

Chromosome Conservation in the Bovidae

D. S. Gallagher, Jr., and J. E. Womack

The chromosomes of 12 bovid species were harvested from fibroblast cultures after incorporation of bromodeoxyuridine into early replicating DNA. Q-band karyotypes were constructed, and, when possible, autosomal arms were numbered according to the cattle standard karyotype. Diploid chromosome number ranged from 30 to 60, yet, based on band similarity, chromosome-arm homologies were extensive. Employing the cattle karyotype as the standard, autosomal-arm differences indicative of possible syntenic disruption were noted for only chromosomes 3, 9, and 14. While chromosome-arm homologies were extensive, shared homologous biarmed chromosomes were rare. The commonness of monobrachially homologous biarmed chromosomes among some bovids (e.g., Antilopinae) suggested that reproductive isolation and speciation in some instances might have resulted from centric fusion events.

The family Bovidae is the most diverse of the nine Artiodactyla families with 45 extant genera and 124 extant species (Vaughan 1986). The diploid chromosome number ($2n$) of the Bovidae ranges from 30 to 60, but the autosomal arm number (NAA) is relatively constant at 56–58 for most karyotyped bovids. Wurster and Benirschke (1968) speculated that the constancy in NAA was indicative of centric fusions and that bovid chromosomal evolution has proceeded from a primitive karyotype of 58 acrocentric autosomes, a condition seen in the domestic cow, domestic goat, and many other bovids. Todd (1975) argued that centric fission was equally plausible. Todd saw the putative ancestral bovid karyotype as consisting of 28 biarmed autosomes (B), two acrocentric autosomes, and an X and Y, and suggested that the two acrocentric autosomes were involved in an autosome to X fusion. Thus, the ancestral bovid karyotype proposed by Todd was 28B + XX for females and 28B + XY1Y2 for males, a condition found in some antelope species (e.g., *Antilope cervicapra*). These early speculations regarding the mode of bovid chromosomal evolution were based on analyses of nonbanded chromosome preparations.

Since the early 1970s, banding studies have been performed on numerous bovid species, and considerable chromosome-band similarities have been found. Evans et al. (1973) demonstrated extensive chromosome-band homology for goat, sheep,

and ox. Their comparative cytogenetic study was extended to nine additional bovid species (Buckland and Evans 1978). They determined diploid numbers ranging from 31 to 60, found numerous monobrachially homologous biarmed chromosomes, and proposed, as did Wurster and Benirschke (1968), that bovid chromosomal variability is primarily the result of centric fusions.

Hediger (1988) proposed that the extensive chromosome conservation of the Bovidae should allow the extrapolation of chromosomal localization of genes in one bovid species to chromosomes of other bovids, and he provided support for his hypothesis by localizing the genes for the major histocompatibility complex, keratin alpha, and keratin beta to sheep and cattle chromosomes with homologous banding. The cattle physical gene map is being rapidly developed. It currently consists of 140 loci assigned to 30 syntic groups, with syntic groups U3, U4, U9, U15, U19, U20, U21, and U24 assigned to *Bos taurus* chromosomes 5, 21, 18, 6, 15, 23, 19, and 14, respectively (Womack 1990). The cattle gene map will be the template for gene mapping in other bovids.

This comparative cytogenetic study is related to our interest in cattle gene mapping and to an interest in testing the general hypothesis that bovids are chromosomally conservative. Since the domestic cattle gene map is the best developed of the Bovidae and likely to be the prototype,

From the Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas 77843-4463. We thank Elaine Owens for her assistance with the establishment and maintenance of fibroblast cultures, and Dr. William Modi and Dr. John Ellison for technical suggestions related to chromosome banding. We are indebted to Dr. Oliver Ryder, Arlene Kumamoto, and Marlys Houck of The Zoological Society of San Diego, Center for Reproduction of Endangered Species, for providing fibroblast lines. We thank Steve Kingswood, Dr. Kenneth Fletcher, Robert Evans, Dr. Mel Richardson, and Wayne Trammel of the San Antonio Zoological Gardens and Aquarium; Mark Thallman, Lee Jones, and Mike Wilson of Granada; and Dr. Doug Armstrong of the Henry Doorly Zoo, Omaha, Nebraska, for providing skin biopsies of bovids. This research was supported by USDA grant no. 90-CSRS-37266, the Texas A&M University Institute of Biosciences and Technology, and the Texas Advanced Technology Program.

Journal of Heredity 1992;83:287–298; 0022-1503/92/\$4.00

we have produced QFH-band karyotypes of other bovid species and have made direct cytogenetic comparisons with domestic cattle chromosomes numbered according to the Reading Conference (1980) GTG-band standard. This approach is significant because it is an attempt at standardizing the numbering of homologous chromosomal arms among bovids, and it couples bovid comparative cytogenetics with a developing physical gene map.

Materials and Methods

Specimens Examined

Each species name is followed in parentheses by the identification number or name of the individual from which the fibroblast line was established. Fibroblast lines of the following species were provided by the Zoological Society of San Diego, Center for Reproduction of Endangered Species: male *Syncerus caffer caffer* (3212-451), male *Rupicapra rupicapra* (6389), male *Gazella granti* (6431), and female *G. granti* (6477). Skin biopsies of the following species were provided by the following institutions: San Antonio Zoological Garden and Aquarium—male *Tragelaphus strepsiceros* (ISIS 860850), *Oryx tao* (ISIS 880467), *Oryx gazella* (ISIS 880248), *Kobus ellipsiprymnus* (ISIS 770799), and *Damaliscus lunatus jimela* (ISIS 810932); Henry Doorly Zoo—female *Bos gaurus* (4863); Texas Veterinary Medical Center—male *Bos taurus* (Billy) and female *Antilope cervicapra* (Bb 5); and Granada—male *Bos indicus* (Y2194).

Culture and Slide Preparation

We grew fibroblast cultures to confluence in Dulbecco's Modified Eagle Medium supplemented with 10% bovine serum (SA 500 Cell Culture Laboratories 20-1050-50, Cleveland, Ohio), 1× nonessential amino acids (Gibco 321140), 1× MEM vitamins (Gibco 320-1120), 1× sodium pyruvate (M. A. Bioproducts 13-115A), and 1× L-glutamine (M. A. Bioproducts 17-605A). On reaching confluence, the cell lines were then subcultured, and we immediately added 5-bromo-2'-deoxyuridine (BrdU) to the medium at a final concentration of 45 µg/ml. We removed medium containing BrdU at the beginning of the mitotic wave (approximately 24 h after subculture), washed the cells twice with calcium and magnesium-free Hank's balanced salt solution, and added fresh medium that contained thymidine (10⁻⁵ M). Six h after removal of BrdU and addition of thymidine, we removed the medium and shook off the mitotic cells into prewarmed 0.075 M KCl.

