Phylogeography of Three Closely Related African Bovids (Tribe Alcelaphini)

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The phylogeography of three species of African bovids, the hartebeest (*Alcelaphus buselaphus*), the topi (*Damaliscus lunatus*), and the wildebeest (*Connochaetes taurinus*), is inferred from sequence variation of 345 sequences at the control region (d-loop) of the mtDNA. The three species are closely related (tribe Alcelaphini) and share similar habitat requirements. Moreover, their former distribution extended over Africa, as a probable result of the expansion of open grassland on the continent during the last 2.5 Myr. A combination of population genetics (diversity and structure) and intraspecific phylogeny (tree topology and relative branch length) methods is used to substantiate scenarios of the species history. Population dynamics are inferred from the distribution of sequence pairwise differences within populations. In the three species, there is a significant structuring of the populations, as shown by analysis of molecular variance (AMOVA) pairwise and hierarchical differentiation estimations. In the wildebeest, a pattern of colonization from southern Africa toward east Africa is consistent with the asymmetric topology of the gene tree, showing a paraphyletic position of southern lineages, as well as their relatively longer branch lengths, and is supported by a progressive decline in population nucleotide diversity toward east Africa. The phylogenetic pattern found in the topi and the hartebeest differs from that of the wildebeest: lineages split into monophyletic clades, and no geographical trend is detected in population diversity. We suggest a scenario where these antelopes, previously with wide pan-African distributions, became extinct except in a few refugia. The hartebeest, and probably also the topi, survived in refugia north of the equator, in the east and the west, respectively, as well as one in the south. The southern refugium furthermore seems to have been the only place where the wildebeest has survived.

Introduction

Phylogenetic studies performed at the population level (intraspecific phylogeography; Avise et al. 1987) can be relevant for the understanding of evolutionary processes in many respects (see Crandall and Templeton 1996). The distribution of genetic variability within and among populations is affected both by recurrent factors and by historical events. Using a fast-evolving marker such as the control region (d-loop) in animal mtDNA, population genetics and phylogenetic methods can be combined to infer species recent history and evolution despite some inherent drawbacks. For instance, historical fragmentation causes departures from some assumptions of population genetics theories and leads to overestimated levels of population differentiation. Conversely, true ancestry may be obscured by current gene flow. One way to assess the occurrence and magnitude of a historical event is to compare the patterns observed in several codistributed species and determine the extent of their biogeographic congruence (Zink 1996; Bernatchez and Wilson 1998). We undertook a comparative study of three closely related and codistributed species of African bovids (tribe Alcelaphini), and we present here work pertaining to sequence variation at the mitochondrial d-loop.

Alcelaphus buselaphus (hartebeest), *Damaliscus lunatus* (topi), and *Connochaetes taurinus* (wildebeest) are all gregarious species that are confined to habitats related to savannah. There are slight differences among and within species, with the hartebeest, for example, being more closely associated with the forest ecotone, while the topi prefers short grass and flood plains. The wildebeest in southern Africa can be found from open savannah woodland (Okavango, Botswana) to semidesert (Kalahari, Botswana), whereas the east Africa populations mainly prefer open savannah (Kingdon 1984, 1997; Haltenorth and Diller 1988; Skinner and Smithers 1990). Habitat requirements of these species overlap so extensively that large-scale environmental changes can be expected to have similar influences on each of them. One major reason for the recent and rapid decline in numbers and range fragmentation is resource competition with domestic cattle. In spite of this, a few very large populations do persist. In the recent past, both larger population size and more continuous distribution have been described (Kingdon 1984), asserting that alcelaphines were a very successful group.

The earliest recorded alcelaphine fossil, with an estimated age of 5 Myr, was found in South Africa (Vrba 1995). Paleontological records provide evidence for a distribution of the tribe across the entire African continent. First-appearance data (FAD) of the studied species are (according to Vrba 1995, appendix 27.2) 0.74 Myr (Ethiopia) for *Alcelaphus buselaphus buselaphus*, 0.5 Myr (Zaire) for *Alcelaphus buselaphus lichtensteini*, 1.5 Myr (Tanzania) for *Connochaetes taurinus*, and 0.7 Myr (South Africa) for *Damaliscus lunatus*, respectively. *Connochaetes taurinus*, which in recent history has not occurred north of equator, is reported in North Africa until the late Pleistocene (Gentry 1978). In spite of possible taphonomic bias, these data suggest recent origins of the studied taxa: their appearance, geographical expansion, and subsequent evolution have thus been under the influence of Pleistocene conditions. In southern and

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east Africa, climatic changes during this period are associated with higher-latitude cyclic ice sheet variation superimposed on the direct monsoonal climate (deMenocal 1995). Also, regional tectonic activity affected east and southern African climate and environment (Partridge, Wood, and deMenocal 1995). The vegetation change related to cyclic cooling and drying is likely to have promoted colonization of new areas, while habitat fragmentation would have resulted from alternate wetter phases combined with the topographical relief.

In an attempt to trace back the species histories, we explored the abilities of the three molecular data sets to reflect geographical isolation versus dispersion events. Although vicariance is difficult to assess, we would expect congruence (1) with geographical taxonomic subdivisions of the species, at least for allopatric subspecies, among which similar levels of genetic diversity should moreover be observed, and (2) among species across their common range. However, the patterns may be obscured if fragmentation was only transient and was followed by spatial expansion allowing secondary contacts. Such processes may have occurred several times during the Pleistocene. Moreover, African savanna is an unstable habitat in which drastic changes may be frequent. Populations may then evolve under a metapopulation model, with transiently occupied areas rather than true in situ extinction. This would also have the effect of erasing traces of ancient significant events.

A colonization process can be emphasized by the observation of various consequences of the associated founder effect. Compared with a vicariance event, we expect (1) higher divergence between contemporaneous populations as a consequence of the theoretically increased divergence between derived and original populations (see Thorpe et al. 1994); (2) larger differences in population genetic diversity (lower levels in newly established populations), depending, however, on the time elapsed since foundation, the subsequent population growth rate (Nei, Maruyama, and Chakraborty 1975), and the degree of relatedness among founders (Wade and McCauley 1988); (3) larger differences in the apparent ages of lineages, for bottlenecks reduce the time to expected monophyly (lineages from recently colonized areas are likely to be monophyletic, while ancestral ones should be paraphyletic; Marko 1998); and (4) as the number of individuals involved in dispersion is usually small, newly established populations should show indications of subsequent demographic expansion (see Slatkin and Hudson 1991). Comparatively, the historical demography of vicariated populations would be steadier in the absence of other factors.

Materials and Methods

Species and Populations Studied

Alcelaphus buselaphus, D. lunatus, and *C. taurinus* were sampled from the main part of their area of distribution (fig. 1). Antelopes are generally very difficult to sample. As a consequence, sample size varies extensively among localities, and analyses performed at the population level are restricted to a subset of data for each species (table 1). Intraspecific taxonomy, although subject to disagreements, describes different subspecies on the basis of geographical range and morphology. In the hartebeest, seven living subspecies are generally admitted (from west to east and south): *Alcelaphus buselaphus major, Alcelaphus buselaphus lelwel, Alcelaphus buselaphus swaynei, Alcelaphus buselaphus tora* (not included in the present study), *Alcelaphus buselaphus cokei, A. b. lichtensteini,* and *Alcelaphus buselaphus caama.* However, the Lichtenstein's hartebeest is either given its own genus, *Sigmoceros lichtensteinii* Peters 1849, placed within the *Alcelaphus* group as a distinct species, or considered a subspecies of *A. buselaphus* (Haltenorth 1963). Five subspecies of topi, of which only two are included here (the southern *Damaliscus lunatus lunatus* and the eastern *Damaliscus lunatus jimela*), and five subspecies of wildebeest, all included in the present study (from south to east Africa: *Connochaetes taurinus taurinus, Connochaetes taurinus cooksoni, Connochaetes taurinus johnstoni, Connochaetes taurinus mearnsi,* and *Connochaetes taurinus albojubatus*), are generally recognized. Numbers of individuals, sampling localities, and subspecies are given in table 1 and on figure 1. Samples were obtained either as small skin biopsies from free-ranging individuals or from dried skins and skulls (approximately half of the samples were obtained in each way).

