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Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation

Øystein Flagstad[1,3], Per Ole Syvertsen[1]†, Nils Chr. Stenseth[1]* and Kjetill S. Jakobsen[2]

[1]*Division of Zoology, and* [2]*Division of General Genetics, Department of Biology, University of Oslo, PO Box 1050, Blindern, NO-0316 Oslo, Norway*

[3]*Department of Evolutionary Biology, Uppsala University, Nordbyvägen 18D, SE-752 36 Uppsala, Sweden*

Global climate fluctuated considerably throughout the Pliocene–Pleistocene period, influencing the evolutionary history of a wide array of species. Using the phylogeographic patterns within the hartebeest (*Alcelaphus buselaphus* (Pallas, 1766)) complex, we evaluated the evolutionary consequences of such environmental change for a typical large mammal ranging on the African savannah. Our results, as generated from two mitochondrial DNA markers (the D-loop and cytochrome *b*), suggest an origin of the hartebeest in eastern Africa from where the species has colonized other parts of the continent. Phylogenetic analyses revealed an early diversification into southern and northern hartebeest lineages, an event that may be related to the formation of the Rift Valley lakes. The northern lineage has further diverged into eastern and western lineages, most probably as a result of the expanding central African rainforest belt and subsequent contraction of savannah habitats during a period of global warming. The diversification events appear to have coincided with major climatic changes and are highly correlated in time. These observations strongly suggest that large-scale climatic fluctuations have been a major determinant for the species' evolutionary history and that hartebeest evolution has mainly taken place in isolated yet environmentally favourable refugia during periods of global warming. Indications of sudden population expansion for two putative ancestral hartebeest populations provide further support for a refugia-based explanation of the diversification events. Reciprocal monophyly between southern and northern lineages may suggest that reproductive barriers exist and that the hartebeest complex comprises two different species.

Keywords: allopatric diversification and speciation; habitat fragmentation; mitochondrial DNA; phylogenetic analysis; refugia

1. INTRODUCTION

Climatic variation may affect evolutionary patterns by altering rates of speciation and extinction through changing availability of appropriate habitats. Two major changes in the global climate have occurred over the past few million years (deMenocal & Bloemendal 1995). A profound global cooling from 3.0 towards 2.5 million years before present (Myr BP) culminated in full glaciation of the Poles (the onset of the 'modern ice age') (Shackleton *et al.* 1984). After 0.9 Myr BP, glacial maxima became even more extreme and the dominant periodicity of glacial–interglacial cycles shifted from 41 to 100-thousand years (e.g. Hooghiemstra *et al.* 1993). The fossil record indicates substantial biotic turnover associated with these climatic episodes (Vrba 1995*a*). Such observations are concordant with the relay model proposed by Vrba (1995*b*)—a model assuming geographical isolation as a prerequisite for speciation (cf. Mayr 1942, 1954, 1963). According to this model, the range of a species having a continuous distribution is fragmented by the appearance of tectonic and climatic barriers (Croizat *et al.* 1974), which subsequently leads to speciation and extinction. Such a scenario may give rise to a stasis-plus-punctuation pattern of evolution (cf. Eldredge & Gould 1972) where bursts of extinction, speciation and other evolutionary changes are associated with and caused by major changes in the physical environment, followed by periods of negligible evolutionary change (see also Stenseth & Maynard Smith 1984).

Vrba (1983, 1984) used species and subspecies diversity among the bovid tribes Alcelaphini and Aepycerotini in order to illustrate such an evolutionary scenario. The alcelaphines, including the genera *Alcelaphus*, *Beatragus*, *Connochaetes* and *Damaliscus*, are the youngest of all African bovid tribes (first appearance date 5 Myr BP). Nevertheless, the group has exhibited remarkable diversification. On the other hand, the tribe Aepycerotini, comprising only the impala *Aepyceros melampus* (Lichtenstein, 1812), seems to have existed more or less unchanged for the last 5 Myr (Vrba 1984). These contrasting patterns may be seen as a response to differences in habitat requirements. Vrba (1983, 1984) emphasized that specialist species (such as the alcelaphines) may be more sensitive to environmental change as they are restricted to a particular habitat type, while generalist species (e.g. the impala) may range across several different habitat types. Thus, as suitable habitat (open savannahs and grasslands) will appear fragmented to the alcelaphines during periods of unfavourable environmental conditions, allopatric populations displaced in isolated refugia may be expected to diverge rapidly. On the other hand, proper habitat may remain

*Author for correspondence (n.c.stenseth@bio.uio.no).
†Present address: Rana Museum, PO Box 1301, NO-8602 Mo i Rana, Norway.

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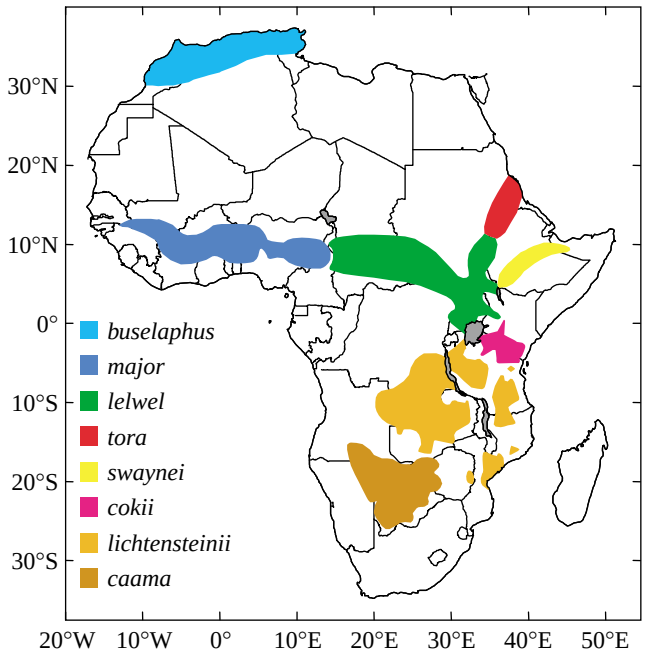


Figure 1. General historic distributions of recent hartebeest subspecies (redrawn after Kingdon 1997). We recognize two main geographical distribution ranges, hereafter referred to as the southern and northern hartebeest distributions. The southern distribution includes the two subspecies located south of the Equator, *caama* and *lichtensteinii*, while the northern range comprises the rest of the hartebeest complex. The north can further be subdivided into western and eastern distribution ranges. The west comprises subspecies *major* and the extinct *buselaphus*, while the east is represented by the remaining hartebeest subspecies *lelwel*, *cokii*, *tora* and *swaynei*.

more or less constant through time for the impala, sustaining a fairly continuous distribution with limited intraspecific diversification.