Table 1. Translocations identified during this study listed by species and numbered according to the Reading Conference (1980) cattle standard

Species	Translocations
<i>Bos gaurus</i>	t(2;28)
<i>Syncerus caffer caffer</i>	t(1;3), t(2;3), t(5;20), t(11;29)
<i>Tragelaphus strepsiceros</i>	t(2;25;24), 2(4;5), t(1;27), t(3;10), t(6;20), t(8;17), t(12;16), t(7;18), t(9;22), t(11;23), t(14;26), t(15;28), t(19;21), t(Y;13)
<i>Rupicapra rupicapra</i>	t(1;3)
<i>Oryx tao</i>	t(1;29)
<i>Oryx gazella</i>	t(1;29), t(2;17)
<i>Kobus ellipsiprymnus</i>	t(1;19), t(2;29), t(5;17), t(6;18), t(7;11)
<i>Damaliscus lunatus jimela</i>	t(5;6), t(1;10), t(4;14), t(7;9), t(8;17), t(2;29), t(12;16), t(11;23), t(18;24), t(3;19), t(13;15), t(20;22)
<i>Antilope cervicapra</i>	t(4;19), t(12;16), t(1;29), t(8;14), t(6;24), t(2;25), t(3;27), t(7;20), t(9;17), t(13;18), t(10;28), t(15;23), t(11;22), t(21;26), t(X;5)
<i>Gazella granti</i>	t(1;24), t(11;22;?), t(2;15), t(7;?), t(4;28), t(8;21), t(6;29), t(16;19), t(13;20), t(10;23), t(17;18), t(9;26), t(14;25), t(12;27), t(X;5), t(Y;16)

Translocations shared among two or more species are in bold type.

After the 20–30 min hypotonic treatment, the cells were fixed and air-dried slides were prepared in standard fashion.

Slide Staining

We stained the chromosomes of each species for 15 min with the A-T specific fluorochrome Hoechst 33258 (50 µg/ml ddH₂O), counterstained them for 10–20 minutes with the G-C specific label actinomycin D (0.3 µg/ml sodium phosphate buffer, pH 7.0; Schweizer 1981), and mounted them in 2× SSC (pH 7.0). To enable comparison of QFH- to QFQ-bands, domestic cow chromosomes were stained for 30–60 s with the A-T specific fluorochrome quinacrine dihydrochloride (0.00005% in ddH₂O), counterstained for 10–20 min with actinomycin D (0.3 µg/ml), and mounted in sucrose (50 g/50 ml ddH₂O, 0.05 M Na⁺ cacodylic acid; Dr. John Ellison, personal communication).

Photography

We evaluated chromosome band quality by inspection. Storage of slides for several hours to several days often improved band contrast. Photographs of the fluorescent chromosomes were taken on Kodak Technical Pan Film 2415 (ASA 800–1,600, developed 3 min in Kodak Dektol) with an Olympus Vanox-T equipped for epifluorescence (QFQ-bands: filter set B, excitation 450–490 nm; QFH-bands: filter set U, excitation 300–400 nm). The negatives were printed on Kodak Polycontrast RCIII paper at a contrast level of three.

Karyotype Preparation

Karyotypes are composites prepared from two or more cells. Chromosome arms were numbered according to the Reading Conference (1980) cattle GTG-band standard.

Results

Bos taurus and *B. indicus* (taurus and indicus cattle), Bovinae

The taurus cattle karyotype possessed the expected 58 acrocentric autosomes, submetacentric X, and submetacentric Y (Reading Conference, 1980). Staining with quinacrine or Hoechst 33258 produced comparably banded chromosomes (Figure 1). The male *B. indicus* was chromosomally equivalent to *B. taurus* (karyotype not shown), except for the presence of an acrocentric Y chromosome. This result corroborated earlier observations that *B. taurus* and *B. indicus* karyotypes differed by the presence of a submetacentric versus an acrocentric Y chromosome, respectively (Basrur and Gilman 1964; Märki and Robinson 1984).

Bos gaurus (gaur), Bovinae

The gaur female had a diploid number of 58 with one biarmed autosomal pair, which was consistent with an earlier report for this species (Wurster and Benirschke 1968). Examination of the gaur and cattle karyotypes (Figure 2) revealed extensive QFH-band similarities and showed that the arms of the gaur biarmed autosomal pair are orthologous with cattle 2 and 28 (Table 1).

Syncerus caffer caffer (African buffalo), Bovinae

The male African buffalo had a diploid number of 52 with four biarmed autosomal pairs (Table 1), which was identical with other published results for this taxon (Wurster and Benirschke 1968). Examination of the African buffalo and cattle chromosomes (Figure 3) revealed extensive band homologies. The buffalo X chro-

