

Genetic Divergence in South African Wildebeest: Comparative Cytogenetics and Analysis of Mitochondrial DNA

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The blue and the black wildebeest, *Connochaetes taurinus* and *C. gnou*, are currently classified as congeneric, but previous reports have placed *C. taurinus* in its own genus, *Gorgon*. To further clarify the evolutionary relationship between these two species, we examined and compared their mitotic chromosomes and mitochondrial DNA (mtDNA). No species-specific G-banded or C-banded chromosomal markers were found, and we conclude that the karyotypes are invariant at the level of resolution obtained. An evolutionary divergence time of approximately 1 million years was calculated from mtDNA restriction fragment data, indicating a close phylogenetic relationship for the two wildebeest species. The low nucleotide diversity detected within the black wildebeest (0.09%) is thought to reflect the recent population bottleneck to which the species has been subjected. In contrast, the limited heterogeneity (0.02%) within the South African blue wildebeest herds sampled in this study was surprising, and we argue that for many populations, especially those on smaller reserves, this may reflect common descent from a small number of animals through management-controlled translocations.

The blue and the black wildebeest, *Connochaetes taurinus* and *C. gnou*, belong to the subfamily Alcelaphinae (family Bovidae), which includes the wildebeest, hartebeest, tsessebe, bontebok, and blesbok. The two species are phenotypically distinct, differing considerably in horn and skull morphology, size, and color (Smithers 1983). There has long been controversy surrounding the taxonomic and evolutionary relationships of the wildebeest species, and, although both taxa are currently regarded as congeneric (Ansell 1974; Corbet and Hill 1980; Honacki et al. 1982), based on morphological characteristics, the blue wildebeest was previously classified as *Gorgon taurinus* (Roberts 1951; Shortridge 1934).

Evidence in support of a relatively close evolutionary and taxonomic relationship for the wildebeest is, however, forthcoming from reports of interspecific hybridization between the two species (Ansell 1974; Fabricius et al. 1988; Sidney 1965; Van Ee 1962; Zukowsky 1967). In fact, reports of hybrid fertility (Ansell 1974) led Ansell (1974) to conclude that *Gorgon* could not be maintained as a separate genus (see also Fabricius et al. 1988). By examining and comparing the mitochondrial DNA (mtDNA) and chromosomes of these two species we hoped to shed some

additional light on the evolutionary relationship of these gregarious African bovids.

The use of cytogenetic parameters in this investigation was prompted by the extensive karyotypic divergence found in the Bovidae, with respect to both diploid number and heterochromatic variation (Buckland and Evans 1978a,b; Mayr et al. 1985; Wallace 1977; Wurster and Benirschke 1968). Although the G-banded and C-banded chromosomes of *C. taurinus* have been published (Buckland and Evans 1978a,b), to our knowledge, comparable data on *C. gnou* are still outstanding. Restriction endonuclease analysis of mtDNA is widely used in evolutionary studies (reviews by Avise 1986; Wilson et al. 1985) because of its rapid rate of mutational change (Brown et al. 1979, 1982), its strict pattern of maternal inheritance (Hutchison et al. 1974; Lansman et al. 1983), and its lack of recombination (Wilson et al. 1985). It has proved to be a useful tool when examining closely related species and thus was considered to be particularly appropriate to the study of the wildebeest.

Our aims in this investigation were two-fold. First, we analyzed the G-banding and C-banding patterns of *C. taurinus* and *C. gnou* in an attempt to determine whether evolutionary divergence was reflected in

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Journal of Heredity 1991;82:447–452; 0022-1503/91/\$4.00