The hartebeest (*Alcelaphus buselaphus* (Pallas, 1776)) complex constitutes a particularly diverse mixture of different forms among the alcelaphines, with seven or eight currently recognized subspecies (Kingdon 1997; East 1999) (figure 1). The complex has been treated as both a single polytypic species (e.g. Haltenorth 1963; Kingdon 1997) and two separate species, with the central African woodland-ranging form *lichtensteinii* (Peters, 1849) forming a separate group either associated with *Alcelaphus* (e.g. Dorst & Dandelot 1972; Gentry 1990; East 1999) or placed in a monotypic genus *Sigmoceros* (e.g. Vrba 1979, 1995*a*; Grubb 1993). The red hartebeest of southern Africa is today usually considered conspecific with the northern savannah lineages, but has been treated as a separate species (*Alcelaphus caama* (G. Cuvier, 1804)) by some authors (e.g. Dorst & Dandelot 1972).

Arctander *et al.* (1999) reported on the phylogeographic pattern for three alcelaphine species, including the hartebeest. They suggested that the three species' evolutionary history was primarily determined by climatic variability during the Pleistocene period. Here, we focus on the hartebeest complex and extend the study of Arctander *et al.* (1999) by analysing a larger and more complete set of data (more representative sampling, a longer D-loop sequence and a 417 base pair (bp) cytochrome *b* (cyt *b*) sequence for all extant hartebeest subspecies). We discuss the centre of origin for the species and outline the most likely colonization routes across the African continent. Particular attention is paid to climatic conditions in examination of the different factors responsible for the evolution within the hartebeest complex. In this connection, we explicitly test the relay model proposed by Vrba (1995*b*) by relating phylogenetic and phylogeographic patterns to available fossil and climatic evidence.

2. MATERIAL AND METHODS

(a) *Sampling and DNA extraction*

(i) *Material collected in the field*

Faeces samples of hartebeest belonging to the form *swaynei* Sclater, 1892 were collected from three different populations in Ethiopia as described by Flagstad *et al.* (2000). In addition, we obtained blood samples collected from the largest surviving *swaynei* population in 1988 (Mattravers Messana 1993). Fresh blood samples from individuals of *caama* (originating from Namibia) were provided from South African sources (cf. Flagstad *et al.* 2000).

Faecal DNA was extracted by employing the magnetic beads protocol as described by Flagstad *et al.* (1999). A standard chloroform:phenol protocol (Sambrook *et al.* 1989) was applied for DNA extraction from blood.

(ii) *Museum specimens*

Dry tissue and skin samples from museum specimens, representing all recent hartebeest subspecies, were provided by the Natural History Museum in London and by the Powell–Cotton Museum and Quex House & Gardens in Kent. DNA was extracted using the same chloroform:phenol protocol as for the blood samples. For a detailed description of all samples employed in this study see electronic Appendix A available on The Royal Society's Web site.

(b) *Amplification and DNA sequencing*

A 486 bp region of the mitochondrial D-loop adjacent to the $tRNA^{pro}$ gene was targeted for polymerase chain reaction (PCR) and solid-phase DNA sequencing for the faeces and blood samples (Hultman *et al.* 1989). The primer sequences employed were L15394 (which is specially designed for hartebeest and targeting the $tRNA^{pro}$ gene) (see Flagstad *et al.* 2000) and H15947 (a 'mammalian' primer targeting CSB-D) (cf. Southern *et al.* 1988). The PCR and sequencing protocols were described by Flagstad *et al.* (2000). A total of 48 D-loop sequences, 27 from faeces and 21 from blood, were obtained using this approach.

The DNA extracts obtained from the museum specimens varied considerably in quality, ranging from large amounts of fairly intact DNA to minute quantities of severely degraded DNA. Extracted DNA was amplified using the same primers as for the faeces and blood samples. However, some of the samples yielded DNA that was too degraded for amplification by these primers. Two nested primers, H15730 (5′-TGTACTTGCTTATATGCGTGGGG-3′) and L15673 (5′-GACATAATATGAATATACTACATTAC-3′), were designed, thus enabling us to amplify the entire 486 bp region in two separate PCR reactions (L15394 paired with H15730 and L15673 paired with H15947). Amplifications were performed in 25 µl volumes with a standard buffer containing 1.35 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer and 0.5 units of AmpliTaq DNA polymerase (AB Applied Biosystems, Foster City, CA, USA). Thirty-five cycles with 40 s at 94 °C, 40 s at 60 °C and 40 s at 72 °C were preceded

by a 4 min pre-denaturation step and followed by an additional 7 min extension step for primer set L15394/H15947. We used 45 cycles of amplification with the same profile as described above for primer sets L15394/H15664 and L15638/H15947, except for an annealing temperature of 50 °C. DNA sequencing was carried out using a ThermoSequenase kit for [γ-33P]ATP-labelled dideoxy-nucleotides following standard procedures as described in the manual (Amersham Pharmacia, Uppsala, Sweden). A total of 34 D-loop sequences were obtained from the dry tissue samples.

In addition, cyt *b* sequences were obtained for a few individuals of each subspecies; 11 altogether. Primers LH14725 (Irwin *et al.* 1991) and HH15149 (Kocher *et al.* 1989) were applied for 40 cycles of amplification using the same PCR profile and conditions as described above. DNA sequencing was carried out using the ThermoSequenase kit.

(c) *Data analysis*

Sequences were aligned by eye using the sequence editor software SeqApp (D. G. Gilbert; seqpup@bio.indiana.edu). Maximum-likelihood (ML) genetic distances were calculated according to Felsenstein (1981) and phylogenetic relationships between D-loop and cyt *b* sequences were estimated from the quartet puzzling (ML) algorithm (Strimmer & vonHaeseler 1996) as implemented in PAUP* 4.0b2a (Swofford 1999). A minimum spanning network (MSN) was constructed for the cyt *b* data, using the program MINSPNET (L. Excoffier; http://www.anthro.unige.ch/~excoffie/default.htm). Nucleotide diversity (Nei 1987) was estimated for the major groups as revealed from the phylogenetic analysis. Assuming that major clades in the hartebeest phylogeny represent distinct ancestral hartebeest populations, we tested for sudden population expansion using the 'lineages-through-time' approach as implemented in the software package EndEpi (Rambaut *et al.* 1997). Putative expansion events were dated using the average number of nucleotide differences as a basis for the estimate (Schneider & Excoffier 1999).