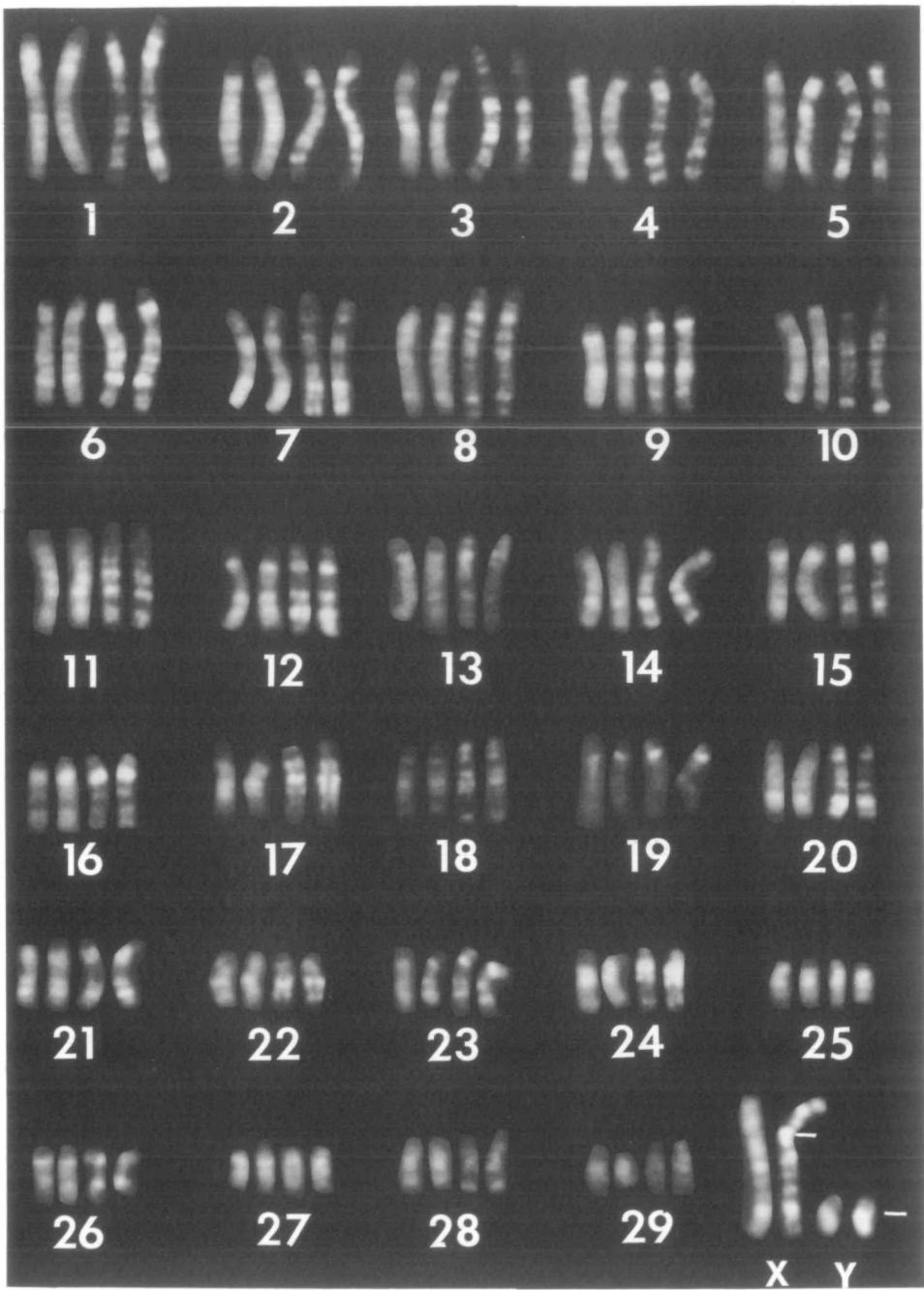


Figure 1. A comparison of *Bos taurus* (taurus cattle) QFQ- and QFH-band chromosomes ($2n = 60$), consisting of 58 acrocentric autosomes, a submetacentric X, and a submetacentric Y, numbered according to the Reading Conference (1980) cattle standard. QFQ- and QFH-banded autosomal pairs are positioned to the left and right, respectively. The QFQ- and QFH-band comparisons of the X and Y chromosomes are positioned in like fashion. White lines mark the centromere position of the X and Y.

mosome is acrocentric, thus the buffalo and cattle X chromosomes differed by a pericentric inversion. Also, the buffalo Y chromosome was noticeably smaller than the cattle Y.

Tragelaphus strepsicerus (greater kudu), Bovinae

The male greater kudu karyotype (Figure 4; $2n = 31$) was consistent with other reports for this species (Buckland and Evans

1978). Cattle and greater kudu chromosome banding homologies were extensive, with cattle equivalent chromosomes forming the arms of the kudu biarmed autosomal pairs (Table 1). The chromosome designated X1 was considered the ancestral X because of banding similarity with the telomeric two-thirds of cattle Xq. The greater kudu X appeared to be a larger chromosome and differed from the cattle X chromosome by at least a pericentric

inversion. Autosomal material homologous to cattle 13 comprised a portion of the greater kudu Y (Buckland and Evans 1978). Greater kudu X2 is homologous to cattle 13, although it appeared to be a slightly larger chromosome, with additional pericentromeric chromatin.

Rupicapra rupicapra (chamois), Caprinae

The female chamois karyotype ($2n = 58$) with one biarmed autosomal pair (Table 1) was in agreement with a previous report for this species (Mayr et al. 1987). QFH-band similarity between chamois and cattle was obvious (Figure 5). A noteworthy difference was the presence of a bright centromeric band for chamois 14 absent for cattle 14, and the presence of a bright centromeric band for cattle chromosome 9 absent for chamois 9. The QFH-band pattern of the cattle and chamois X chromosome were similar in appearance for the region corresponding to the telomeric one-half of cattle Xq, but differed by at least a pericentric inversion since the chamois X was acrocentric.

Oryx tao and *O. gazella* (scimitar-horned and gemsbok), Hippotraginae

The female scimitar-horned (Figure 6; $2n = 58$) and gemsbok oryx (Figure 7; $2n = 56$) karyotypes were consistent with earlier cytogenetic characterizations of these species (Buckland and Evans 1978; Wurster 1972). QFH-band similarities with cattle were extensive, including the biarmed chromosomes (Table 1), although noteworthy differences existed. The cattle and oryx X chromosomes were quite different in appearance, with the pericentromeric region of the oryx X chromosomes being dullly fluorescent (G-C rich, and light staining). Another band difference involved oryx chromosome 21, which was a larger chromosome than its cattle equivalent. The size difference was the result of additional A-T rich DNA in the pericentromeric region of chromosome 21, which was seen in both oryx species. Also, the oryx chromosomes 9 and 14 were banded as those of the chamois. The gemsbok karyotype differed from that of the scimitar-horned oryx by an additional biarmed autosomal pair, and the X chromosomes showed subtle QFH-band differences.

Kobus ellipsiprymnus (waterbuck), Reduncinae

The karyotype of the waterbuck ($2n = 50$), with five biarmed autosomal pairs (Table