DNA Extraction, Amplification, and Sequencing

Total DNA was phenol-extracted following standard procedures (e.g., Sambrook, Fritsch, and Maniatis 1989) or CTAB-extracted (modified from Doyle and Doyle 1987) using the QuiaAmp tissue kit. An ~400-bp-long fragment of the variable 5′ part of the mtDNA control region (d-loop) was PCR-amplified using the primers MT4 (Arnasson, Gullberg, and Widergren 1993) and BT16168H (Simonsen, Siegismund, and Arctander 1998). Symmetrical PCR amplifications were carried out in 50-μl reaction volumes containing 5 μl (10 μM) of each primer, 200 μmol dNTPs, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl (pH 8.3), and 1 U *Taq* polymerase. We used 1 cycle of denaturation at 94°C for 2 min followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 40 s. Double-stranded products were cleaned through QuiaQuick PCR purification kit columns, 3 μl of purified PCR product was used as template for dRhodamine terminator cycle sequencing (ABI kit) following the manufacturer's instructions. Both strands were run on an ABI377 DNA sequencer. For the *Connochaetes* sequences from Amboseli and Masai Mara and half the sequences from Nairobi, see Arctander et al. (1996). Sequences were deposited in the GenBank database (accession numbers AF167720–AF167978 and AF176682–AF176685).

Sequence Analysis

Phylogenetic Relationships

Sequences were aligned both by eye and using the software SEQUENCHER. Maximum-likelihood distances were calculated between haplotypes using a transi-

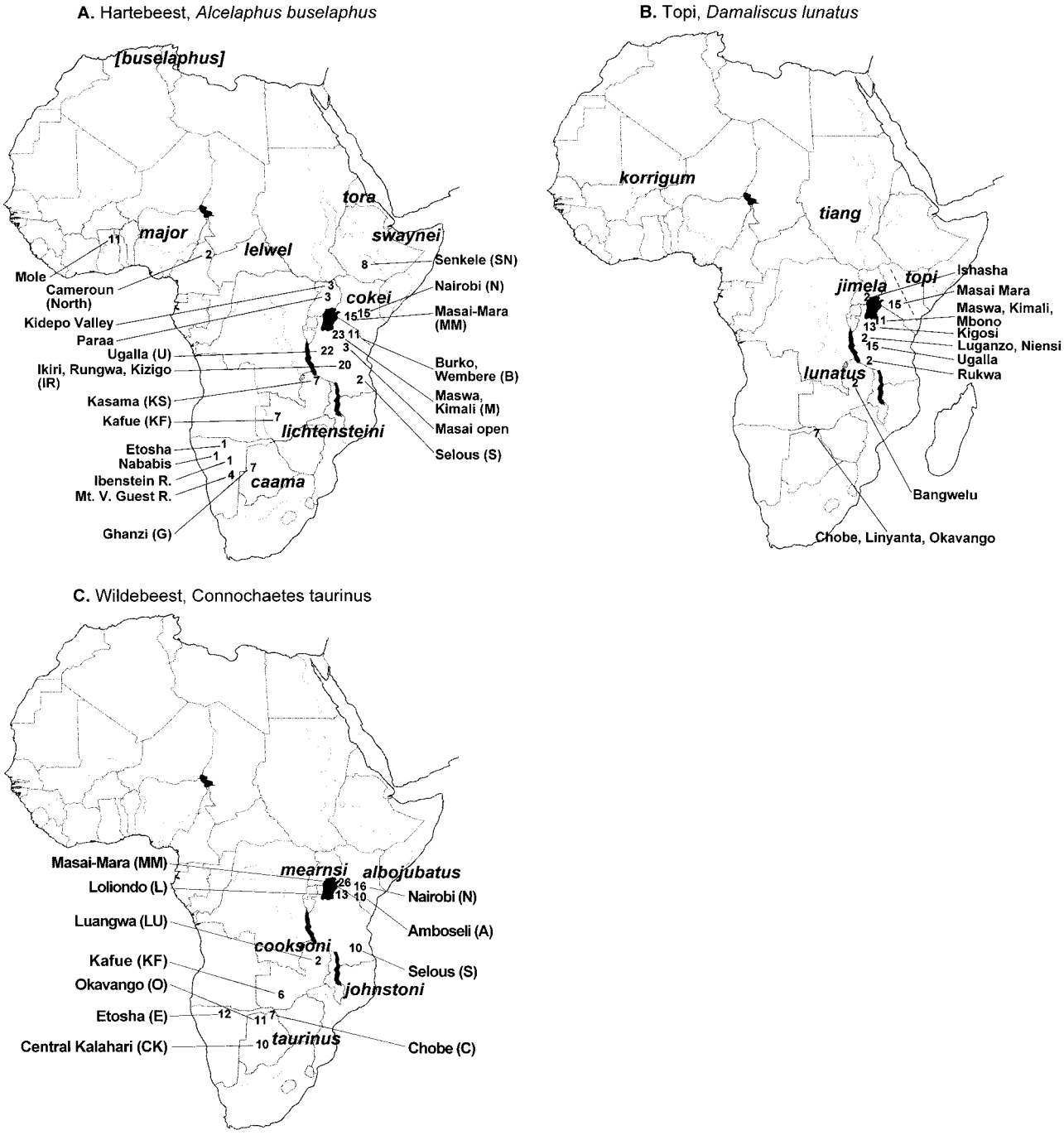

FIG. 1.—Maps of the species distribution and sampling localities. The range of subspecies is also shown. *A, Alcelaphus buselaphus. B, Damaliscus lunatus. C, Connochaetes taurinus.*

tion/transversion ratio estimated from the data, and among-site heterogeneity for the mutation rate that we assumed to be Γ distributed, with four discrete classes (Tamura and Nei 1993). The estimated value of α, the shape parameter of the Γ distribution, was always slightly less than 0.5, indicating that the rate of mutation varied extensively among sites. Distances were used to draw neighbor-joining (NJ) trees (Saitou and Nei 1987) and minimum spanning networks (MSNs). The latter are preferable for intraspecific studies (Crandall and Templeton 1996) because operational taxonomic units (OTUs) are not forced to occupy tip positions (ancestral lineages are not assumed to be extinct) and multifurcations are allowed. However, in polytypic species, we may expect heterogeneous relationships among lineages, so both methods were used. Distance matrices and NJ trees were calculated using PAUP*, Version 4.0b1 (Swofford 1998). Trees were rooted with different representatives of the tribe. Branch support was obtained using 1,000 bootstraps. Networks were performed with the program MINSPNET, provided by Laurent Excoffier: a minimum spanning tree (MST) with $n - 1$ con-

Table 1
Geographic Origins, Subspecific Taxonomies, and Numbers of Sequences Studied in *Alcelaphus buselaphus* (*A. b.*), *Damaliscus lunatus* (*D. l.*), and *Connochaetes taurinus* (*C. t.*) (see also fig. 1)

Species	Subspecies	Country	Locality	Locality Code	Sample Size
A. b.	*swaynei*	Ethiopia	Senkele	SN	8
	lelwel	Uganda	Paara	—	3
			KVNP	—	3
	major	Ghana	Mole	—	6
		Cameroun	Unknown	—	2
	cokei	Kenya	Nairobi	N	15
			Masai-Mara	MM	15
		Tanzania	Maswa-Kimali	M	18
			Other localities		3
	lichtensteini	Tanzania	Burko-Wembere	B	9
			Ikiri-Rungwa	IR	19
			Ugalla	U	22
			Selous	S	5
			Other	—	4
		Zambia	Kafue	KF	6
			Kasama	KS	7
	caama	Botswana	Ghanzi	G	6
		Namibia	Different localities	—	7
D. l.	*jimela*	Kenya	Masai-Mara	MM	15
		Tanzania	Maswa-Kimali	M	11
			Kigosi	KI	13
			Ugalla	U	13
			Other	—	2
		Uganda	Ishasha	—	1
	lunatus	Botswana	Okavango	0	7
		Zambia	Bangwelu	—	2
C. t.	*albojubatus*	Kenya	Amboseli	A	10
			Nairobi	N	16
	mearnsi	Kenya	Masai-Mara	MM	26
		Tanzania	Loliondo	L	13
	johnstoni	Tanzania	Selous	S	10
	cooksoni	Zambia	Luangwa	—	2
	taurinus	Zambia	Kafue	KF	6
		Botswana	Okavango	O	11
			Chobe	C	7
			Centre Kalahari	CK	10
		Namibia	Etosha	E	12

nections for *n* haplotypes, to which alternative links (ambiguities) were added when they occurred. In this case, several equivalent MSTs result in a construction that we call an MSN. When different clades were identified from the NJ tree, we did not include them in the same MSN, for their common ancestor is likely to be extinct. Some ambiguities can be resolved by applying a frequency criterion proposed by Crandall and Templeton (1993): from their study of 29 data sets, frequent haplotypes occur preferentially at node positions while rare haplotypes have a higher probability of occupying tips. To see whether this criterion fits our data, we compared the mean haplotypic frequency between nonambiguous tips and nodes on a given MSN with a nonparametric Kruskal-Wallis test. Although the frequency was always higher at node positions, the difference was significant only for *C. taurinus* (table 2). Therefore, ambiguities were kept, but we represent as dashed curves the links that were less or equally probable (see fig. 3).