The age of the deepest root in the hartebeest phylogeny was estimated from the total number of nucleotide substitutions in the cyt *b* sequence, assuming a saturation effect with time (cf. figure 2). The substitution rate in the cyt *b* gene appears to be fairly constant across bovid taxa (Mathee & Robinson 1999). Our estimate was therefore calibrated against a broad spectrum of bovine taxa where divergence times were assumed known from either the fossil record or molecular data (figure 2) (see Vrba 1979, 1984; Janecek *et al.* 1996; Hassanin & Douzery 1999; Mathee & Robinson 1999). The remaining time-estimates were based on the D-loop sequences. Since diversification events within species are in most cases relatively recent events, we assumed a linear relationship between genetic distance and divergence time (cf. Tamura & Nei 1993). Point estimates were arrived at from a topologically constrained UPGMA (unweighted pair-group method using arithmetic averages) tree (cf. Georgiadis *et al.* 1990).

All available raw data relevant to our study, a total of 96 D-loop sequences and seven cyt *b* sequences (cf. electronic Appendix A), were imported from GenBank and included in our analyses.

3. RESULTS

(a) *Sequence patterns*

The 417 bp cyt *b* region showed moderate variation, with 47 variable sites (11.3%) comprising 45 transitions and only two transversions. Forty-two of the detected nucleotide substitutions were found at third codon positions, while first and second codon positions were represented by four and one substitutions, respectively.

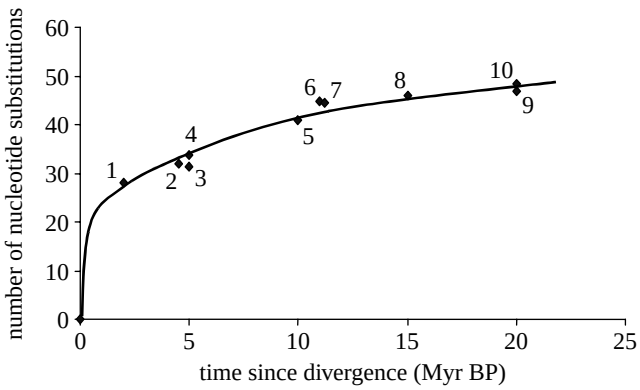


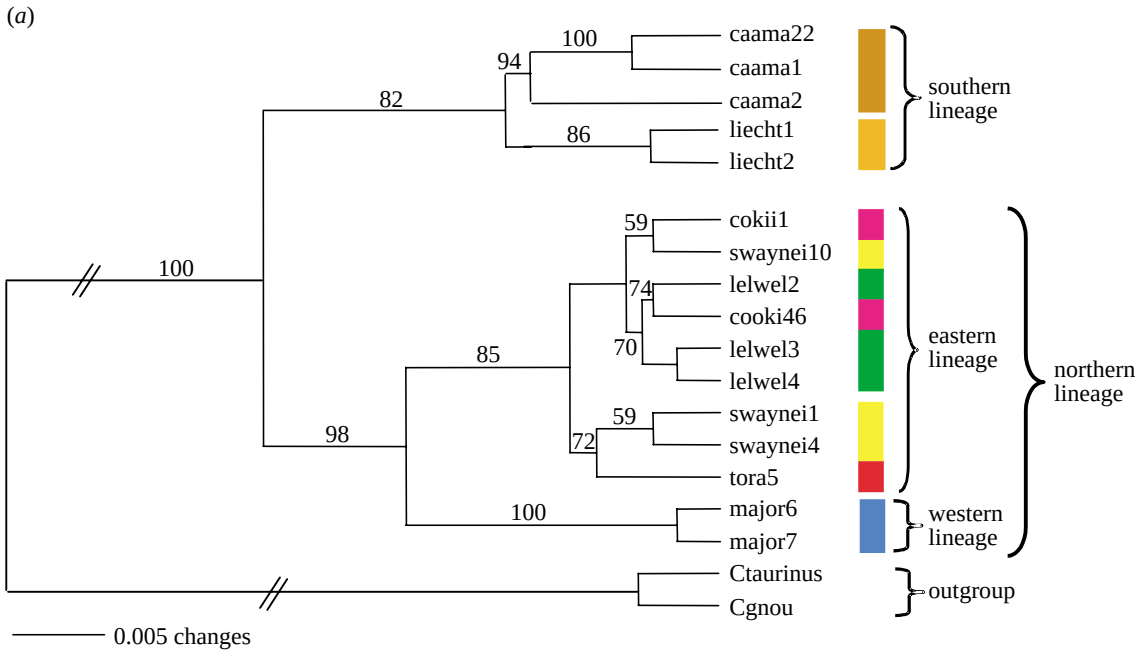
Figure 2. Total number of nucleotide substitutions against time for pairs of bovid taxa where the divergence times are assumed known from the fossil record. The visualized model was linearized by log transforming both axes (adjusted $R^2 = 0.94$ for the resulting model) and the deepest root in the hartebeest phylogeny was estimated by fitting the average number of nucleotide substitutions between the two main hartebeest lineages into the model. Data points indicate the different pairs of taxa: (1) *Bos taurus*/*Bos javanicus* (fossil evidence) (Janecek *et al.* 1996), (2) *Alcelaphus* sp.–*Damaliscus* sp. (fossil evidence) (Vrba 1979, 1984), (3) *Bubalis* sp.–*Syncerus* sp. (fossil evidence) (Janecek *et al.* 1996), (4) *Capra* sp.–*Ovis* sp. (fossil evidence) (Janecek *et al.* 1996), (5) and (6) tribes *Alcelaphini*–*Hippotragini* (molecular evidence) (Hassanin & Douzery 1999), (7) *Tragelaphus strepsiceros*–*Tragelaphus imberbis* (molecular evidence) (Matthee & Robinson 1999), (8) *Bos* sp.–*Boselaphus* sp. (fossil evidence) (Janecek *et al.* 1996), (9) *Bos* sp.–*Capra* sp. (fossil evidence) (Janecek *et al.* 1996), and (10) *Bos* sp.–*Ovis* sp. (fossil evidence) (Janecek *et al.* 1996).