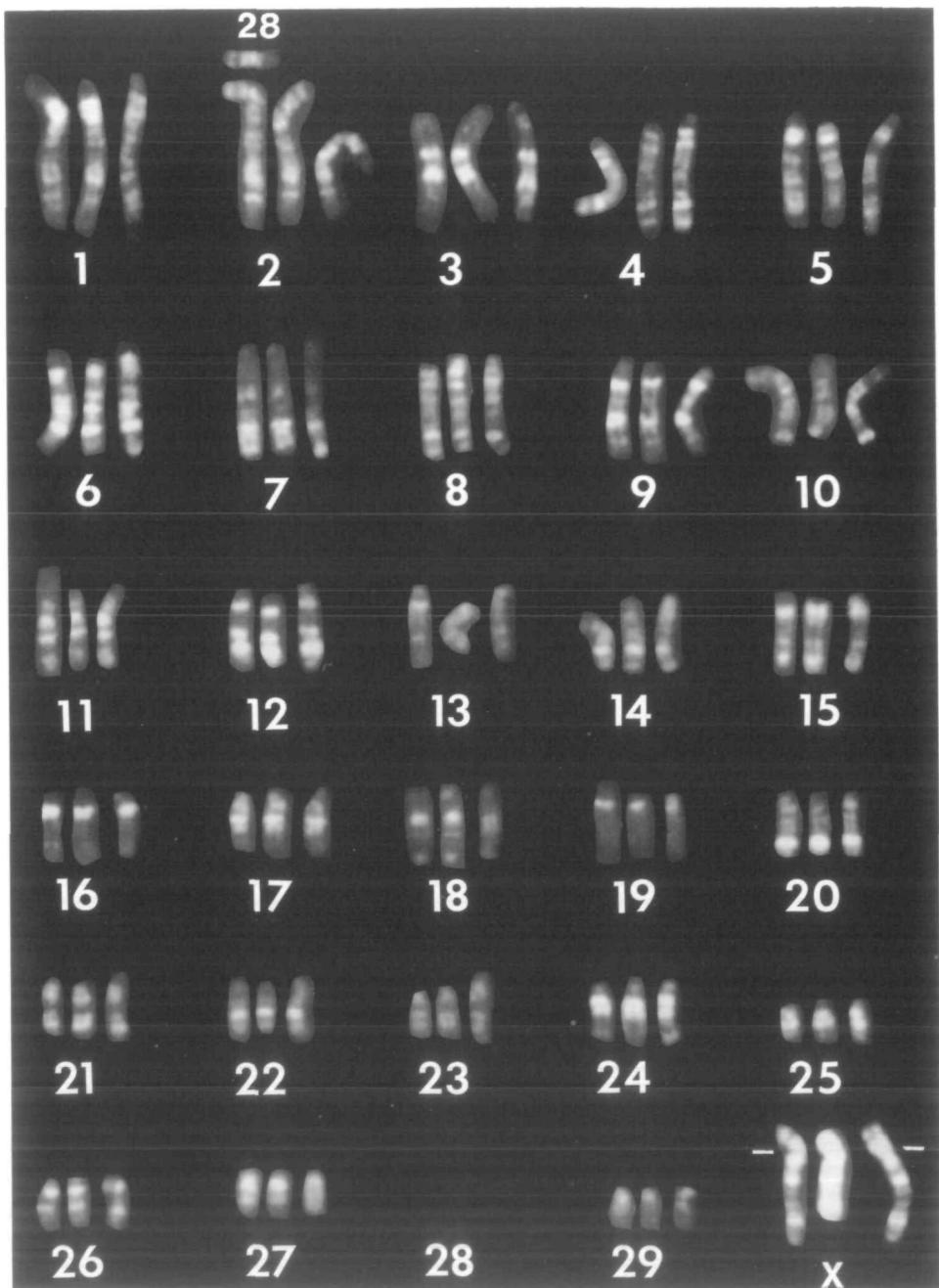


Figure 2. A female *Bos gaurus* (gaur) QFH-band karyotype ($2n = 58$) consisting of 54 acrocentric autosomes, two biarmed autosomes, and two biarmed X chromosomes, numbered according to the Reading Conference (1980) domestic cow standard. The gaur autosomal pairs are positioned above each chromosome number, and the cattle equivalent chromosomes are positioned to the right of each gaur pair (cattle 28 is inverted). The sex chromosomes arranged from left to right are the gaur early and late replicating X, followed by the cattle X. White lines mark the centromere position of the X chromosomes.

1), was comparable to an earlier cytogenetic report by Wurster and Benirschke (1968), although substantial intraspecific karyotypic variation has been reported (Ryder et al. 1990). Examination of chromosome comparisons between the waterbuck and cattle (Figure 8) revealed a great deal of QFH-band homology, although waterbuck 9 and 14 chromosomes were banded as those of the chamois. The QFH-band

patterns of cattle and waterbuck X chromosomes appeared quite different.

Damaliscus lunatus jimela (topi), Alcelaphinae

Chromosome comparisons between cattle and topi demonstrated extensive QFH-band homology (Figure 9; Table 1). However, the topi chromosome arms 3p and 4p, shown to be homologous to cattle

chromosomes 14 and 9, respectively, were banded like those of the chamois, and the topi X chromosome banded differently from that of cattle. The centromeric half of the topi X chromosome banded similarly to the centromeric one-third of the scimitar-horned and gemsbok oryx X chromosomes. We are unaware of a previously published karyotype for this species, but the $2n = 36$ for the topi is similar to the $2n = 38$ reported for *D. dorcus* (blesbok; Wurster and Benirschke 1968).

Antilope cervicapra (blackbuck), Antilopinae

The female blackbuck karyotype (Figure 10; $2n = 30$) consisted exclusively of biarmed autosomes. A portion of the X chromosome was autosomally derived (Effron et al. 1976) and homologous with cattle chromosome 5. The blackbuck X chromosome less the autosomal material was obviously larger than the cattle X. The short arm of the blackbuck X has been reported as consisting largely of constitutive heterochromatin (Effron et al. 1976), which would account for the size difference relative to the cattle X. Chromosome banding similarities between cattle and blackbuck demonstrated that cattle acrocentric autosomes were homologous with p and q arms of the blackbuck autosomes (Table 1). Cattle equivalents 9 and 14 (blackbuck 9q and 4p, respectively) banded as those of the chamois.

Gazella granti (Roosevelt's gazelle), Antilopinae

Female ($2n = 30$) and male ($2n = 31$) diploid numbers were the same as those reported by Effron et al. (1976). As seen for the blackbuck, cattle equivalent chromosome 5 comprised a portion of the gazelle X chromosome (Figures 11 and 12). The male karyotype exhibited what was believed to be an autosome to Y chromosome translocation (see Y1). The male individual was apparently heterozygous for a t(16;19), gazelle chromosome 8, and the autosomal material representing 8q (cattle 16) was translocated to Y1. The translocated autosomal material appeared to be rearranged. QFH-banding allowed us to detect homologous chromosomes in the Roosevelt's gazelle representing all cattle chromosomes except 3. It appeared that the homolog to cattle 3 had been disrupted in the gazelle karyotype, and was represented by gazelle 3p and the telomeric two-thirds of 2p. Cattle equivalent 9 and 14, gazelle 12q and 13q, respectively, banded as those of the chamois. The standard

karyotype of the blackbuck and Roosevelt's gazelle appeared quite similar, but on examination of the QFH-bands, we found many monobrachially homologous biarmed chromosomes (Table 1). Our findings differed from a report by Effron et al. (1976), which indicated that the two species shared five homologous biarmed autosomal pairs.

Discussion

Chromosome Banding and Nomenclature

We routinely use Hoechst 33258 to produce Q bands, because the contrast between negative and positive bands tends to be greater after BrdU incorporation during S1 than when using quinacrine (see Figure 1). BrdU incorporated during S1 quenched the early replicating bands, which improved band contrast between early (negative) and late replicating (positive) bands. We are confident that the tissue culture protocol used resulted in the incorporation of BrdU during S1, because the GBG bands produced were comparable in appearance to the GTG bands of the Reading Conference (1980) cattle standard (data not shown). This result would be expected if BrdU had been incorporated during S1.

It has been suggested to us that because BrdU was incorporated during early synthesis we should refer to the resulting bands as GBH-type rather than QFH-type (Leopoldo Iannuzzi, personal communication), but in our opinion the protocol that we followed resulted in an enhanced Q-band pattern. If a specific nomenclature must be used, the acronym QBH more precisely represents the band type.