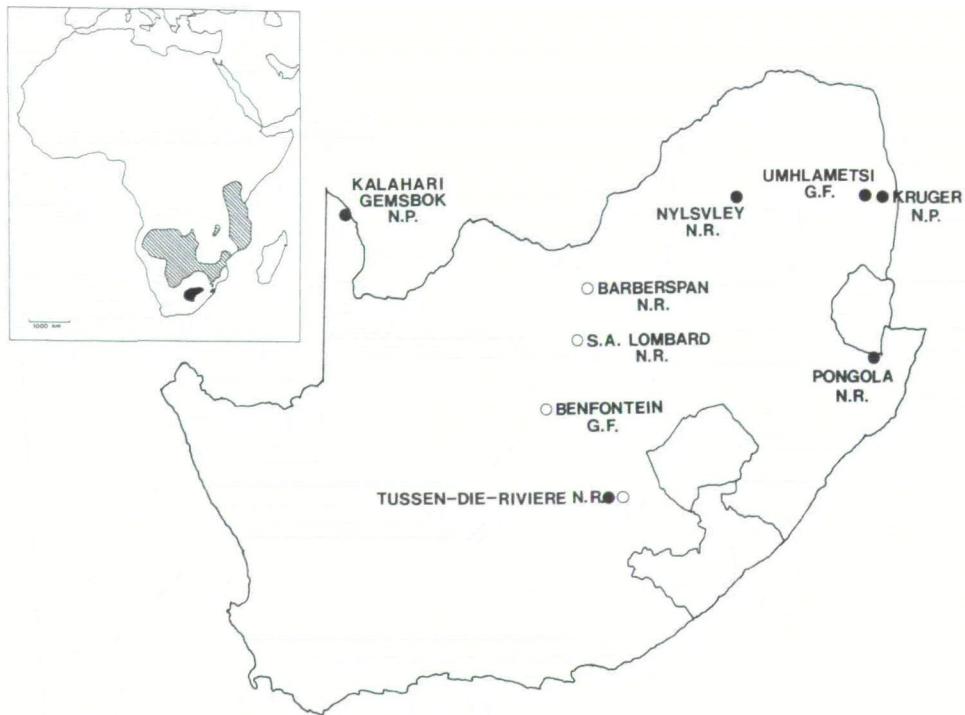


Figure 1. Distribution of collection localities within South Africa for the blue wildebeest, *C. taurinus* (●), and the black wildebeest, *C. gnou* (○). Inset illustrates the geographic distributions in Africa of the two species (redrawn from Smithers 1983): the cross-hatched area depicts the distribution of *C. taurinus*, and the solid black area that of *C. gnou*.

gross changes in karyotype and to present standardized karyotypes for the two species. Second, we examined the magnitude of mtDNA divergence between these two closely related, yet phenotypically distinct, African bovids and compared the estimate of genetic divergence in mtDNAs of the two species to divergence times obtained from the fossil record.

Materials and Methods

Sample Collection

We analyzed samples from 15 *C. taurinus* and 15 *C. gnou* specimens collected from various localities throughout South Africa (Figure 1). In most instances we collected liver and heart samples from fresh carcasses resulting from culls and trophy hunts, while, in a number of cases, we obtained ear biopsies from immobilized animals.

Unlike many of the smaller African antelope that are widely used in game ranching, the larger ungulates, such as the black and blue wildebeest, are cropped far more restrictively. With the unavoidable use of relatively small sample sizes, we reasoned that mtDNA heterogeneity could best be assessed by sampling from as many geographically discrete herds as possible. Sample numbers and collection localities were:

- *C. taurinus* Kalahari Gemsbok National Park ($n = 4$); Kruger National Park ($n = 3$); Nyfsvley Nature Reserve ($n = 1$); Pongola Nature Reserve ($n = 1$); Tussen-die-Riviere Nature Reserve ($n = 5$); Umhlametsi Game Farm ($n = 1$).

- *C. gnou* Barberspan Nature Reserve ($n = 6$); Benfontein Game Farm ($n = 2$); S. A. Lombard Nature Reserve ($n = 3$); Tussen-die-Riviere ($n = 4$).