Population Genetic Variability and Structure

Population nucleotide diversity and standard deviation (Nei 1987) were estimated. Population subdivision was analyzed using the parameter K_{ST} (Hudson, Boos, and Kaplan 1992), as estimated from sequence data. Significance of K_{ST} values was tested with a permutation procedure, using a program written by Hans Siegismund (personal communication). We also analyzed population structure with analysis of molecular variance (AMOVA; Excoffier, Smouse, and Quattro 1992), which divides the total variance into additive components. With sequence data, the number of mutations between haplotypes is taken into account. Populations were grouped according to the clades identified or according to geography. The hierarchy yielded three sources of variation: within populations, among populations within groups, and among groups. Population pairwise Φ_{ST} values were also calculated. Significance of all Φ values was assessed using permutations. AMOVAs were performed using the program ARLEQUIN (Schneider et al. 1997).

Population Demography

Population historical dynamics was inferred from the distribution of the number of differences between pairs of sequences (mismatch distribution; Rogers and Harpending 1992). Multimodal distributions are consistent with demographic stability, while sudden expansion would generate unimodal patterns (Slatkin and Hudson

Table 2
Comparison of Mean Haplotype Frequencies Between Nonambiguous Tip and Node Positions on the Minimum Spanning Networks Drawn *Alcelaphus buselaphus* (*A. b.*), *Damaliscus lunatus* (*D. l. jimela*), and *Connochaetes taurinus* (*C. t.*) (see fig. 3)

	Mean Frequency (SD)		Test			
	Tip	Node	*H*	*n*	df	*P*
A. b. clade II	1.33 (0.88)	1.65 (1.31)	0.67	32	1	0.412
A. b. clade III	1.08 (0.27)	1.42 (0.94)	2.58	62	1	0.109
D. l. jimela	1.21 (0.42)	1.61 (0.98)	2.85	42	1	0.091
C. t.	1.04 (0.20)	1.55 (1.59)	4.89	87	1	0.027

Note.—Clade II = *cokei-swaynei-lelwel* and clade III = *lichtensteini-caama,* as inferred from the neighbor-joining trees. *n* = number of used haplotypes; *P* = type 1 error probability associated with *H* values (Kruskal-Wallis test). *H* calculated with adjustment for ties.

1991). Departures of the observed distributions from that expected under the expansion hypothesis were tested with a χ^2 test of goodness of fit using ARLEQUIN (Schneider et al. 1997).

Results

Detecting Clades Within Species—Subspecies Relationships

Alcelaphus buselaphus

One hundred fifty-eight studied sequences revealed 112 different haplotypes. Sequence length was 386 bp, including indels. No haplotype was shared among subspecies, while some haplotypes were found in different populations of the same subspecies (e.g., *lichtensteini, cokei*). Three major features emerge from the NJ tree (fig. 2*a*). First, the group is monophyletic. Second, we observe three clades, although they are unevenly supported by the bootstrap test: clade I (all *major* haplotypes but one), clade II (*cokei, swaynei,* and *lewel* subspecies), and clade III (*lichtensteini* and *caama*). Third, within-clade relationships are contrasted. Within clade II, *lewel* and *swaynei* haplotypes are either isolated or well integrated to *cokei* clusters. Within clade III, *lichtensteini* and *caama* subspecies are almost reciprocally monophyletic, except for three basal *lichtensteini* haplotypes (IR06, IR07, and U10 from Tanzania). A bootstrap value of 89% supports the monophyly of *caama* lineages.

Separate MSNs are presented for clades II and III (fig. 3*a* and *b*). Within clade II, *swaynei* haplotypes are found either as tips connected to *lewel* nodes or as nodes connecting *cokei* tips (fig. 3*a*). Most of the *cokei* haplotypes are found to share recent ancestry (short paths), and some of them have high frequencies. The MSN of clade III reveals a homogeneous group composed mainly of *lichtensteini* samples from Tanzania (fig. 3*b*). *Lichtensteini* haplotypes from Zambia (KS, KF) are closer related to their Tanzanian counterparts than to southern *caama* lineages. *Caama* and *lichtensteini* subclades are connected via three Tanzanian haplotypes, found basal on the NJ tree.

Damaliscus lunatus

Fifty-one haplotypes were found among the 64 sequences analyzed. Aligned sequences were 370 bp long including indels. They split up into two well-supported clades which perfectly match subspecific taxonomy (NJ tree, fig. 2*b*). Branches are slightly longer in *D. l. lunatus* than in *D. l. jimela,* within which no phylogenetic signal is detected. Weak divergence within *jimela* subspecies is further illustrated by the MSN (fig. 3*c*): most connections are short and many ambiguities are found, regardless of geographical origin.

Connochaetes taurinus

Ninety-six haplotypes were observed among the 123 sequences studied, with a total length of 388 sites. The haplotype NJ tree is asymmetric, and branches become more shallow from southern Africa towards northeastern regions (fig. 2*c*). No clear-cut pattern between subspecies is observed, except for most of the *johnstoni* haplotypes (S, Tanzania). Rather, we identify a geographical subdivision composed mainly of eastern populations (bootstrap value = 94) and including *C. t. mearnsi, albojubatus,* and *johnstoni* plus three haplotypes from Zambia (KF [*C. t. taurinus*] and LU [*C. t. cooksoni*]). Due to this complexity, a single MSN was built on the whole data set (fig. 3*d*). In east Africa (Tanzania, Kenya), most of the connections are short. *Johnstoni* samples (S) link east and southern Africa via a haplotype from Nairobi (N13, *albojubatus*) on one side and a haplotype from Chobe (C, *taurinus*) on the other side. A single *taurinus* haplotype (KF1, Zambia) is found within the ''northeastern clade.'' *Cooksoni* samples (LU, Zambia) exhibit close affinities with the Kafue atypic haplotype (KF1) and with *johnstoni* samples.

Population Analysis

Populations from some localities were discarded due to sample size (see table 1). The numbers of populations studied for *A. buselaphus, D. lunatus,* and *C. taurinus* were 10, 5, and 10, respectively. Summary statistics are presented per population or per subspecies in table 3. Nucleotide diversity is generally higher in *A. buselaphus* (0.015–0.054) than in *C. taurinus* (0.005–0.039) or *D. lunatus* (0.013– 0.025).

Population Differentiation

For *D. lunatus* and *A. buselaphus,* the hierarchy reflected the clades, as identified from the NJ tree (excluding *A. b. major* and *A. b. lelwel* due to small sample size). In *C. taurinus,* we used a geographical hierarchy that grouped southern (KF, O, C, E, CK) versus eastern (A, N, M, L, S) populations. Φ_{ST} values are very high in the three species (table 4). All levels of differentiation