Robertsonian Translocations

Since Wurster and Benirschke (1968) speculated that chromosomal variation in the Bovidae was primarily the result of centric fusions, no cytogenetic study of bovids has disputed their hypothesis. Buckland and Evans (1978) conducted the most complete cytogenetic survey of the Bovidae to date and found considerable monobrachial G-band homologies, but few biarmed chromosome homologies. They suggested that the most parsimonious explanation of their data was that bovid chromosomal evolution had proceeded from a karyotype of 58 acrocentric autosomes ($2n = 60$), and that centric fusion played an important role in reducing the ancestral diploid number to the range of values currently seen. Effron et al. (1976) and Bunch and Nadler (1980) noted many

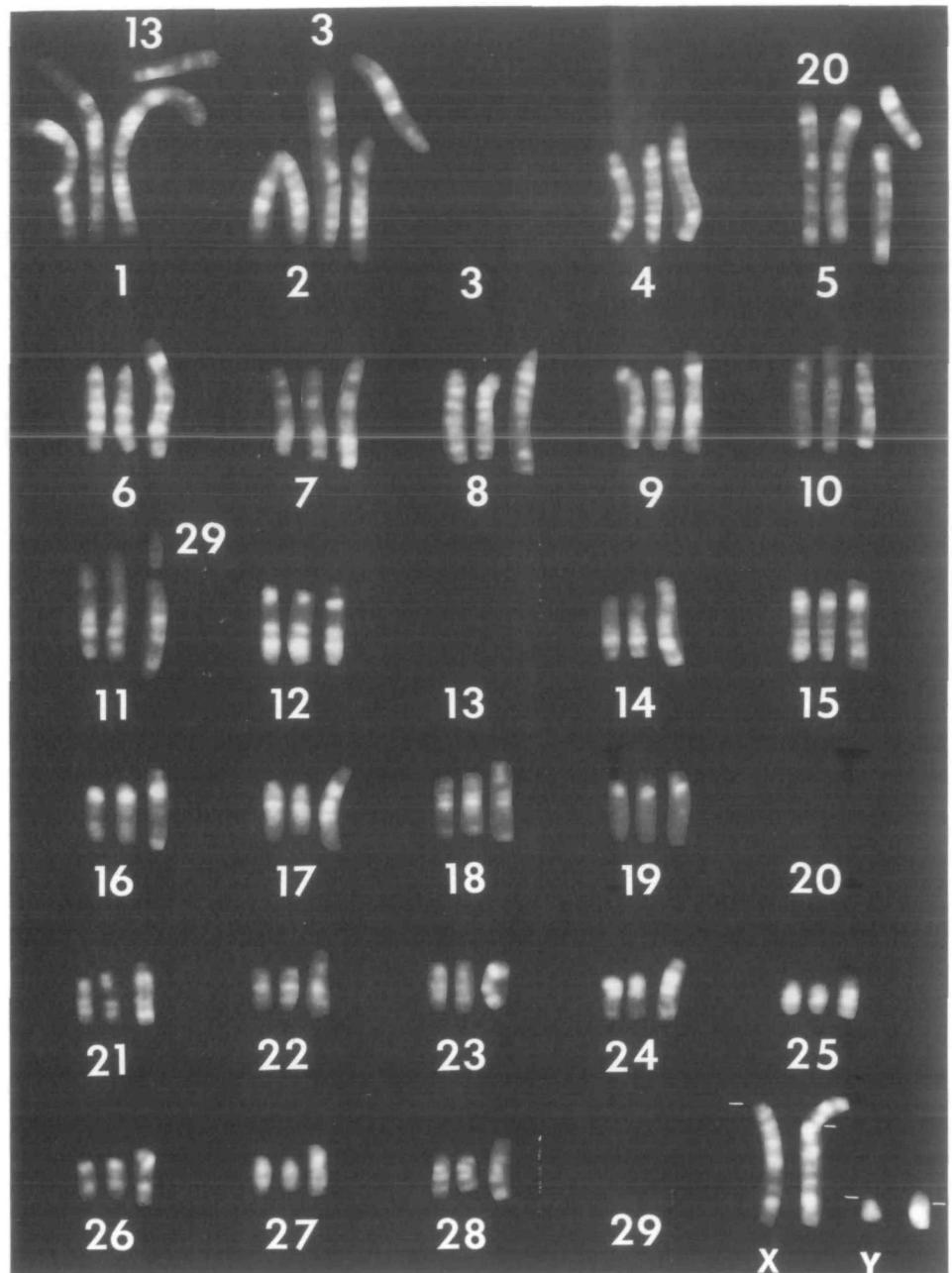


Figure 3. A male *Syncerus caffer caffer* (African buffalo) QFH-band karyotype ($2n = 52$) consisting of 42 acrocentric autosomes, eight biarmed autosomes, and an acrocentric X and Y, numbered according to the Reading Conference (1980) cattle standard. The African buffalo autosomal pairs are positioned above each chromosome number. The cattle equivalent chromosomes are positioned to the right of each African buffalo autosomal pair, with cattle chromosomes 3, 13, 20, and 29 inverted. The cattle biarmed X and Y chromosomes are positioned to the right of the buffalo acrocentric X and Y. White lines mark the centromere position of the sex chromosomes.

monobrachial homologies but few homologous biarmed chromosomes among species of the Antilopinae and Caprini, respectively. They concluded that their data supported the Wurster and Benirschke (1968) hypothesis of bovid chromosomal change. We also noted extensive monobrachial homologies and few homologous biarmed chromosomes (Table 1); these data support the Wurster and Benirschke (1968) hypothesis.

Buckland and Evans (1978) questioned the usefulness of Robertsonian translocations in predicting bovid phylogeny, because species might share Robertsonian chromosomes by convergence. They noted that the greater kudu and hartebeest shared two Robertsonian translocations, but they recognized these as homoplastic events, primarily because of banding differences involving cattle equivalent chromosomes 9 and 14 (bovine-type versus