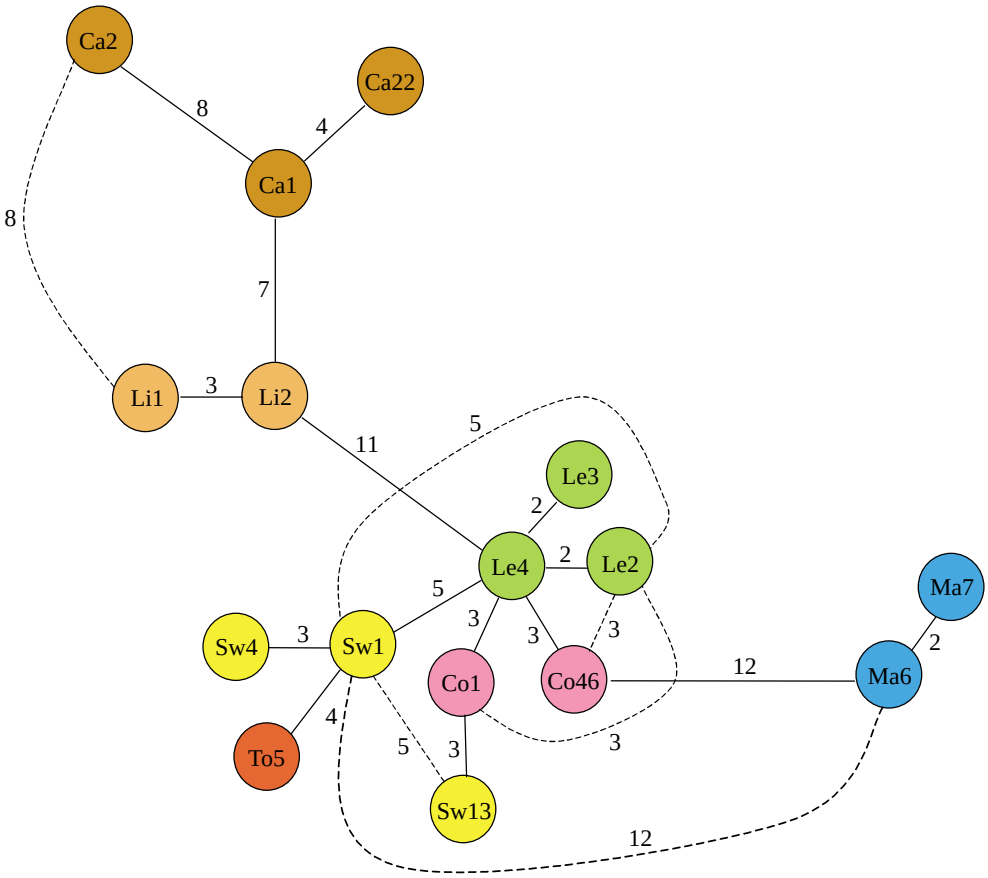
The D-loop showed extensive variability among the 170 individuals examined (see electronic Appendix A). A total of 205 (41.2%) variable sites were found, comprising 148 transitions and 29 transversions. Twenty-eight sites displayed both substitution categories. One hundred and forty-five different haplotypes were detected, out of which 117 were found only once. Sixteen insertions/deletions, ranging from 1 to 8 bp, were interspersed throughout the sequenced region.

(b) *Phylogenetic relationships*

Figure 3*a* depicts the phylogenetic relationships within the hartebeest complex as estimated from the cyt *b* data. There is strong evidence for two major clades, one southern and one northern, reflecting the two main distribution ranges of the hartebeest. The southern clade is composed of *caama* and *lichtensteinii*, while the northern one comprises the rest of the hartebeest complex (see also figure 1). This latter clade further divides into two subclades, one consisting of the western-distributed *major* (Blyth, 1869) and the other comprising the forms distributed throughout eastern Africa. The eastern subclade displays a complex pattern where most of the internal branches are only moderately supported.

The MSN supports the existence of two main hartebeest lineages. There are three well-defined haplotype clusters within these, representing easterly, westerly and





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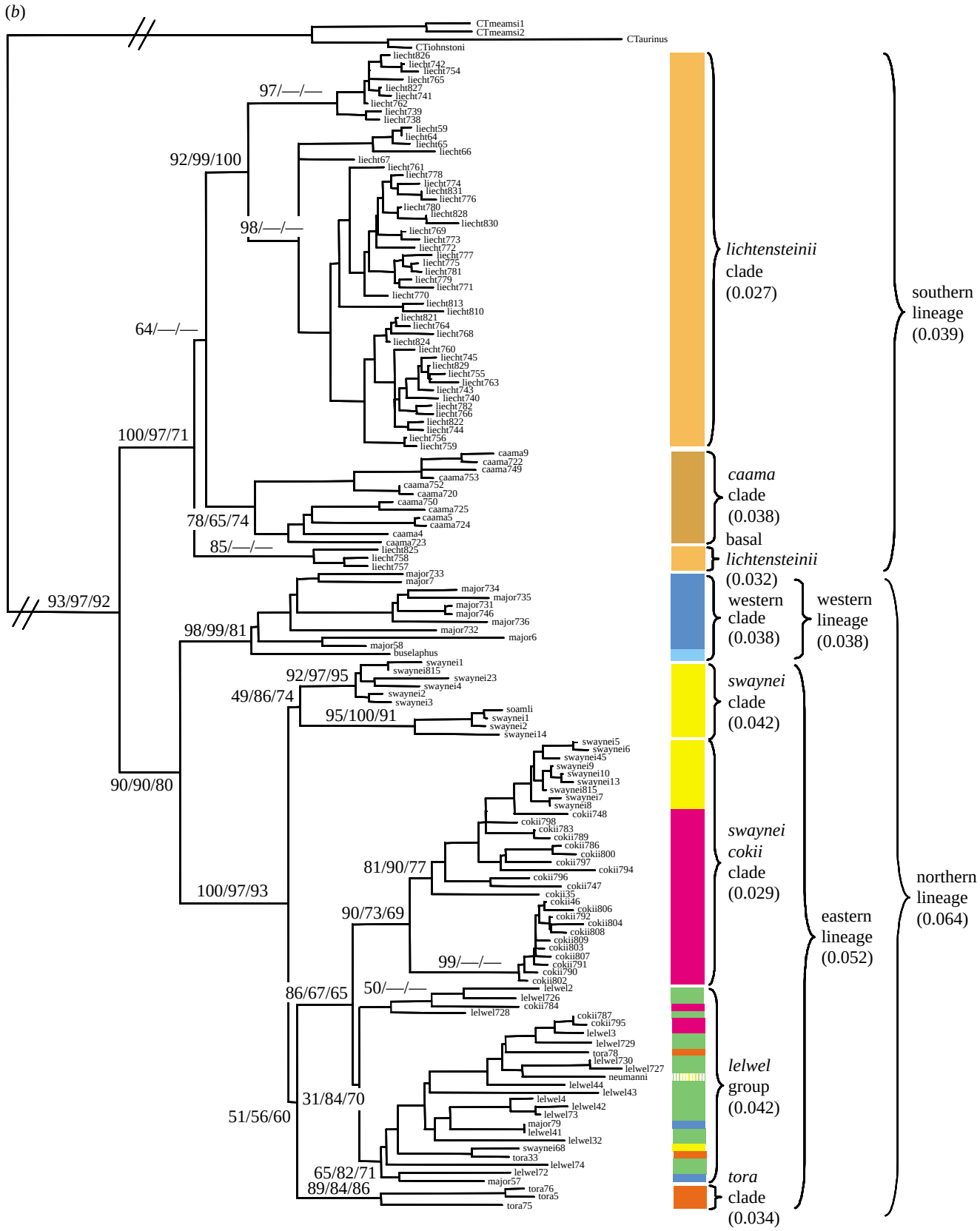