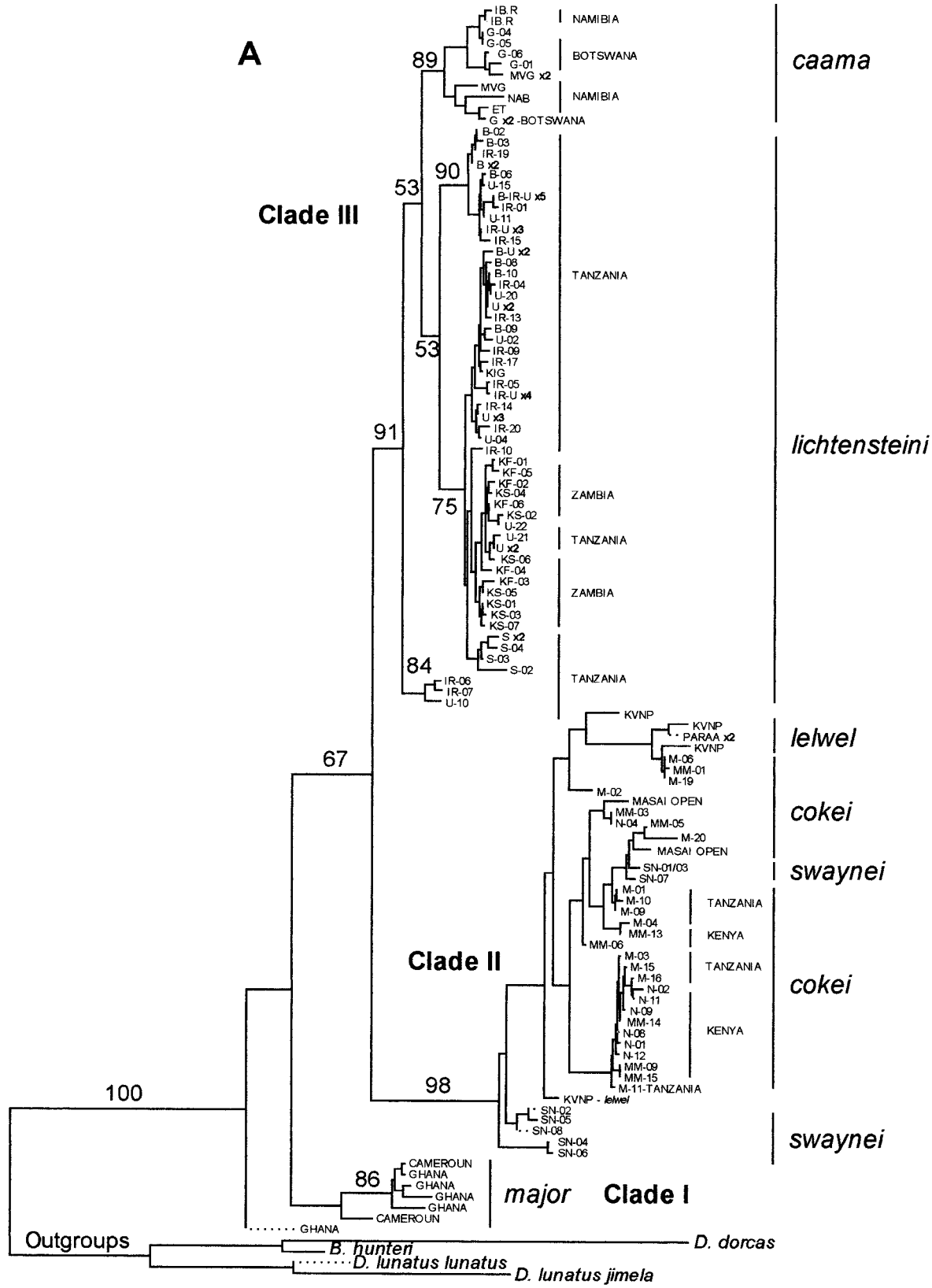

FIG. 2.—Neighbor-joining trees of the maximum-likelihood distances calculated among conspecific haplotypes using a transition/transversion ratio estimated from the data and allowing for among-site heterogeneity (Γ distribution). Branch support was obtained from the bootstrap procedure with 1,000 replicates. Haplotype codes indicate either the population used for the population genetics study or the serial number of the database and the country of origin (see table 1), the number of haplotypes is indicated by **x**n when there is more than one. *A, Alcelaphus buselaphus* (outgroup: *Beatragus hunteri, Damaliscus lunatus,* and *Damaliscus dorcas*). *B, Damaliscus lunatus* (outgroup: *Beatragus hunteri*). *C, Connochaetes taurinus* (outgroup: *Connochaetes gnu*).

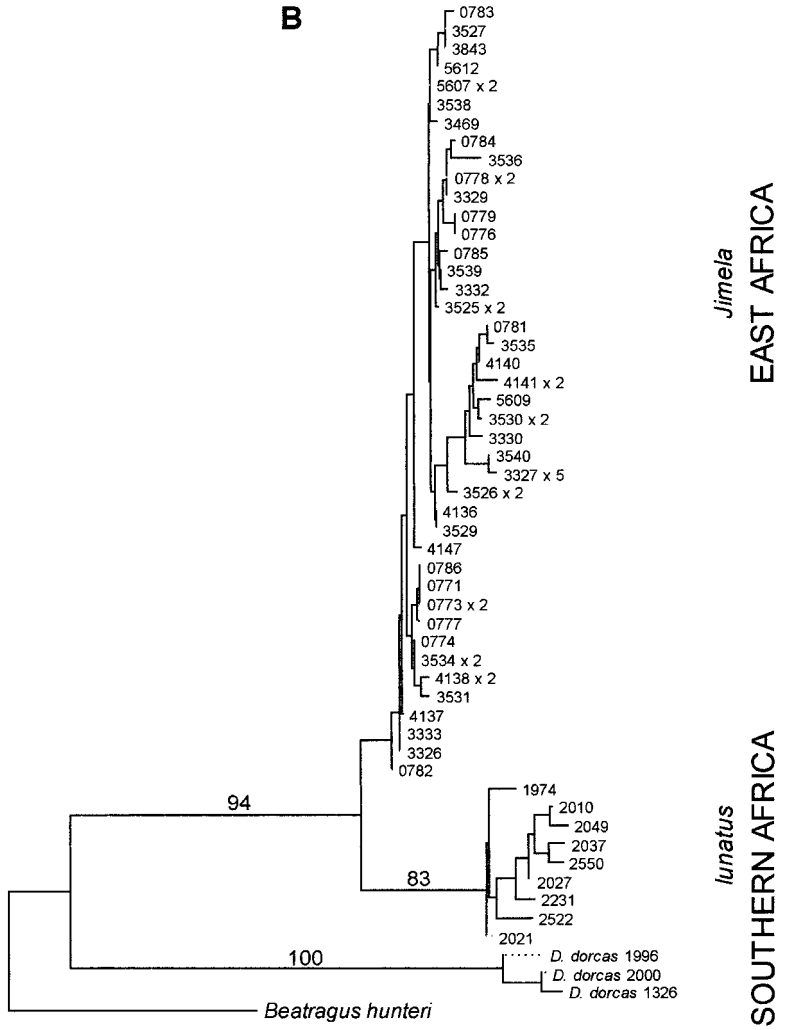

FIG. 2 *(Continued)*

are significant, except among groups in *D. lunatus*. The percentage of variation found within groups (Φ_{SC}) is higher in *C. taurinus* (15.05 %) than in *A. buselaphus* (6.68%) or *D. lunatus* (4.51%). Moreover, in all cases, Φ_{SC} values are lower than Φ_{CT} and Φ_{ST} values, reflecting more homogeneity within the defined groups than among them or than among populations as a whole.

As significance tests performed on pairwise K_{ST} and Φ_{ST} values gave the same qualitative results, we present Φ_{ST} estimates only (tables 5–7). For *A. buselaphus*, all comparisons of populations from two different clades or two subspecies (including *swaynei–coeki* comparisons) are significant. Among *lichtensteini* populations, two homogeneous groups are significantly differentiated, i.e., southern (KF and KS) and eastern (U, B, IR) populations, respectively. Among *cokei* populations, only one comparison is significant (N/M). For *D. lunatus*, the *D. l. lunatus* population (Okavango, Botswana) is significantly differentiated from all the others. No clear geographical pattern is observed within the *jimela* group. For *C. taurinus*, most pairwise Φ_{ST} values are significant: only 4 of 45 comparisons are nonsignificant, and in all cases, they involve populations from the same region (A/N, M/L, O/C, and C/CK).

Population Demography

For *A. buselaphus* and *C. taurinus* populations, no evidence for sudden expansion is given by the mismatch distribution (χ^2 test; fig. 4). Unfortunately, the test was impossible to perform in three cases, due either to many low values of the expected frequencies (*C. taurinus*, [Kafue] and *A. buselaphus*, [Ghanzi]) or to a variance higher than the mean (*C. taurinus*, population of Nairobi). In the latter case, however, the mismatch distribution is unimodal. The Masai Mara population shows a similar trend despite significant departure from unimodality. For *D. lunatus*, nonsignificant χ^2 values suggest sudden expansion in two out of five populations (Maswa-Kimali and Okavango-Chobe; fig. 4*c*).