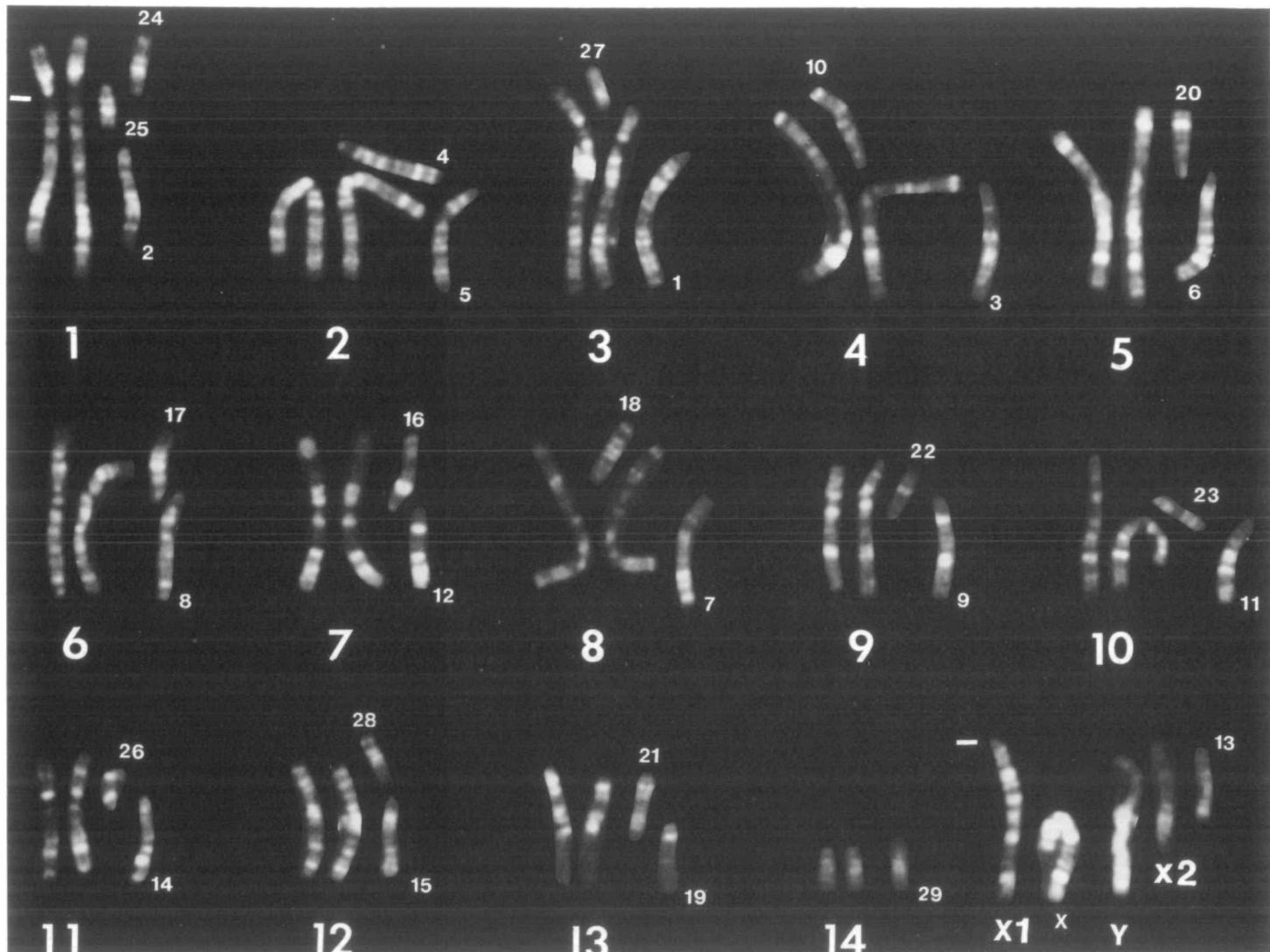


Figure 4. A male *Tragelaphus strepsiceros* (greater kudu) QFH-band karyotype ($2n = 31$) consisting of 26 biarmed autosomes, three acrocentric autosomes (one labeled as X2), an acrocentric X1, and a biarmed Y (the ancestral Y is fused to cattle equivalent chromosome 13). The autosomal pairs are arranged and numbered (large numbers) according to relative size. The domestic cow equivalent chromosomes are arranged to the right of the greater kudu autosomes and are numbered (small numbers are placed toward the telomeric ends of the greater kudu acrocentric chromosomes) according to the Reading Conference (1980) standard. The banding pattern of cattle chromosome 25 does not precisely match the region of kudu chromosome 1 to which we believe it is homologous, but this placement is the only way we found to account for cattle 25 within the kudu karyotype. The greater kudu sex chromosomes and equivalent cattle chromosomes are arranged from left to right as X1 (greater kudu ancestral X), cattle X, greater kudu Y, greater kudu X2, and cattle chromosome 13. White lines are positioned at the centromere of some chromosomes.

caprine-type). [We noted a similar situation between the greater kudu and topi (Table 1).] Thus, by evaluating all available chromosomal data, they resolved homoplasy. Effron et al. (1976) demonstrated that gazelle species share varying numbers of homologous biarmed chromosomes and that karyotypic data may be invaluable in unraveling Antilopinae phylogeny. Buckland and Evans (1978) showed that the greater kudu and eland shared homologous biarmed as well as monobrachially homologous biarmed chromosomes, and Bunch and Nadler (1980) demonstrated a comparable condition among genera of the Caprini. The t(1;3) in the chamois (Mayr et al. 1987) and sheep

(Bruere et al. 1976) indicates a phylogenetic affinity between *Ovis* and *Rupicapra*. The cytogenetic data suggest to us that Robertsonian chromosomes will be valuable as phylogenetic characters in resolving some bovid relationships. Only a more thorough cytogenetic treatment of the Bovidae with appropriate outgroup comparisons will determine how useful these data are in reconstructing bovid phylogeny.

Non-Robertsonian Change

In addition to centric fusions, centromere to telomere translocations have been documented in the Bovidae (Buckland and Evans 1978; Di Berardino and Iannuzzi

1981). We identified a tandem fusion in the greater kudu involving cattle equivalent chromosomes 2 and 25 believed to be the same as that identified earlier in the kudu and eland by Buckland and Evans (1978), and one between cattle equivalent chromosome 22 and a portion of chromosome 3 for Roosevelt's gazelle 2p (Figures 11 and 12).

Chromosome banding homologies are extensive among bovids, although differences have been seen. QFH-band differences are noted for cattle equivalent chromosomes 9 and 14, chromosomes 11 and 12 of Evans et al. (1973), respectively. Buckland and Evans (1978) suggested that the band difference for these two chro-

mosomes might be the result of a reciprocal translocation, referred to these chromosomes as being of the caprine- or bovine-type, and considered the bovine condition to be primitive based on out-group comparison with the giraffe. We noted that species of the Bovinae had bovine-type 9 and 14 and that representatives of the Reduncinae, Hippotraginae, Alcelaphinae, Antilopinae, and Caprinae had the caprine-type 9 and 14. Buckland and Evans (1978) suggested that this constant chromosomal difference marked a dichotomy within the Bovidae. Lowenstein (1986) also noted a dichotomy between species of the Bovinae and those of other Bovidae subfamilies based on immunological distances. Traditionally the Bovidae has been divided into the Boodontia and the Aegodontia based on tooth morphology (Gentry 1978; Vrba 1979), although the dichotomous arrangement of subfamilies differs markedly from that of Lowenstein (1986). Interestingly, recent cytogenetic comparisons of cattle, goat, and sheep chromosomes did not reveal a chromosome 14 band difference (Hayes et al. 1991; ISCNDA 1989), and band differences noted for chromosome 9 are reported as having resulted from a paracentric inversion (Hayes et al. 1991).

Another noteworthy chromosomal difference involves chromosome 21 of the Oryx, which is slightly larger than the homologous chromosomes in the other bovids. This fact was first noted by Buckland and Evans (1978), who proposed that its larger size was the result of a translocation from the small autosome fused to cattle equivalent 1. The larger size of oryx 21 appeared to us to be the result of additional chromatin in the pericentromeric region. We did not note a reduced size for the small autosome translocated to cattle equivalent 1 and are uncertain of the origin of the additional chromatin.

Karyotypes of a male and female Roosevelt's gazelle revealed that cattle equivalent chromosome 3 was not present intact, but rather seemed to have been broken and translocated to other chromosomes. This is believed to be the first documentation of the disruption of cattle equivalent chromosome 3 within a bovid karyotype.