Cytogenetic Analysis

Chromosomes from two blue wildebeest bulls (collected at Tussen-die-Riviere Nature Reserve) and two black wildebeest bulls (collected at S. A. Lombard Nature Reserve) were prepared from fibroblast cultures propagated from ear biopsies using standard techniques. Chromosomes were G-banded and C-banded according to the methods of Wang and Federoff (1972) and Sumner (1972), respectively. For each specimen examined, we photographed and karyotyped a minimum of three G-banded mitotic spreads. Then we selected the best karyotypes (five in *C. taurinus* and three in *C. gnou*) and used them for the standardization of the respective karyotypes, which was based on the percentage contribution of each autosome to the female genome (Lee and Martin 1980). For this, we identified ho-

mologous chromosomes by their banding pattern and measured the position of the centromere and length of the chromosome directly from photographs using a Quantimet 520 Image Analyzer (Cambridge Instruments, United Kingdom). Precise measurements of the short arms of acrocentric chromosomes proved to be inaccurate in many cases, and thus we used only the measurements of the long arms of acrocentric elements in the calculations for determining standardized karyotypes.

mtDNA Isolation and Analysis

We collected liver or heart samples in liquid nitrogen and isolated mtDNA according to the method of Lansman et al. (1981) with minor modification. Mitochondrial DNA samples (20–30 ng) were digested with 10 restriction endonucleases (*Aval*, *Bgl*I, *Eco*RI, *Ksp*I, *Bam*HI, *Hind*III, *Stu*I, *Dra*I, *Xba*I, and *Scal*) following assay conditions recommended by the manufacturers. Samples were subsequently end-labeled with ^{32}P dCTP and separated on a 1% agarose gel at 30 V for 16 h alongside a *Hind*III digest of lambda DNA as a size standard. Gels were vacuum dried at 55°C for 1 h and autoradiographed. Occasionally soft tissue was not available for mtDNA extraction, and in these cases we extracted high molecular weight DNA either from cultured tissue (Miller et al. 1988) or directly from skin biopsies. In these instances, we separated DNA digestions on a 0.8% agarose gel. Southern blots were produced (Southern 1975) and hybridized to an mtDNA probe labeled to high specificity with ^{32}P dCTP (Feinberg and Vogelstein 1984). The mtDNA probe was prepared from soft tissue using the above technique and, prior to labeling, further purified using the BIO 101 Geneclean Kit (Cat. No. 3105, California). Probes prepared from both *C. taurinus* and *C. gnou* samples were found to hybridize equally well to both species.

We measured the distance from the origin of sample and marker fragments using the image analyzer, which was programmed to calculate sample fragment sizes from the position of the marker fragments based on a regression of the reciprocal of the distance migrated. The largest marker fragment (23.13 kb) was excluded from the regression calculation as it greatly increased the error in the regression. Sizes of fragments varying between 23.13 and 9.42 kb (the first and second marker fragments) were calculated according to the logarithm of the distance moved. Because of the inaccuracy in size