Discussion

Our geographical survey of mtDNA variation showed a significant effect of both recurrent and historical factors on the evolution of the three species line-

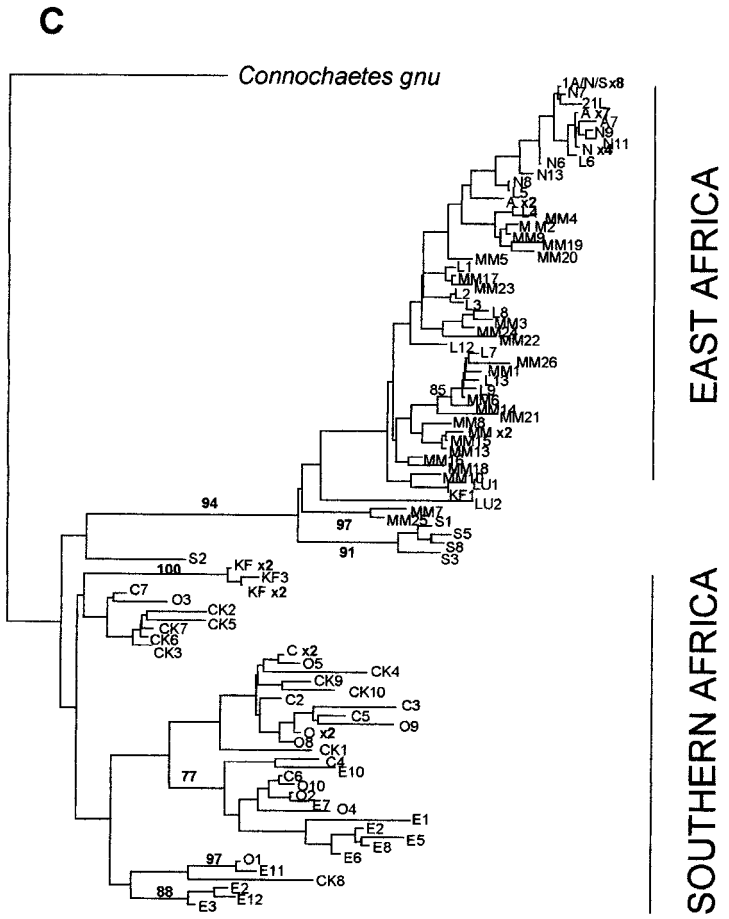

FIG. 2 *(Continued)*

ages. With regard to population history, we tried to explore a set of expectations relative to allopatry, vicariance, and dispersion in order to substantiate our proposed hypotheses.

Population Genetic Structure

The variation of mtDNA sequence reflected significant population structure in the three species. Moreover, nested analyses showed significant differentiation at all levels, except for the among group variation in *D. lunatus.* This result may be due to the permutation scheme, whereby group size varies at each run (Excoffier, Smouse, and Quattro 1992), combined with the disequilibrated grouping of *D. lunatus* populations (4/1). In the hierarchical analysis of *C. taurinus* populations, the greatest part of variation was found among regions, i.e., southern and east Africa. Meanwhile, a marked structure was also detected within regions, and most of the pairwise comparisons were significant. On population genetics grounds, these results suggest that genetic drift is effective at all levels and that genetic exchange via females is insufficient. For *A. buselaphus* and *D. lunatus,* the part of variance found within groups (here clades) was lower. However, for *A. buselaphus,* population pairwise Φ_{ST} values involving different subspecies were significant, even within a clade. Within clade II (*swaynei-lelwel-cokei*), this would mean that the relationships observed among some *swaynei* and *cokei* haplotypes are due to ancestry (NJ tree). Alternatively, gene flow may occur at a low rate, and moreover it might have been underestimated due to small sample size. In clade III, the significant differentiation between *caama* and *lichtensteini* populations is consistent with their phylogenetic split. Moreover, pairwise Φ_{ST} values suggest the isolation of Zambian versus Tanzanian populations of the subspecies *lichtensteini.* Thus, among clade III populations, both historical fragmentation (*caama*/*lichtensteini*) and restricted gene flow (Zambia/Tanzania) can be effective in shaping genetic variation.

The genetic structures of eastern populations differ between species: for *C. taurinus,* significant differentiation is found across the Rift Valley, while populations from the same side are not different (Loliondo, Masai-Mara, and Nairobi, Amboseli, respectively). Moreover, this reflects the taxonomic subdivision (*mearnsi*/*albojubatus*). Contrastingly, in *A. buselaphus,* no differentiation is found among these three populations, which belong to the *cokei* subspecies. Habitat discontinuity has been proposed to be responsible for the fragmentation of *C. taurinus* and *Aepyceros melampus* (impala) populations across the Rift Valley, as inferred from restriction fragment length polymorphism data (Templeton and Georgiadis 1996). For the buffalo, a broader ecological

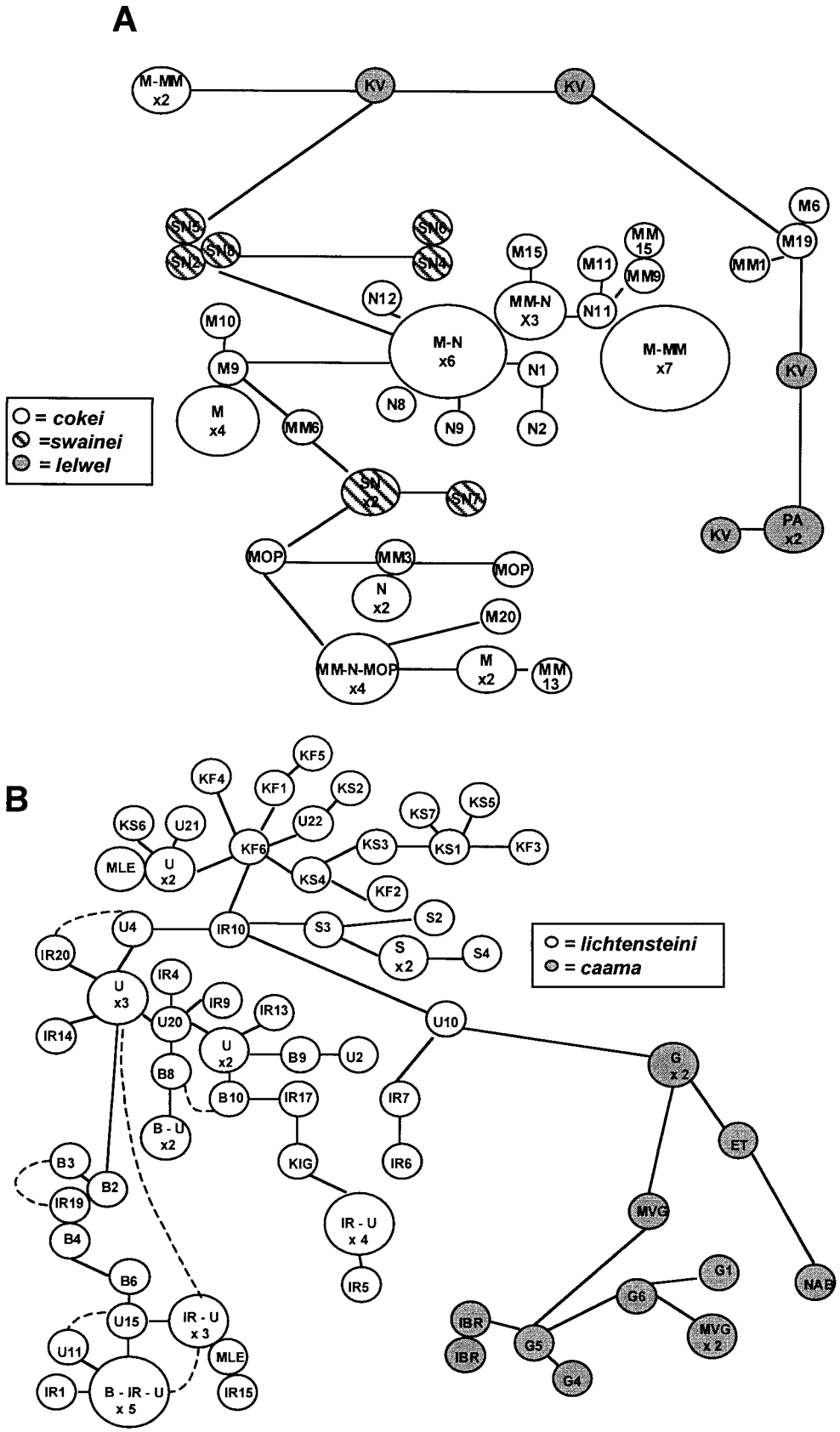

FIG. 3.—Minimum spanning networks based on the maximum-likelihood distances calculated among members of a clade (*Alcelaphus buselaphus, Damaliscus lunatus*) or among all haplotypes (*Connochaetes taurinus*). Dashed curves represent less or equally favored connections relative to full lines (Crandall and Templeton's [1993] criterion, see text). *A, Alcelaphus buselaphus,* clade (II). *B, Alcelaphus buselaphus,* clade (III). *C, Damaliscus lunatus jimela. D, Connochaetes taurinus.*