Chromosome band differences indicative of intrachromosomal arm rearrangements appeared more common for the sex chromosomes than for the autosomes. Variation in the placement of the centromere (e.g., Cape buffalo relative to cattle X) suggested rearrangement of the bovid X chromosome by pericentric inversion.

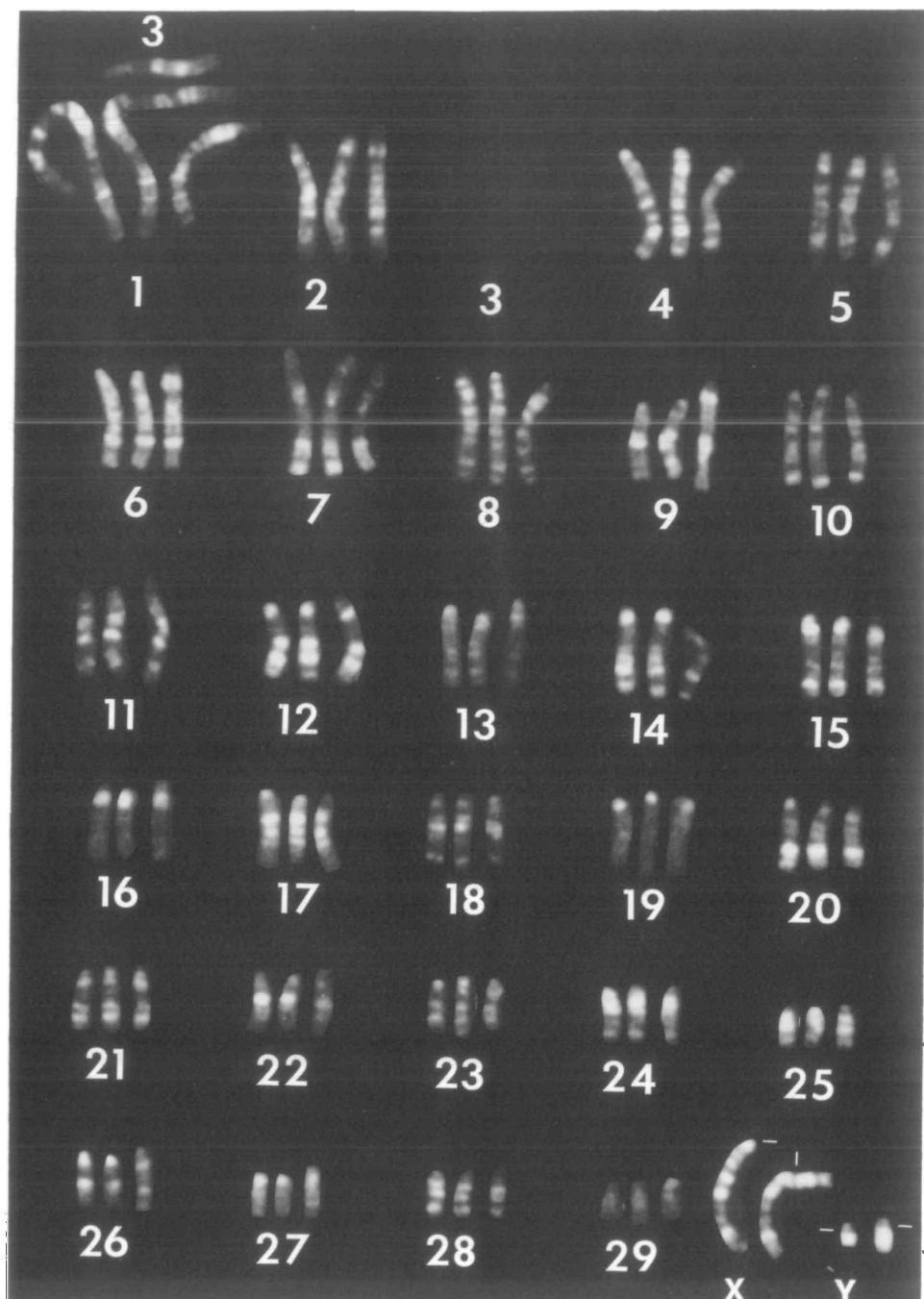


Figure 5. A male QFH-band *Rupicapra rupicapra* (chamois) karyotype ($2n = 58$) consisting of 54 acrocentric autosomes, two biarmed autosomes, an acrocentric X, and a biarmed Y, numbered according to the Reading Conference (1980) cattle standard. The chamois autosomal pairs are positioned above the chromosome number, and the equivalent cattle autosomes are positioned to the right or above each chamois pair. Cattle chromosome 3 is inverted. Cattle biarmed X and Y are positioned to the right of the chamois acrocentric X and biarmed Y, respectively. White lines mark the centromere position of the sex chromosomes. The centromeric bright band of cattle chromosome 9 is missing in the equivalent chamois chromosome, and the centromeric bright band of chamois chromosome 14 is missing in the equivalent cattle chromosome.

There were also obvious size differences for the X chromosome, which indicated the addition of chromatin (e.g., blackbuck and oryx species). Several gazelle species have been reported to have short heterochromatic arms (Effron et al. 1976). The X chromosome pericentromeric region for the scimitar-horned and gemsbok oryx

(Hippotraginae) and topi (Alcelaphinae) was mostly negative staining with a prominent positive band, and generally the X chromosome of these three species appeared quite different from that of the other bovids studied. Buckland and Evans (1978) presented a GTG-band karyotype of the gemsbok oryx that also showed the