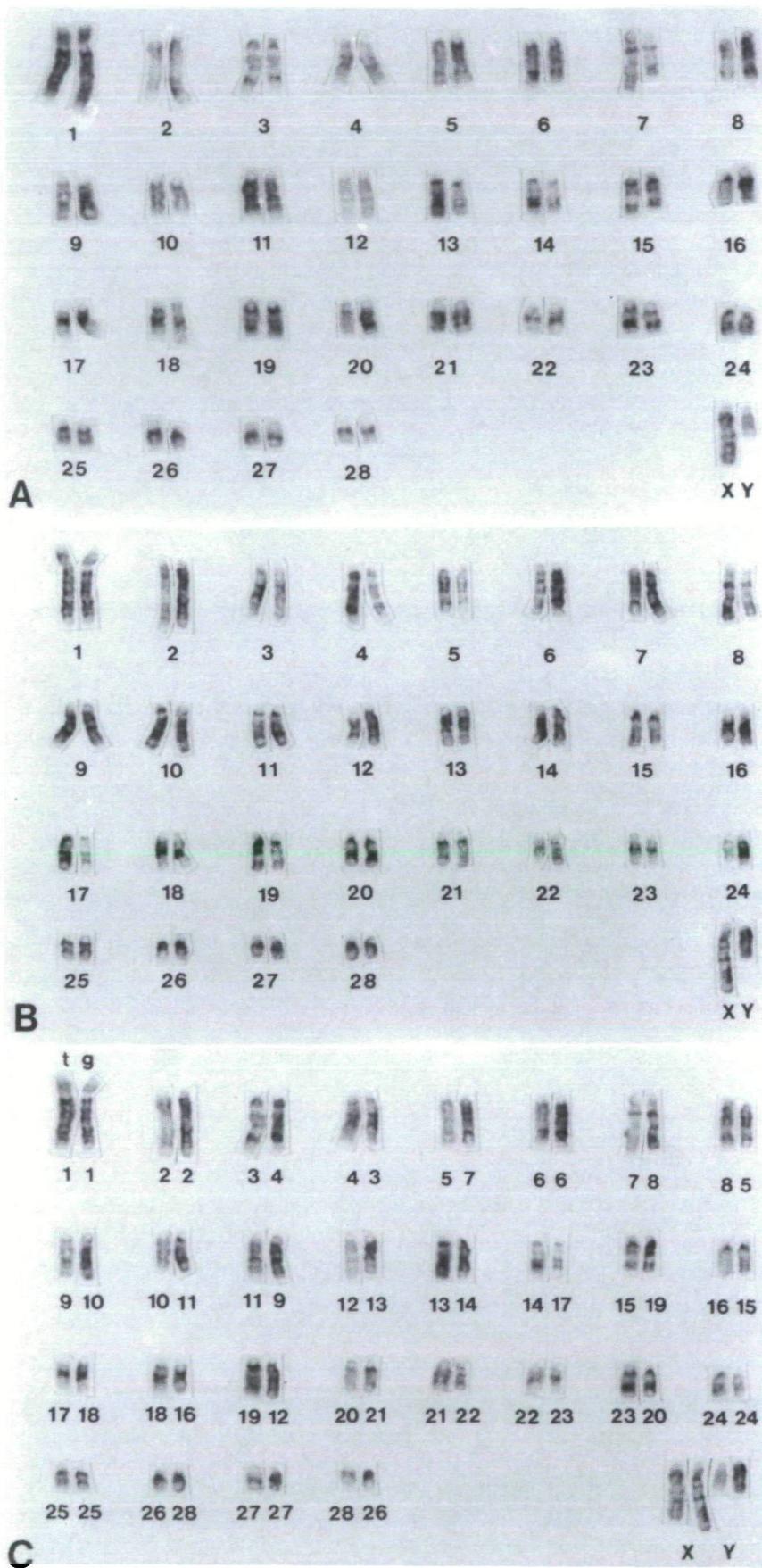


Figure 2. (A) G-banded karyotype of the blue wildebeest, *C. taurinus* ($2n = 58$); (B) G-banded karyotype of the black wildebeest, *C. gnou* ($2n = 58$); (C) Haploid composite of *C. taurinus* (t) and *C. gnou* (g). Chromosome numbers correspond to the standardized karyotypes for these species.

estimates of large fragments, we excluded all patterns with fragments larger than 9.42 kb when calculating the size of the mitochondrial genome.

Following conventional procedures, we assigned each restriction endonuclease a number and each pattern produced by that enzyme a lower case letter. Thus each haplotype or clone (designated by a roman numeral) could be described by a 21-character code. Nucleotide divergence (d) between the two species was calculated using the Site/Fragment Program v1.1 (Nei and Miller 1990), which incorporates intraspecific nucleotide diversity; standard errors were obtained via bootstrapping.

Results

Cytogenetic Analysis

Standardized karyotypes of G-banded chromosomes of *C. taurinus* and *C. gnou* are shown in Figure 2A,B. The diploid number ($2n = 58$) was found to be the same in both species and confirms earlier reports (Buckland and Evans 1978a; Gerneke 1967; Wallace 1978; Wurster and Benirschke 1968). In both instances karyotypes were found to comprise one large pair of submetacentric autosomes (pair 1), 27 pairs of acrocentric autosomes (pairs 2–28), a large acrocentric X, and a small acrocentric Y. The X chromosome constitutes $5.00\% \pm 0.06\%$ of the female genome in *C. taurinus* and $5.43\% \pm 0.14\%$ in *C. gnou*, whereas the Y chromosome contributes $2.62\% \pm 0.08\%$ and $2.76\% \pm 0.07\%$, respectively (Tables 1 and 2). Half karyotypes, comparing the G-bands of the two species, were constructed and are shown in Figure 2C.