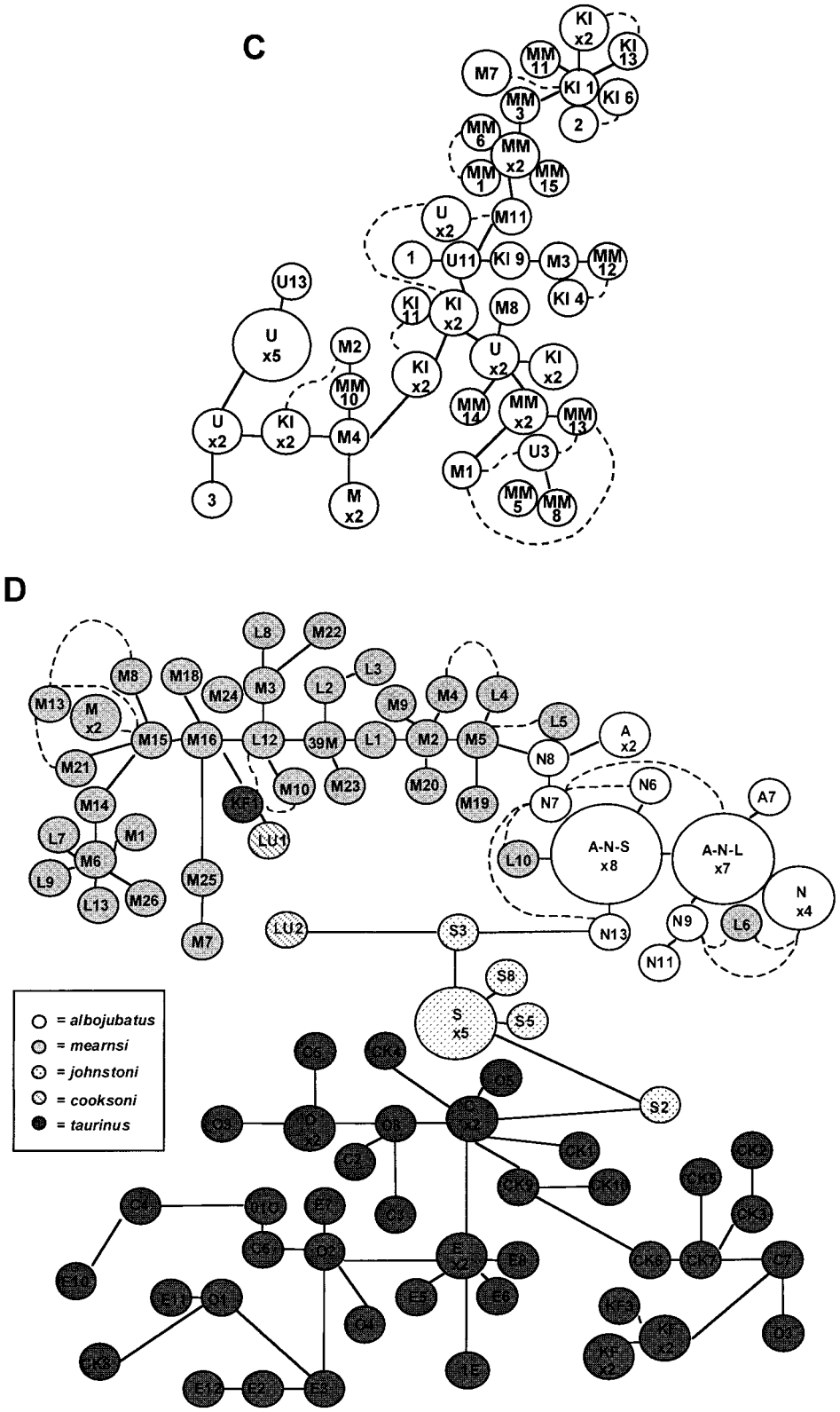

FIG. 3 *(Continued)*

niche is invoked by the same authors to explain the observed pattern of isolation by distance. Low population structure at the regional level was also found for this species using either mtDNA control region sequences or microsatellite loci (Simonsen, Siegismund, and Arctander 1998). In the present study, because ecological requirements do not differ extensively between *C. taurinus* and *A. buselaphus*, the lack of concordance in the

Table 3
Summary of Population or Subspecies Statistics for mtDNA Polymorphism

Species	Subspecies	Locality	n_S	n_H	P	ts/tv	Indel	Π (SD)
Alcelaphus buselaphus	*swaynei*	Senkele	8	7	40	29/0	12	0.054 (0.031)
	cokei	Nairobi	15	10	31	23/4	4	0.021 (0.011)
		Masai-Mara	15	10	59	46/2	14	0.041 (0.022)
		Maswa-Kimali	18	12	54	45/3	6	0.040 (0.021)
	lichtensteini	Burko-Wembere	9	9	27	25/0	2	0.033 (0.019)
		Ikiri-Rungwa	19	16	51	37/9	7	0.043 (0.022)
		Ugalla	22	15	52	43/1	6	0.036 (0.019)
		Kafue	6	6	15	11/0	4	0.015 (0.010)
		Kasama	7	7	15	11/0	4	0.016 (0.010)
	caama	Ghanzi	6	5	36	24/2	10	0.047 (0.028)
	lelwel	—	6	5	34	31/1	2	0.044 (0.027)
	major	—	8	7	36	35/0	2	0.036 (0.021)
Damaliscus lunatus	*jimela*	Masai-Mara	15	14	29	16/1	12	0.013 (0.008)
		Maswa-Kimali	11	10	26	18/2	6	0.025 (0.014)
		Ugalla	7	7	15	12/1	2	0.018 (0.010)
		Kigosi	13	11	23	15/0	8	0.015 (0.009)
	lunatus	Okavango-Chobe	7	7	12	9/1	2	0.014 (0.009)
Connochaetes taurinus	*albojubatus*	Amboseli	10	4	6	6/0	0	0.005 (0.004)
		Nairobi	16	9	7	4/2	2	0.005 (0.003)
	mearnsi	Masai-Mara	26	25	37	33/4	4	0.023 (0.012)
		Loliondo	13	13	24	19/2	4	0.020 (0.011)
	johnstoni	Selous	10	6	31	27/3	2	0.018 (0.010)
	cooksoni		—	—	—	—	—	—
	taurinus	Kafue	6	4	28	26/1	1	0.026 (0.016)
		Okavango	11	10	45	33/7	6	0.038 (0.021)
		Chobe	7	7	34	27/6	4	0.037 (0.022)
		Centre-Kalahari	10	10	42	36/6	0	0.034 (0.019)
		Etosha	12	11	46	35/7	7	0.039 (0.021)

NOTE.—n_S, n_H, P, ts/tv, and indel indicate numbers of sequences, haplotypes, polymorphic sites, transitions/transversion, and insertions/deletions, respectively. Π holds for Nei's (1987) nucleotide diversity.

Rift Valley area would, rather, have a historical explanation. Thus, the high divergence between *A. buselaphus* populations located east of the Rift Valley is likely to reflect the early separation of *cokei* and *lichtensteini* lineages that today live in parapatry. For *C. taurinus*, the Rift Valley may act as a reinforcing factor against gene flow, yet other historical causes such as past colonization eastward via two parallel ways could also be possible.

However, it is to be noted that our inferences are restricted to females and in the case of different levels of philopatry or gene flow between sexes, the total population structure is expected to differ. For example, in *C. taurinus*, a study of four microsatellite loci based on the same samples revealed nonsignificant variation among groups (unpublished data). This suggests a higher contribution from males to gene flow, which may re-

Table 4
Summary of Population Genetic Structure, as Estimated from Hierarchical AMOVAs

	Hierarchy	Variance Component	df	% Total	*F*	*P*
Alcelaphus buselaphus	2 groups	WP	115	25.74	Φ_{ST} = 0.743	0.000[a]
		AP/WG	8	6.68	Φ_{SC} = 0.206	0.000[b]
		AG	1	67.59	Φ_{CT} = 0.676	0.006[b]
		Total	124			
Damaliscus lunatus	2 groups	WP	54	25.46	Φ_{ST} = 0.745	0.000[a]
		AP/WG	3	4.51	Φ_{SC} = 0.150	0.000[b]
		AG	1	70.03	Φ_{CT} = 0.700	0.184[b]
		Total	58			
Connochaetes taurinus	2 regions	WP	111	30.81	Φ_{ST} = 0.692	0.000[a]
		AP/WG	8	15.05	Φ_{SC} = 0.328	0.000[b]
		AG	1	54.14	Φ_{CT} = 0.541	0.013[b]
		Total	120			

NOTE.—For *A. buselaphus* and *D. lunatus*, clades are *caama-lichtensteini*/*coeki-swaynei* and *lunatus*/*jimela*, respectively. For *C. taurinus*, 10 populations are grouped into two regions (south/east). WP = within populations; AP/WG = among populations within groups; AG = among groups. Subscripts are as follows: ST = sample/total; SC = sample/groups; CT = groups/total. Probabilities were calculated from 1,023 permutations.

