The combination of genetic population studies and morphology studies can potentially provide further clues as to how mammal dispersals within Africa shaped the morphological variation observed in the fossil record.

ACKNOWLEDGEMENTS

This research received support from the SYNTHESYS Project http://www.synthesys.info/ which is financed by European Community Research Infrastructure Action under the FP6 'Structuring the European Research Area' Programme. Part of this research derived from my doctoral studies at the Liverpool John Moores University, Liverpool, United Kingdom. Meredith Robinson kindly assisted with the data collection in Johannesburg. I thank the curators of the Northern Flagship Institute (formerly the Transvaal Museum, Pretoria), the Bernard Price Institute, University of the Witwatersrand (Johannesburg), the Natural History Museum, (London), the National Museums of Kenya (Nairobi), Powell-Cotton Museum (Kent) and the University Museum of Zoology (Cambridge) and the museums for access to their comparative collections.

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