The C-banded chromosomes of *C. taurinus* and *C. gnou* are shown in Figure 3. C-bands were similar in both species and were centromeric and small in size. The amount of heterochromatin present in the submetacentric pair was reduced relative to that contained in most acrocentric autosomes.

Mitochondrial DNA Analysis

Four mtDNA clones were observed in each species but, in each case, one genotype was by far the commonest (clone I in *C. taurinus* and clone V in *C. gnou*, Tables 3 and 4). We determined the number of mutational steps (restriction site losses or gains) separating the haplotypes within each species, as well as the changes between the closest clones of each species (clone IV for the blue wildebeest and clone

Table 1. Relative chromosome lengths of the blue wildebeest, *C. taurinus*, expressed as a percentage of the haploid karyotype (A + X)

Chromosome	\bar{X}^a	SE ^b
1	6.528	0.11
2	5.636	0.05
3	4.594	0.05
4	4.454	0.11
5	4.414	0.07
6	4.122	0.11
7	4.112	0.07
8	4.107	0.08
9	3.844	0.06
10	3.625	0.10
11	3.620	0.09
12	3.503	0.12
13	3.274	0.10
14	3.153	0.06
15	3.109	0.07
16	3.104	0.09
17	3.009	0.06
18	2.962	0.06
19	2.863	0.06
20	2.736	0.07
21	2.590	0.07
22	2.589	0.04
23	2.541	0.04
24	2.366	0.05
25	2.076	0.04
26	2.075	0.04
27	2.030	0.04
28	1.971	0.06
X	4.996	0.06
Y	2.620	0.08

^a Measurements were taken from five male G-banded karyotypes. \bar{X} = arithmetic mean.

^b SE = standard error.

Table 2. Relative chromosome lengths of the black wildebeest, *C. gnou*, expressed as a percentage of the haploid karyotype (A + X)

Chromosome	\bar{X}^a	SE ^b
1	6.156	0.05
2	5.543	0.14
3	4.319	0.11
4	4.236	0.09
5	4.181	0.13
6	4.160	0.13
7	4.149	0.06
8	4.009	0.09
9	3.831	0.14
10	3.641	0.07
11	3.375	0.09
12	3.305	0.13
13	3.272	0.15
14	3.188	0.12
15	3.094	0.09
16	3.038	0.06
17	3.037	0.08
18	3.004	0.13
19	2.968	0.09
20	2.818	0.16
21	2.791	0.09
22	2.690	0.12
23	2.652	0.06
24	2.644	0.09
25	2.288	0.10
26	2.102	0.06
27	2.059	0.06
28	2.017	0.10
X	5.428	0.14
Y	2.755	0.07

^a Measurements were taken from three male G-banded karyotypes. \bar{X} = arithmetic mean.

^b SE = standard error.