[a] Random value less than or equal to the observed value.

[b] Random value equal to or more than the observed value.

Table 5
Pairwise Φ_{ST} Values as Calculated from AMOVA Among Populations of *Alcelaphus buselaphus*

	B	IR	U	KF	KS	M	MM	N	SN	G
B	—	NS	NS	*	*	*	*	*	*	*
IR	0.001	—	NS	*	*	*	*	*	*	*
U	0.063	0.006	—	*	*	*	*	*	*	*
KF	0.440	0.300	0.222	—	NS	*	*	*	*	*
KS	0.440	0.312	0.219	0.082	—	*	*	*	*	*
M	0.750	0.722	0.748	0.780	0.792	—	NS	*	*	*
MM	0.741	0.713	0.741	0.772	0.784	0.012	—	NS	*	*
N	0.834	0.781	0.805	0.879	0.883	0.107	0.037	—	*	*
SN	0.698	0.665	0.702	0.755	0.776	0.233	0.214	0.434	—	*
G	0.492	0.418	0.477	0.653	0.661	0.742	0.728	0.837	0.698	—

NOTE.—NS = nonsignificant; * = significant ($\alpha = 0.05$). See table 1 for locality codes. Levels of significance were estimated from 1,000 permutations.

sult from the dispersal mode of the species, described as male-biased (Estes 1991).

Lineage History and Population Demography

Estimates of divergence time based on a control region calibration are questionable, because the reported rates vary extensively among vertebrates (see Hoelzel, Hancock, and Dover 1991). Since fossil data suggest a recent origin of the species (about 1 MYA), we assume that within-species divergence occurred during the Pleistocene.

For *C. taurinus,* a general trend was shown by southern haplotypes to exhibit longer branches compared with Tanzanian and Kenyan lineages. If we assume similar population sizes in both time and space and equivalent rates of evolution, this pattern is expected if southern lineages are on average effectively older than their eastern counterparts. Moreover, southern lineages appear paraphyletic relative to eastern ones. Such relationships are consistent with a colonization scenario (see Thorpe et al. 1994; Marko 1998) from south to east. Alternatively, the pattern may be due to variation in population size. Demographic variation is indeed known to affect the probability of lineage extinction (Avise, Neigel, and Arnold 1984) and the lineage-sorting period (LSP) (Hoelzer, Wallman, and Melnick 1998), with lower probabilities and longer periods in larger populations. Larger population sizes in the south would accordingly be responsible for the retention of ancient polymorphism among the present-day OTUs. The mismatch distribution, when statistically tested, did not indicate demographic expansion in any of the *C. taurinus* populations. Although not tested, the distribution shown by the Nairobi population was unimodal and strongly skewed to the left (few differences). This situation is expected when the population genealogy is starlike (Slatkin and Hudson 1991), and reflects a recent divergence (see MSN). Moreover, in Nairobi and Amboseli (Kenya), the level of genetic variability was substantially lower (0.005) than in any other population. Consequently, we may suspect a pattern of demographic expansion, at least in Nairobi, following founder events associated to recent colonization toward east Africa. Indeed, this hypothesis is in accordance with the evolution of genetic variability when spatial expansion after a bottleneck is rapid, as predicted from simulations (Hewitt 1996). The hypothesis is further strengthened by the observation of a gradual decline in genetic variability going toward east Africa. However, focusing on one sex's evolution, as is the case with mitochondrial DNA, may lead to an overestimation of the bottleneck general effect, because there may be sex-mediated patterns and because the effective population size is one fourth that of nuclear autosomal genes. Furthermore, the outcome of a population bottleneck depends on the severity and duration of the reduction and on the subsequent growth ability (Nei, Maruyama, and Chakraborty 1975).

Table 6
Pairwise Φ_{ST} Values as Calculated from AMOVA Among Populations of *Damaliscus lunatus*

	KI	M	MM	U	O
KI	—	NS	NS	*	*
M	0.073	—	*	NS	*
MM	0.056	0.108	—	*	*
U	0.242	0.078	0.300	—	*
O	0.770	0.726	0.714	0.793	—

NOTE.—NS = nonsignificant; * = significant ($\alpha = 0.05$). See table 1 for locality codes. Levels of significance were estimated from 1,000 permutations.

For *D. lunatus,* our demographic inferences might account for a colonization scenario, since expansion was detected in several populations. However, both *jimela* and *lunatus* clades were involved, so we cannot give a direction to the process. Morever, the tree topology is also insufficient in this respect, because both clades were reciprocally monophyletic, and genetic variability was similar among populations. Compared with the pattern found in *C. taurinus,* the hypothetical colonization or dispersal process either occurred earlier or involved fewer founders, leading to complete sorting of the lineages. As the demographic signature typically expected after colonization is still detectable in *D. lunatus,* we prefer the latter explanation. Up to this point, we assume that the mismatch distribution reflects demography only. However, unimodal distributions have been shown to occur under conditions other than sudden expansion (see Lundstrom, Tavaré, and Ward 1992; Marjoram and Donnelly 1994), so our inferences should generally be viewed with caution. Moreover, results may also be strongly affected by sample size.

Alcelaphus buselaphus lineages exhibit a different pattern: the NJ tree shows a marked tendency to sym-

Table 7
Pairwise Φ_{ST} Values as Calculated from AMOVA Among Populations of *Connochaetes taurinus*

	A	N	M	L	S	KF	O	C	CK	E
A	—	NS	***	**	***	***	***	***	***	***
N	0.085	—	***	***	***	***	***	***	***	***
M	0.301	0.387	—	NS	***	***	***	***	***	***
L	0.216	0.311	0.024	—	***	***	***	***	***	***
S	0.661	0.729	0.514	0.544	—	**	***	***	***	***
KF	0.793	0.839	0.640	0.660	0.660	—	**	***	**	***
O	0.724	0.773	0.647	0.642	0.616	0.385	—	NS	**	**
C	0.753	0.805	0.648	0.651	0.619	0.365	0.072	—	NS	*
CK	0.735	0.784	0.648	0.645	0.630	0.377	0.125	0.095	—	***
E	0.726	0.774	0.666	0.660	0.621	0.447	0.146	0.158	0.326	—

NOTE.—NS = nonsignificant; * = significant ($\alpha = 0.05$). See table 1 for locality codes. Levels of significance were estimated from 1,000 permutations.

metry, compared with *C. taurinus.* Three clades are identified that do not overlap geographically. Moreover, no evidence was found for population demographic expansion. These results are consistent with an early split of the species lineages followed by their independent evolution. In accordance with this, *A. buselaphus* shows a high degree of morphological differentiation. To illustrate, the *lichtensteini* lineage is characterized by a high divergence of the two horn bony cores, while in other subspecies, these unite to form a pedicel whose development is maximal in the subspecies *lewel* (Gentry 1978). *Lichtensteini* and *caama* lineages, in spite of obvious morphological differences (horn cores and coat color), do share a character state consistent with our finding that they form one clade: their facial hair is oriented upward from muzzle to eye, while in all other subspecies it is directed downward (Haltenorth and Diller 1988).

Distinct patterns were found within *A. buselaphus* clades. The difference in cladogenesis within clades II and III may be related to contrasted past population dynamics. Indeed, the time to reach reciprocal monophyly between daughter populations is related to the effective population size—it is expected to be achieved within $4N_{f(e)}$ generations (four times the female effective population size) for mtDNA lineages (Neigel and Avise 1986). More precisely, in clade III, severe population reduction or long-term small N_e may have been effective in reducing the LSP between *lichtensteini* and *caama* lineages, whereas ancient polymorphism would have been retained in larger populations to the east (clade II). Since the genetic variability in neither *lichtensteini* nor *caama* populations shows any sign of reduction, the hypothesis of long-term small N_e appears unlikely.