Table 1. *Dating of the major evolutionary events within the hartebeest complex*

(The standard deviations were estimated from the genetic distances between pairs of sequences from different clades. The estimated times since evolutionary events are approximated to the nearest 1000 years. The total number of nucleotide substitutions in the cyt *b* gene was used as the genetic distance measure for the first row of the table. The genetic distances for the bottom rows of the table are the average genetic distances measured within the clade.)

evolutionary event	taxa involved	genetic distance	s.d.	estimated time since evolutionary event (years)
diversification	southern versus northern hartebeest clade	18.650	3.209	495 000 ± 85 000
diversification	western versus eastern hartebeest subclade	0.145	0.015	389 000 ± 41 000
diversification	*A. b. caama* versus *A. b. lichtensteinii*	0.079	0.009	212 000 ± 24 000
diversification	*A. b. swaynei* versus *A. b. lelwel–A. b. cokii–A. b. swaynei*	0.075	0.014	201 000 ± 38 000
diversification	*A. b. tora* versus *A. b. lelwel–A. b. cokii–A. b. swaynei*	0.086	0.011	230 000 ± 30 000
diversification	*A. b. lelwel* versus *A. b. swaynei–A. b. cokii*	0.070	0.009	188 000 ± 25 000
diversification	within unique *A. b. swaynei* lineage	0.049	0.005	130 000 ± 14 000
diversification	*A. b. cokii* versus *A. b. swaynei–A. b. cokii*	0.048	0.009	128 000 ± 24 000
sudden population explosion	*A. b. lelwel*	0.051	0.015	137 000 ± 41 000
sudden population expansion	*A. b. major*	0.052	0.018	138 000 ± 50 000

southerly distributed hartebeest forms (see figure 1). The eastern haplotypes appear as a cluster of tightly linked haplotypes, bridging the western and southern haplotype clusters. Haplotype Le4 occupies the most evident node position, indicating an ancestral state in the hartebeest phylogeny. Considering southern and eastern groups separately, as was done based on D-loop data in Arctander *et al.* (1999), does not reveal any major differences in the topology of the networks from the two different markers.

The D-loop data (figure 3*b*) essentially reveal the same phylogeny as deduced from the cyt *b* analysis, although an even more complex pattern emerges within the northern clade. There is again strong evidence for two major clades, one southern and one northern. The western subclade within the northern clade comprises the form *major* as well as the extinct North African form *buselaphus*, while the eastern subclade divides into four main lineages. Two of the four eastern lineages are homogenous for the mainly Sudanese form *tora* Gray, 1873 and the Ethiopian *swaynei*, respectively. The latter has moderate support when all sequences are included in the analysis, but is strongly supported when excluding the GenBank sequences (Arctander *et al.* 1999). These are *ca.* 20% shorter than the full-length sequences, presumably leading to a poorer resolution of the major clades when included. The third lineage, which is highly supported, comprises all but three *cokii* Günther, 1884 haplotypes. In addition, approximately one-third of the *swaynei* haplotypes are found within this lineage. The last lineage within the eastern subclade is poorly supported when including the shorter Genbank sequences, but statistical support increases significantly when restricting the analysis to full-length sequences. This lineage comprises all haplotypes found in the geographically widely distributed *lelwel* Heuglin, 1877. Representatives of all the remaining eastern subspecies as well as the western form *major* are also contained within this lineage.

The low support at the deepest node within the eastern subclade (figure 3*b*) suggests that there is no clear-cut relationship between the four main groupings. This suggests that the eastern subclade comprises a star-like phylogeny and that all groups diverged more or less simultanously.

(c) *The evolutionary history of the hartebeest*

(i) *Diversification events*

The earliest known *Alcelaphus* in the fossil record is from *ca.* 740 000 years BP (Vrba 1995*a*). Our estimates suggest a deepest root within the hartebeest complex at 495 000 ± 85 000 years BP (table 1), reflecting an early diversification into southern and northern lineages. The latter appears to diverge into eastern and western lineages at 389 000 ± 41 000 years BP (table 1). Assuming a true star phylogeny within the eastern clade (figure 3*b*), all lineages contained therein seem to have diverged from a common ancestor at *ca.* 200 000 years BP, an estimate corresponding well with the diversification event within the southern clade (table 1).

(ii) *Population expansion*

Figure 4 shows lineages-through-time plots (Nee *et al.* 1995) for each of the six major groupings as recognized from the D-loop-based phylogenetic tree (figure 3*b*). Most

Figure 3. Phylogenetic relationships within the hartebeest complex as estimated from (*a*) cyt *b* and (*b*) D-loop sequence data. *Connochaetes* sp. was chosen as an outgroup in order to retain the main topology of the phylogenetic trees across the two markers. The relationships for the cyt *b* data are presented as both a phylogenetic tree (top) (statistical support > 50 indicated at the branches) and a minimum spanning network (bottom) (direct count distances at the branches). The support for the D-loop tree is based on three different analyses where (i) all sequences were included with no gamma correction, (ii) all GenBank sequences were excluded, thereby only retaining full-length sequences with no gamma correction, and (iii) only full-length sequences with gamma correction were included ($a = 0.376$). The colour codes are as follows: *caama*, brown; *lichtensteinii*, orange; *major*, blue; *buselaphus*, turquoise; *tora*, red; *swaynei*, yellow; *lelwel*, green; *cokii*, pink. Nucleotide diversity indices (in parentheses) are given for all subspecies as well as for the three main hartebeest lineages.