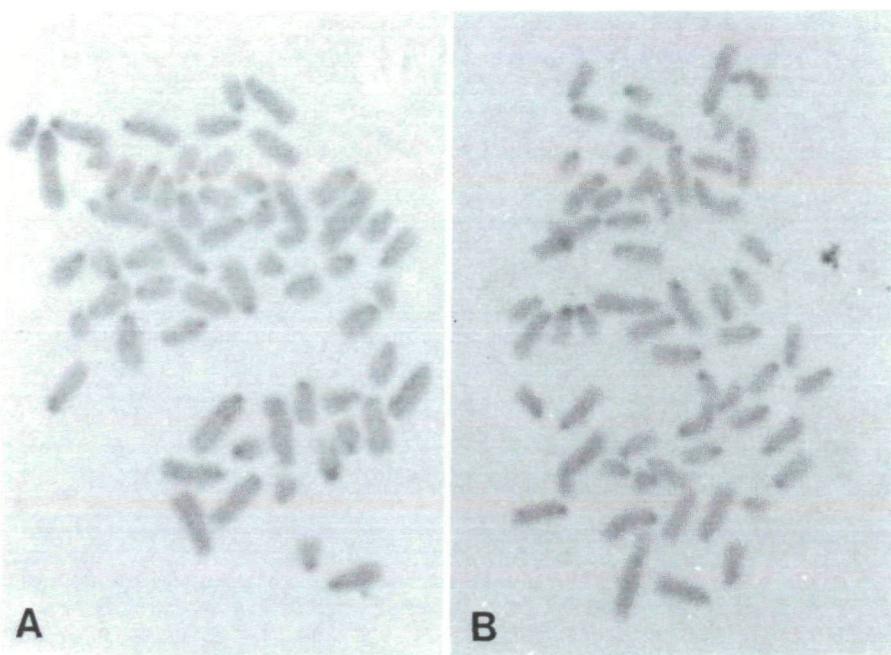


Figure 3. C-banded chromosomes of the wildebeest illustrating centromeric heterochromatin: (A) C-banded metaphase cell of *C. taurinus*; (B) C-banded metaphase cell of *C. gnou*.

V for the black wildebeest, Figure 4A,B). The total number of fragments produced by the 10 enzymes was 56 in *C. taurinus* and 54 in *C. gnou* with an average of 5.0 and 4.5 restriction fragments per enzyme, respectively. Representative restriction profiles are shown in Figure 5. The size of the mitochondrial genome did not differ significantly between the two species and

was found to be 16.21 ± 0.11 kb. On average, we examined approximately 1.8% of the mitochondrial genome. The nucleotide divergence (d) between the two species was $2.2\% \pm 0.4\%$, and the proportion of shared fragments (F) was calculated at 0.675 ± 0.04 . Intraspecific nucleotide diversity was 0.02% in *C. taurinus* and 0.09% in *C. gnou*.

Table 3. Mitochondrial DNA genotypes in the blue wildebeest, *C. taurinus*

Clone no.	Genotype ^a	No. of individuals	Collection locality ^b
I	1c 2b 3b 4b 5a 6a 7b 8c 9b 10b	1	NNR
		5	TDR
		3	KNP
		1	PNR
II	1c 2c 3b 4b 5a 6a 7b 8c 9b 10b	1	UGF
III	1c 2b 3b 4b 5a 6a 7b 8c 9c 10b	3	KGNP
IV	1b 2b 3b 4b 5a 6a 7b 8c 9c 10b	1	KGNP

^a 1 = *Aval*; 2 = *EcoRI*; 3 = *Xba*I; 4 = *Bgl*I; 5 = *Bam*HI; 6 = *Ksp*I; 7 = *Scal*; 8 = *Hind*III; 9 = *Stu*I; 10 = *Dra*I. Lowercase letters designate restriction patterns.

^b NNR = Nylsvley Nature Reserve; TDR = Tussen-die-Riviere Nature Reserve; KNP = Kruger National Park; PNR = Pongola Nature Reserve; UGF = Umhlanga Game Farm; KGNP = Kalahari Gemsbok National Park.

Table 4. Mitochondrial DNA genotypes in the black wildebeest, *C. gnou*

Clone no.	Genotype ^a	No. of individuals	Collection locality ^b
V	1a 2a 3a 4a 5a 6a 7a 8a 9a 10a	3	SAL
		2	TDR
		5	BNR
		2	TDR
VI	1b 2a 3a 4a 5a 6a 7a 8a 9a 10a	2	BGF
VII	1b 2a 3a 4a 5a 6a 7a 8b 9a 10a	2	BNR
VIII	1b 2a 3a 4a 5a 6a 7a 8d 9a 10a	1	BNR

^a 1 = *Aval*; 2 = *EcoRI*; 3 = *Xba*I; 4 = *Bgl*I; 5 = *Bam*HI; 6 = *Ksp*I; 7 = *Scal*; 8 = *Hind*III; 9 = *Stu*I; 10 = *Dra*I. Lowercase letters designate restriction patterns.

^b SAL = S. A. Lombard Nature Reserve; TDR = Tussen-die-Riviere Nature Reserve; BNR = Barberspan Nature Reserve; BGF = Benfontein Game Farm.