Searching Concordant Patterns Among Species—East/South Relationships

From our data, despite distinct specific patterns, the split between southern and eastern lineages appears to be a feature shared by the three species. The absence of an obvious geographical barrier that is strictly impossible to circumvent between the two regions indicates that true vicariance would not be involved. Rather, environmental variation during the Pleistocene would have prevailed in shaping the three species' evolution within each area. Such variation is related in Africa to a combination of climatic changes associated with glacial-interglacial periods, direct climate change, and tectonic activity. For example, there is evidence for a humid period in the Kalahari in the late Pleistocene (Lancaster 1979). However, the climate of southern Africa seems to have oscillated only from arid to semiarid since the end of the Miocene (Lancaster 1984). To the east, the east Africa rift was well established by the mid-Miocene, but uplift continued in some areas until the terminal Pleistocene (see Partridge, Wood, and deMenocal 1995).

In response to the resulting changes, each region may have served as a refugium with alternate expansion and contraction of the lineages, owing to the inconstancy of favorable conditions (aridity). This hypothesis would explain the phylogeny of *D. lunatus* and *A. buselaphus* (for the latter, west Africa was probably a third refugium). Comparatively, for *C. taurinus*, our analyses suggest a colonization from south to east, reflecting a more continuous process. The first recorded remains of *C. taurinus* (1.5 Myr old) were found in east Africa (Olduvai bed, Tanzania) (Vrba 1995), the region that appears from our data to be the most recently colonized. A possible explanation for the opposition between DNA and fossil data would be that some lineages occurred earlier in southern Africa (consistent with our tree), but no fossils have been recorded. Alternatively, the present phylogeographical survey describes a secondary colonization toward east Africa, which implies that the lineages initially established in this region (consistent with paleontology) became extinct at some time during the last 1.5 Myr. Consequently, the present-day populations of the wildebeest in east Africa would not directly descend from the lineage dated at 1.5 MYA. *Connochaetes taurinus* FAD would suggest that the species' appearance predates the last highly significant ''FAD pulse'' of bovids (a cluster in time of first appearances of distinct morphologies), which occurred between 0.9 and 0.7 MYA according to Vrba (1995). *Alcelaphus buselaphus* and *D. lunatus* would more likely have arisen within this pulse. The hypothesis of a previous extinction of *C. taurinus* in this region is not in contradiction to the 0.9–0.7 Myr FAD pulse, as long as this measure effectively reflects an evolutionary turnover pulse, which includes not only speciation, but also extinction

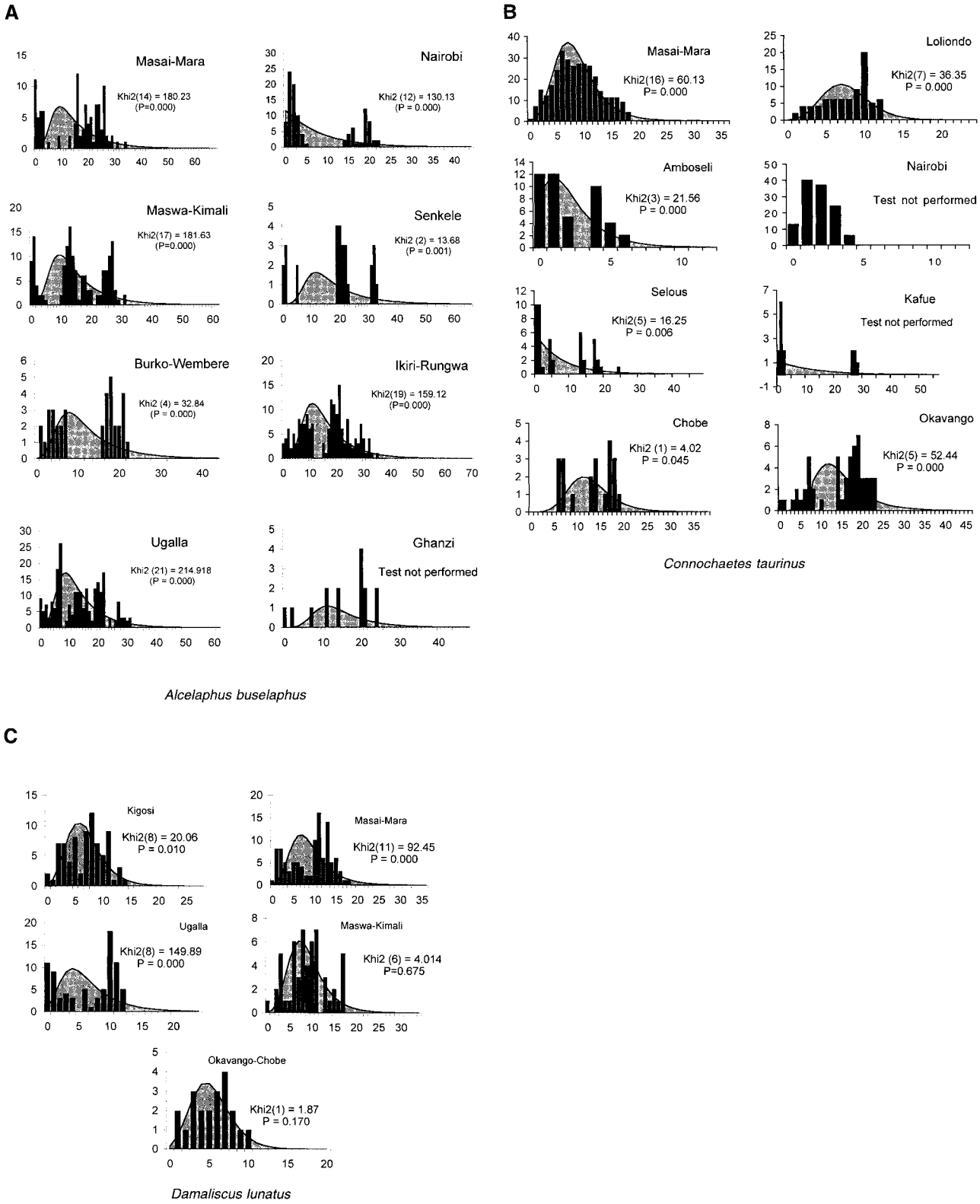

FIG. 4.—Mismatch distribution per population: observed values (bars) and expected curve under the expansion hypothesis. Departures were tested with a χ^2 test of goodness of fit. *A, Alcelaphus buselaphus. B, Connochaetes taurinus. C, Dasmaliscus lunatus.*

and migration (see Vrba 1995). In east Africa, the composition of paleosol carbonates reveals a relatively recent development of savanna grasslands, with C4 plants being dominant (>50% of the biomass) only in the last 0.6 Myr (Cerling 1992), although some previous brief dominances were also detected (at about 1.7 and 1.2 Myr). Our hypothesis that initial colonization of east Africa by *C. taurinus* before 1 MYA was unsuccessful may correlate with a contraction of the savanna at that time. The second wave of colonization would have given rise to the current populations, which appear more recent than those from southern Africa. Detailed chronology of the fossil records in east Africa are needed to further support this hypothesis. Last, it is to be noted that without invoking any colonization process, simply larger population sizes over long periods of time to the south may be sufficient to explain the observed pattern.

To summarize, a phylogeographic scenario can be proposed for the evolution of the three species during the Pleistocene. The occurrence of geographic refugia in the west, east, and south, characterized by the persistence of drier conditions during the interglacial phases that induced a wetter climate in other parts of the continent, is supported by the phylogeny of *A. buselaphus.* Moreover, the high divergence found in the eastern clade (*cokei*, *swaynei*, and *lewel*) may have resulted partly from colonization processes from one refugium (west Africa) to another (east Africa) or a split into a mosaic of several refugia in the east. For *D. lunatus*, our sampling does not cover the central part of Sahel, where two other subspecies are described. According to the proposed scenario, we would expect them to diverge significantly from the two clades identified (subspecies *lunatus* and *jimela*). However, further sampling of this species would be of importance for testing the occurrence of a ''bovid'' western refugium. Comparatively, *C. taurinus* is not currently found in west Africa, yet its former range covered the region. It is therefore likely that the extinction of this species in western areas took place during the Pleistocene and that populations might have been isolated there but failed to survive. Under the colonization hypothesis, our data also suggest an extinction of populations previously established in east Africa. The specific pattern observed among *C. taurinus* mitochondrial lineages and the evolution of their geographical range suggests that this species was less successful than *D. lunatus* and *A. buselaphus* in facing the Pleistocene environmental changes. Further comparisons with other herbivorous species are currently being conducted in order to draw a more general biogeographic scenario of the recent evolution in African ungulates (including 17 species; unpublished data).

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