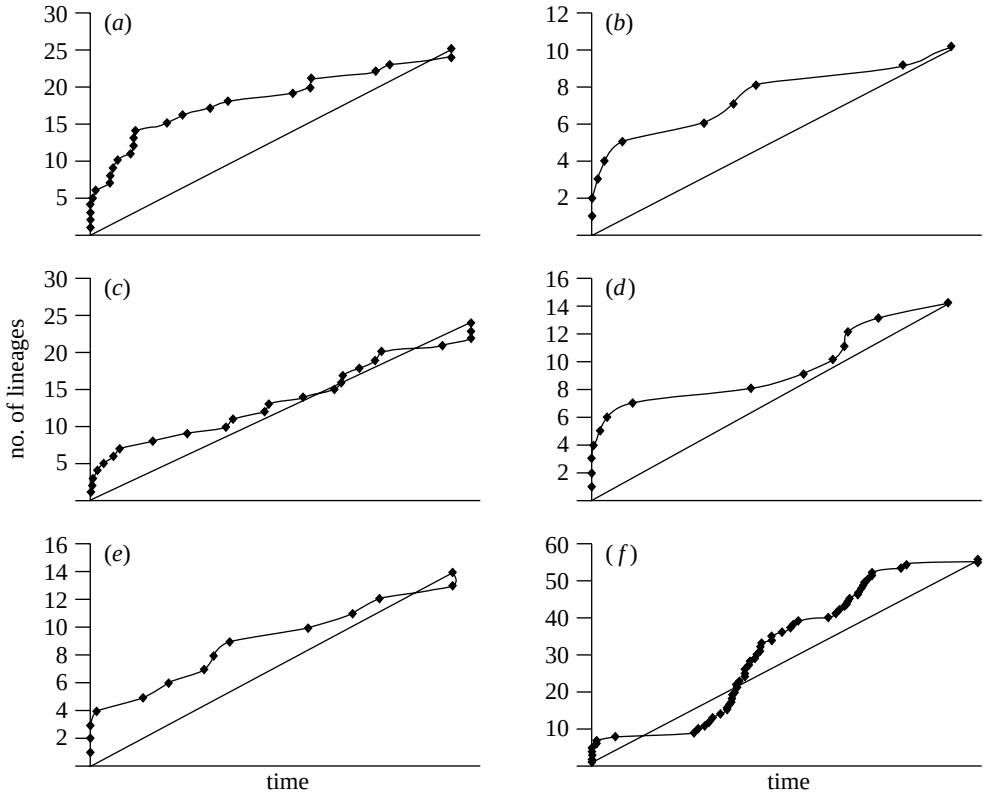


Figure 4. Number of D-loop lineages (*y*-axis) through time (*x*-axis) (endemic transformation) for the main clades in the phylogenetic tree (figure 2*b*). Numbers in parentheses provide results from a uniform conditional test (*U*-test) given that the null hypothesis equals linearity, as expected if population sizes have been near constant through time. (*a*) *lelwel* group ($U = -3.27$ and $p < 0.01$), (*b*) western clade ($U = -2.66$ and $p < 0.05$), (*c*) *swaynei–cooki* clade ($U = -1.06$ and $p > 0.05$), (*d*) *swaynei* clade ($U = -0.811$ and $p > 0.05$), (*e*) *caama* clade ($U = -0.981$ and $p > 0.05$) and (*f*) *lichtensteinii* clade ($U = -1.70$ and $p > 0.05$).

of the groups show a pattern that is not significantly different from linearity (figure 4), suggesting that most ancestral hartebeest populations had fairly stable sizes through time. However, the *lelwel* grouping exhibits a different pattern. A significant deviation from linearity ($p < 0.01$) is consistent with what would be expected with a population history characterized by sudden bursts of population increase. Such a scenario is also indicated by the many short internal branches as compared to the terminal ones in the phylogenetic tree (figure 3*b*). The putative population increase has apparently been accompanied by geographical range expansion, as indicated from the presence of all northerly distributed extant hartebeest subspecies in the *lelwel* clade. There is also some weak indication that an ancestral western hartebeest population has undergone a sudden population increase ($p < 0.05$ under the null hypothesis of stable population size through time). The expansion events were estimated to have occurred at *ca.* 140 000 years BP (table 1).