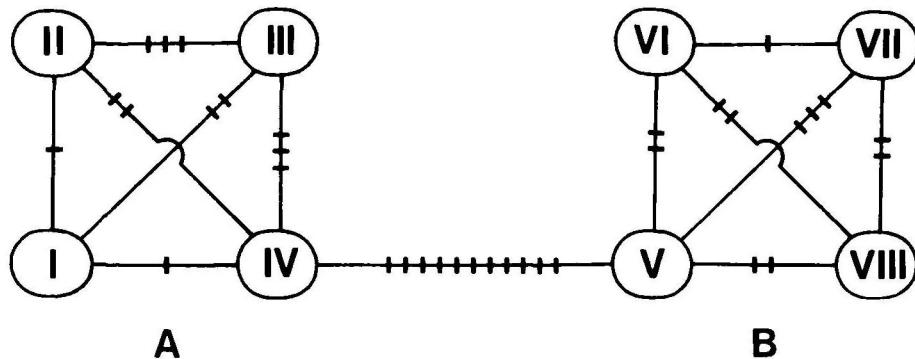


Figure 4. Parsimony network of the mtDNA clones observed in the blue (A) and the black wildebeest (B). Solid lines crossing branches of the network represent numbers of observed restriction site changes between haplotypes within each species. Clones are designated by roman numerals and correspond to those in Tables 3 and 4. Eleven mutational steps separate the closest clones in the two species (IV in *C. taurinus* and V in *C. gnou*).

Discussion

Fundamental and diploid chromosome numbers have been published for many species of the Bovidae (Buckland and Evans 1978a), and from these data it is

clear that Robertsonian change has played a major role in genome restructuring within this group. However, with respect to *C. gnou* and *C. taurinus*, the two species investigated here, the karyotypes were determined to be invariant at the resolution obtained. This karyotypic conservatism extends to both the euchromatic and heterochromatic portions of the genome. Consequently, although phenotypically distinct, morphological divergence does not, in this case, appear to have been accompanied by structural karyotypic change. It should be noted, however, that although we were unable to detect consistent differences in the banding patterns of the two wildebeest species, standardization of their karyotypes resulted in minor differences in the placement of chromosomes for each species (Tables 1 and 2). We feel that this is unlikely to reflect meaningful differences in chromosome length but is more probably a result of small sample size, particularly in *C. gnou*, and the subtle gradation in the size of autosomal pairs (Figure 2) in both species.

In contrast to the cytogenetic data, restriction patterns of mtDNA do allow some inferences with respect to the species' evolutionary history. The Alcelaphinae (the hartebeest, wildebeest, and blesbok group) appeared in the fossil record about 5 million years ago and are thought to have been wholly African throughout their evolution. The earliest fossil records of *C. gnou* and *C. taurinus* are estimated at 600,000 and 1.5 million years B.P., respectively, and an evolutionary divergence time of approximately 2 million years B.P. has been estimated (Vrba 1979). In the present study, a nucleotide divergence (d) of 2.2% was calculated between the two species which, assuming a rate of sequence divergence of 2% per million years (Brown et

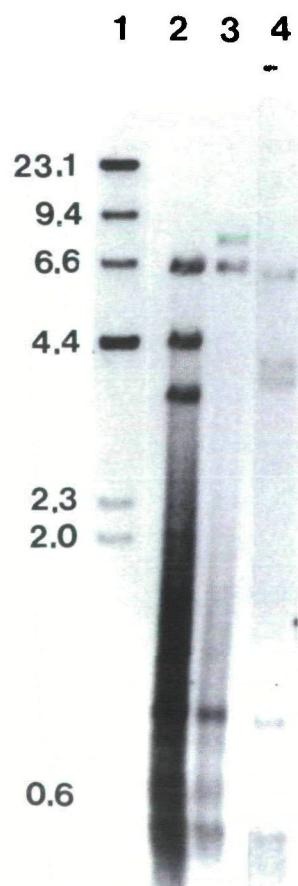


Figure 5. Examples of *Aval* digestion patterns in wildebeest: (Lane 1) *HindIII* digest of lambda DNA as a molecular weight marker; (Lane 2) Restriction profile unique to *C. gnou* comprising five fragments (6.7, 4.4, 3.4, 1.2, 0.5 kb); (Lane 3) Restriction profile common to both species comprising four fragments (7.8, 6.7, 1.2, 0.5 kb); (Lane 4) Restriction profile unique to *C. taurinus* comprising fragments of 6.9, 4.2, 3.8, 1.2, and 0.9 kb, respectively.

al. 1979), may be interpreted as indicating an evolutionary divergence time slightly over 1 million years ago. Although the extrapolation of the standard 2% sequence divergence per million years to bovids may be arguable, given the vagaries of dating techniques and a fossil record that is at best fragmentary, there is nonetheless fairly good concordance between the two data sets.

The low intraspecific mtDNA nucleotide diversity detected in this study for both species (0.02% in *C. taurinus* versus 0.09% in *C. gnou*) was surprising given the differences in the population dynamics of the species in their recent past and allows for some speculation as to possible causes. The black wildebeest, whose distribution is strictly South African (Figure 1 inset), formerly ranged in large numbers throughout much of this country. The species was brought to the point of extinction by overexploitation and agricultural development (Smithers 1983), and, by 1938, it is thought that only 300 animals survived (Kirkman 1938). Furthermore, these 300 animals were restricted to a few small protected herds, which were probably already quite highly related. We would expect that the reestablishment of *C. gnou* populations from such a limited founding stock could result in a substantial reduction in mtDNA diversity, and, consequently, it is not unrealistic to assume that the relatively low mtDNA heterogeneity detected in *C. gnou* merely reflects this.

In contrast, the blue wildebeest was never decimated to the extent of its congener. Historically, the species was widespread throughout much of east and southern Africa with its current disjunct distribution extending from southern Kenya southward through central and eastern Tanzania, northeastern Mozambique, and marginally into Zambia, Zimbabwe, southern Mozambique, and the northern and northeastern reaches of South Africa (Smithers 1983; Figure 1 inset). The species is characterized by large herd sizes in national parks and refuges, particularly in east Africa, Botswana, and the Kruger National Park, and large seasonal aggregations are still witnessed in parts of its range. Although our sampling in this species is representative only of the southern extremes of its range, a factor which obviously precludes extrapolation to the extensive herds in east Africa, the low number of maternal lineages detected in the surveyed South African herds was nonetheless not anticipated. All the blue wildebeest analyzed in this study ($n = 15$), with the exception of

those from the Kalahari Gemsbok National Park ($n = 4$) and the Umhlametsi Game Farm ($n = 1$), share the same maternal lineage (clone I, Table 3). At both the Kalahari National Park and the Umhlametsi Game Farm the extant populations are descended from animals present in these areas prior to fencing. Although the other large population (which was irrefutably "indigenous" to one reserve, the Kruger National Park) lacked mtDNA heterogeneity, it was characterized by the same mtDNA lineage (clone I) as that found for the Nylsvley Nature Reserve, the Tussen die-Riviere Nature Reserve, and the Pongola Nature Reserve (Table 3).

Although we could not verify the ancestry of the majority of the blue wildebeest herds sampled in this investigation, it seems likely that many of the herds currently existing on smaller reserves are descended from animals originally derived from the Kruger National Park area, which were subsequently relocated in recent times into areas where local populations had disappeared. In other words, the lack of mtDNA heterogeneity reflected in the South African sample populations merely reflects common descent from a small number of animals through management-controlled translocations. If this hypothesis holds, it may be anticipated that mtDNA analysis of large east African populations will detect elevated levels of intraspecific heterogeneity within the blue wildebeest. This would probably result in a further reduction in estimates of nucleotide divergence between *C. taurinus* and *C. gnou* and even more clearly underscore the lack of genetic support for the classification of the blue wildebeest in a separate genus (Shortridge 1934).

In summary, the karyotypes of *C. taurinus* and *C. gnou* were found to be invariant and may possibly offer no structural impediment to interspecific hybridization and therefore lend further credence to reports of crossbreeding in the wildebeest. The mtDNA analysis is in rough concordance with estimates of evolutionary divergence times obtained from the fossil record and provides strong support for the current

congeneric status of these two species. In addition, the unexpectedly low intraspecific nucleotide diversity found in the South African blue wildebeest herds could have some implications for future management of herds in this region. Should this indeed reflect the repeated translocations of animals from the same stock to establish subpopulations, inbreeding in the blue wildebeest may be relatively high. Investigation into variation in the nuclear genome would shed further light on this aspect of our findings.

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