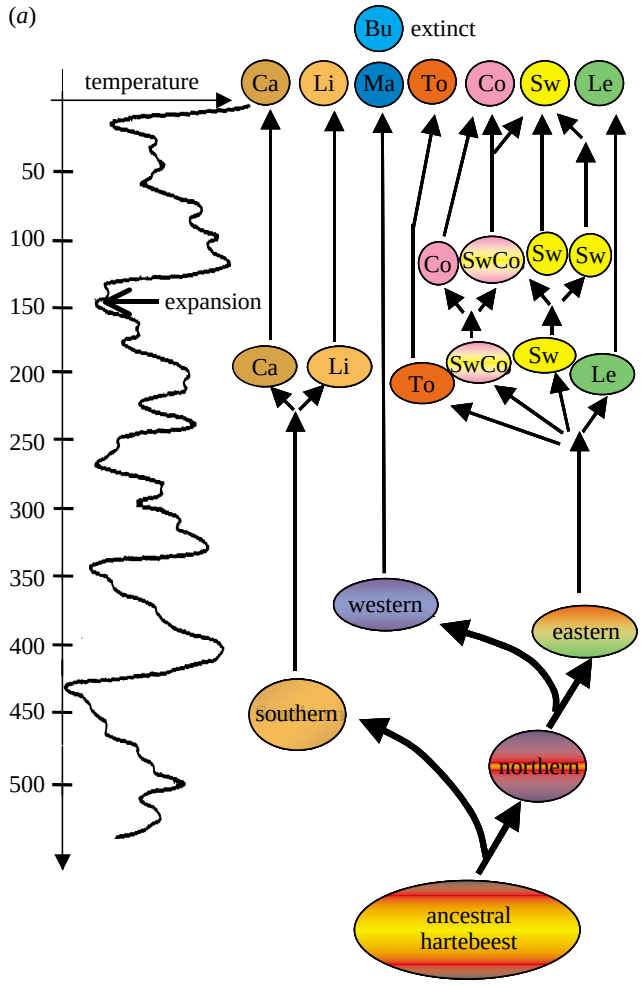
4. DISCUSSION

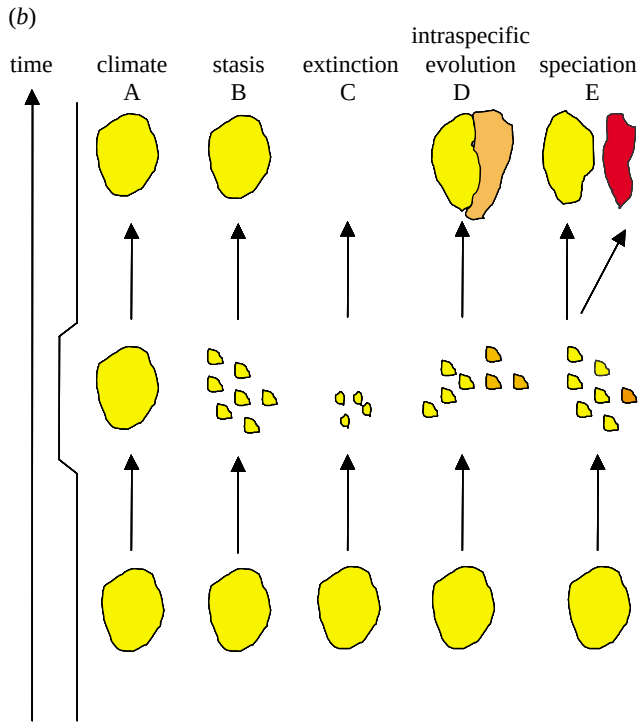
(a) *Hartebeest phylogeny: evidence for reproductive barriers*

Our phylogenetic analyses (figure 3*a*,*b*) show that the hartebeest complex comprises two main lineages, one southern and one northern. Furthermore, we see a subdivision into eastern and western lineages to the north. This is largely in agreement with Arctander *et al.* (1999). However, in contrast to Arctander *et al.* (1999), our data demonstrate paraphyly between the eastern and western lineages. On the other hand, the southern and northern lineages are reciprocally monophyletic even though the two forms *cokii* (northern lineage) and *lichtensteinii* (southern lineage) have partly overlapping distribution ranges. The lack of current physical barriers suggests that previously existing topographic barriers efficiently prevented gene flow over extensive periods of time, leading to reproductive isolation of the two lineages. This observation may suggest that the hartebeest complex today comprises two different species that are represented through the southern and northern lineages, respectively.

(b) *Geographical origin of the hartebeest*

The clustering of all eastern haplotypes in a tight network together with their central position in the MSN bridging the western and southern haplotype clusters (figure 3*a*) point towards an eastern origin of the species (cf. Crandall & Templeton 1993; Castelloe & Templeton 1994). An eastern origin is further supported by the high nucleotide diversity found within the eastern lineage, as well as within subspecies and even single populations (cf. Arctander *et al.* 1999; Flagstad *et al.* 2000). The star-like appearance of haplotypes branching off from the *lelwel* haplotype Le4 may suggest a centre of origin within the distribution range of this subspecies. In fact, current *lelwel* populations are distributed in a region where savannah habitat is particularly widespread. It may therefore seem





likely that this region has provided a more or less constant refugium for the species.

Thus, it seems likely that the first known hartebeest fossil, which was found in eastern Africa and dated to 740 000 years BP (Vrba 1995*a*), is representative of an ancestral hartebeest form. The monophyly of the southern lineage suggests that the south was successfully colonized only once. To the north, subspecies *major* appears paraphyletic, suggesting several bursts of gene flow directed from the east towards the west (figure 3*b*). All eastern subspecies except *lelwel* also appear as paraphyletic groups (figure 3*b*), indicating bursts of gene flow from a constant refugium within the distribution range of *lelwel*.

(c) *Biogeographical patterns and environmental variability*

Figure 5*a* summarizes our interpretation of the genetic data. We see that the last 500 000 years have been characterized by strong oscillations in global temperature with four recognizable periods of global warming. Similarly, the evolutionary history of the hartebeest has been characterized by periods of stasis followed by bursts of evolution. Although the exact dating of diversification events should be interpreted with caution (see Templeton (1993) and references therein), the apparent covariation between diversification intensity and climatic change strongly suggests that climatic changes have been important in shaping evolution within the hartebeest complex.

Figure 5*b* provides an outline of several hypothetical evolutionary scenarios under significant environmental change (Vrba 1983, 1993). Whether a change in environmental conditions will lead to extinction, intraspecific evolution or speciation depends on the intensity as well as the temporal extent of the new conditions. In the following, we interpret our data in light of these possible scenarios.

(i) *Topographic barriers*

The earliest diversification within the hartebeest complex is estimated to have occurred around 500 000 years BP (table 1 and figure 5*a*), reflecting an early colonization event and a subsequent split into southern and northern lineages (scenario D or, alternatively, E in figure 5*b*). This estimate corresponds well with the first appearance date for *lichtensteinii* in the fossil record (Vrba 1995*a*); hence, it is possible that the fossil actually reflects

Figure 5. (*a*) Major evolutionary events within the hartebeest complex as related to climatic variability during the upper Pleistocene period as adopted from Berger *et al.* (1996). The evolutionary events depicted are those being inferred from our genetic data. The colour codes for the different subspecies are as in figure 3. Abbrevations: Ca, *caama*; Li, *lichtensteinii*; Ma, *major*; Bu, *buselaphus*; Sw, *swaynei*; Co, *cokii*; To, *tora*; Le, *lelwel*. (*b*) Hypothetical scenarios (A–E) under evolutionary change as predicted from the turnover pulse hypothesis (Vrba 1983, 1993, 1995*b*). Scenarios A and B are typical for a generalist species such as the impala, while scenarios C–E are typical outcomes for a specialist species such as the hartebeest.

the diversification of the two main hartebeest lineages. Several earlier studies have invoked the Rift Valley lakes, which were formed less than 1 Myr BP (Cromie 1982), as a topographic barrier leading to distinct populations of several species in eastern and southern Africa (e.g. Ashley *et al.* 1990; Girman *et al.* 1993). Similarly, it seems that the earliest split within the hartebeest complex may be related to this particular topographic change. A raise in the water level, as expected during periods of global warming (cf. figure 5*a*), would constitute an efficient barrier between southern and eastern hartebeest populations and, together with the massive central African rainforest belt, delimit gene flow between the two main hartebeest lineages.

(ii) *Climatic barriers*

Almost all of the diversification events that have contributed to the current subspecies diversity within the hartebeest complex (scenario D in figure 5*b*) appear to have occurred during a relatively short time interval *ca.* 200 000 years BP (table 1 and figure 5*a*). At this time, all eastern subspecies seemed to form their own lineages. Simultaneously, *caama* and *lichtensteinii* diverged within the southern lineage. This congruence among different geographical regions strongly suggests that some external factor, such as climatic change, was responsible for diversification events within the hartebeest complex throughout Africa. Since there is strong evidence that a prolonged period of global warming occurred at 250 000–190 000 years BP (figure 5), we would expect a significant contraction of savannah habitat under such environmental conditions (e.g. Colinvaux 1997; Clark *et al.* 1999; Hewitt 2000).

Our results suggest a true star phylogeny with four main lineages within the eastern lineage (figure 3*b*), suggesting a comprehensive fragmentation of suitable habitat accompanied by the establishment of a few allopatric hartebeest populations within environmentally favourable refugia (see figure 5*a*,*b*). Four refugial populations are indicated and these might be seen as the ancestors of the current east African subspecies (scenario D in figure 5*b*). As discussed above, subspecies *lelwel* seems to have originated within the most important East African refugium from where the rest of the continent was colonized. Several subsequent bursts of gene flow have influenced all the other easterly as well as westerly distributed subspecies. Two smaller refugia are indicated by the monophyletic *tora* and *swaynei* clades (figure 3*b*). The last refugium seems to have been more extensive and indicates a common ancestry of subspecies *swaynei* and *cokii*. It appears that *cooki* has its origin within this refugium, but also that the present *swaynei* population is strongly influenced by genes having their origin here.

The woodland-ranging *lichtensteinii* within the southern lineage has probably had a history of origin rather different from that of the eastern subspecies. Facing profound environmental change, one or several populations may have been isolated under non-optimal habitat conditions (i.e. woodland instead of open savannah). Presumably, rapid adaptation to the surrounding habitat saved one or, alternatively, several ancestral populations from extinction (avoidance of scenario C in figure 5*b*) (see also Maynard Smith 1989) and *lichtensteinii* appears to be the only extant alcelaphine that currently is truly adapted to woodland conditions.

The diversification between the western and eastern hartebeest lineages at an estimated 389 000 years BP corresponds well with a global temperature peak at *ca.* 400 000 years BP (figure 5*a*). Under the assumptions of the relay model (Vrba 1995*b*), we would expect the unfavourable climatic conditions to have promoted diversification between the eastern and western lineages (scenario D in figure 5*b*). We suggest that the western lineage was isolated in refugia north and west of an expanding central African rainforest belt (cf. Clark *et al.* 1999) while the eastern lineage was displaced in refugia to the east.

The last array of recognizable diversification events seems to have occurred at *ca.* 130 000 years BP, an estimate that may correspond to the exceptionally rapid global warming occurring at 125 000–120 000 years BP (figure 5*a*). This dramatic environmental change led to rapid contraction of savannah habitat (cf. Clark *et al.* 1999), which in turn would have generated independent evolution among allopatric hartebeest populations. However, none of these population have given rise to new subspecies (scenario B in figure 5*b*), possibly reflecting the short time interval before the global temperature declined again (figure 5*a*).

(iii) *Sudden population expansion*

Following a period of global warming, one would expect ancestral hartebeest populations displaced in small, isolated refugia to have increased rapidly in size when environmental conditions again improved. Our results do indeed suggest sudden population expansion within the *lelwel* group (figure 4*a*). Moreover, although less evidently, there are indications that subspecies *major* underwent some expansion (figure 4*b*). The putative expansion events are estimated to have occurred at *ca.* 140 000 years BP, corresponding well with a prolonged period characterized by cool and arid environmental conditions at 175 000–125 000 years BP (figure 5). Under these conditions, we would expect an expansion of savannah habitat, accompanied by increasing hartebeest populations. The presence of all extant northern hartebeest subspecies within the *lelwel* clade (figure 3*b*) suggests that the demographic expansion was accompanied by geographical expansion in virtually all directions from the presumed centre of *lelwel* origin. To the west, it is conceivable that North Africa was colonized in connection with a similar geographical expansion and that the now extinct *buselaphus* was isolated from *major* in connection with an expansion of the Sahara desert. However, the available data do not allow us to draw any firm conclusions.

In contrast, no evidence was found for sudden expansion in any of the other subspecies. A combination of environmental and habitat-related factors may explain this incongruity for some of the subspecies. There is evidence that the climate in southern Africa was more stable as compared to the rest of the continent (Lancaster 1984), suggesting that *caama* populations may have been demographically stable through time (scenario A in figure 5*b*). On the other hand, the subspecies *tora* and *swaynei* inhabit a region where savannahs are interspersed with mountains and where savannah habitat has probably

never been similarly widespread. The habitat in these areas may never have reached the degree of continuity required to support large and rapidly growing antelope populations.

(iv) *Differences in subspecies diversity*

If large-scale environmental change has been the single factor determining the evolutionary history of the hartebeest complex, it follows that the amount of diversification should be similar for the northern versus southern lineages and the eastern versus western lineages. Clearly, this is not the case as diversification is much more evident to the north and, in particular, to the east. A difference in topography between the three parts of the African continent is one factor that could explain the deviation in subspecies diversity. The land in West Africa is relatively flat and uninterrupted, allowing similar habitat belts to be widely distributed east–west. On the other hand, East Africa is characterized by strong topographic variation with more pronounced habitat heterogeneity in space and time.

A similar argument could partly explain the low diversity in the south as compared to the north. Climatic evidence suggests that the South African climate may have oscillated between arid and semi-arid since the end of the Miocene period (Lancaster 1984). Savannah habitat has therefore possibly remained widespread through most periods of global warming (scenario A in figure 5*b*) and relatively high levels of gene flow among populations may have prohibited diversification.

5. CONCLUSION

Our results strongly suggest that changes in the physical environment act as a major determinant of evolutionary events. The hartebeest evolutionary scenario may well be applicable to a wide array of specialist mammalian species on the African savannah as these species' sensitivity to environmental change (and the unavoidable, associated habitat fragmentation) may typically lead to bursts of speciation and extinction in more or less environmentally favourable refugia.

On the basis of the relay model and the turnover pulse hypothesis (Vrba 1995*a*), we would predict that the hartebeest complex exhibits a typical punctuated-equilibrium mode of evolution (*sensu* Eldredge & Gould 1972) characterized by periods of stasis followed by bursts of evolution. Although the exact dating of diversification events must be interpreted with caution (e.g. Templeton 1993), our findings are in close agreement with such a view. An elevated global temperature leading to contraction of savannah habitat has presumably been accompanied by independent evolution among allopatric hartebeest populations. Although being partly parapatric today, our data suggest that northern and southern lineages have been isolated by physical barriers over extensive periods of time in the past and that this isolation has culminated in the establishment of reproductive barriers.

The extant hartebeest forms are all likely to have originated through successive dispersal pulses from a permanent refugium in East Africa. We maintain that the existence of such a refugium is crucial to our understanding of the evolution within the hartebeest complex, as well as to our understanding of the evolutionary history of other mammalian species on the African savannah.

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