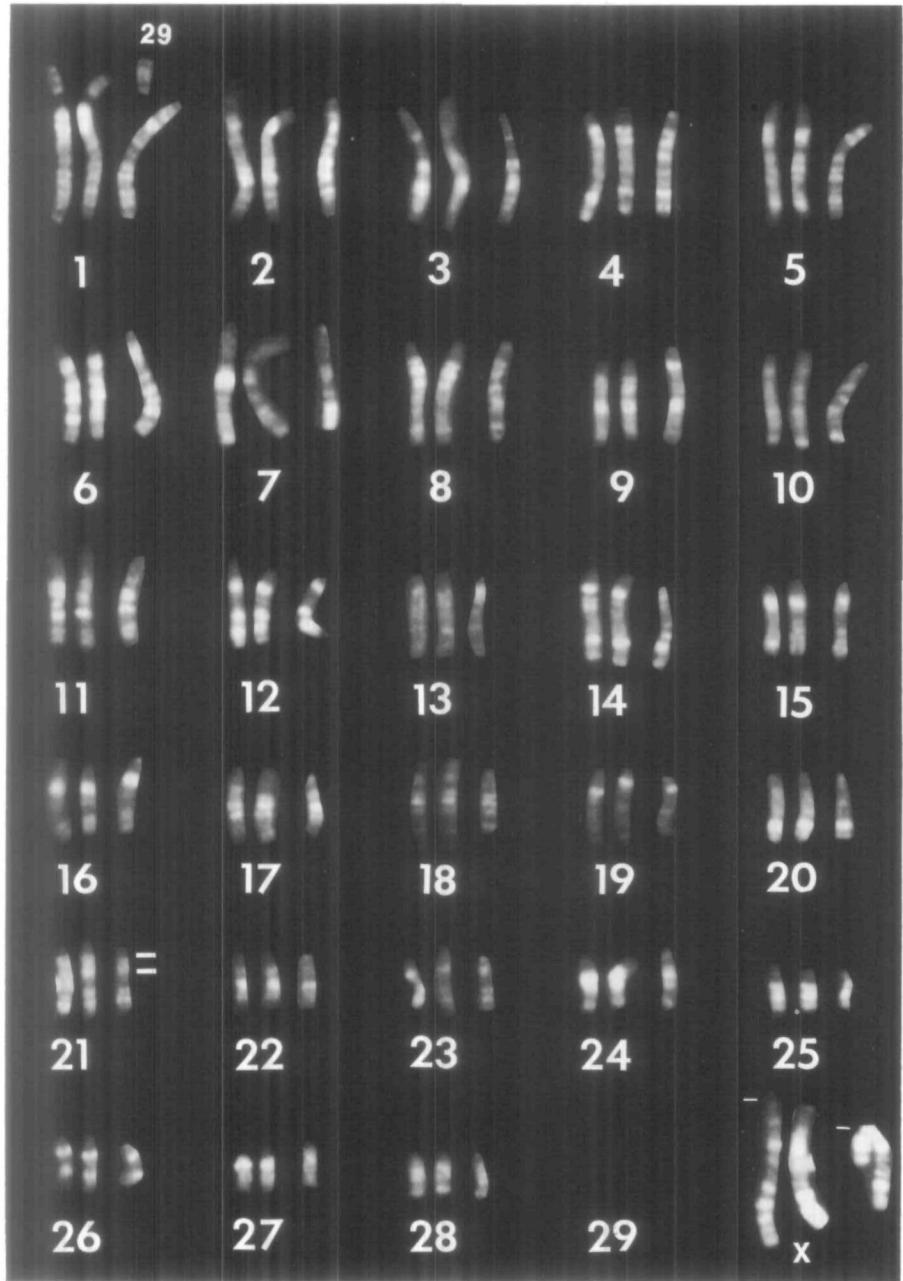


Figure 6. A female QFH-band *Oryx tao* (scimitar-horned oryx) karyotype ($2n = 58$) consisting of 54 acrocentric autosomes, two biarmed autosomes, and two acrocentric X chromosomes, numbered according to the Reading Conference (1980) cattle standard. The equivalent cattle chromosomes are positioned to the right of the scimitar-horned oryx chromosome pair. Cattle 29 is inverted. White lines mark the centromere position of the X chromosomes. Parallel white lines mark the bright pericentromeric region of cattle chromosome 21 that is enlarged in the equivalent oryx autosome. Scimitar-horned oryx 9 and 14 are banded as those of the chamois (Figure 5).

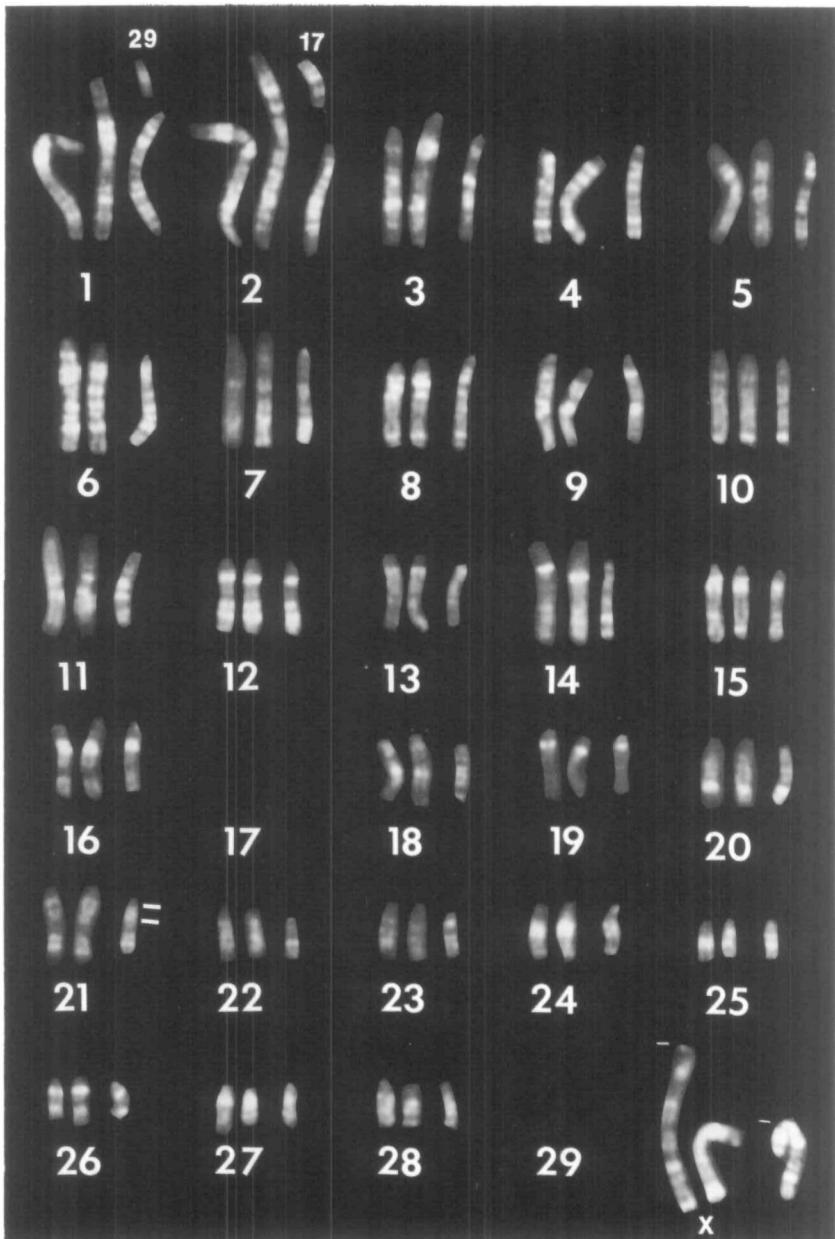


Figure 7. A female QFH-band *Oryx gazella* (gemsbok) karyotype ($2n = 56$) consisting of 50 acrocentric autosomes, four biarmed autosomes, and two acrocentric X chromosomes, numbered according to the Reading Conference (1980) cattle standard. The equivalent cattle chromosomes are positioned to the right of each gemsbok chromosome pair. Cattle chromosomes 17 and 29 are inverted. White lines mark the centromere position of the X chromosomes. Parallel white lines mark the bright pericentromeric region of cattle chromosome 21 that is enlarged in the equivalent gemsbok autosome. Gemsbok 9 and 14 are banded as those of the chamois (Figure 5).

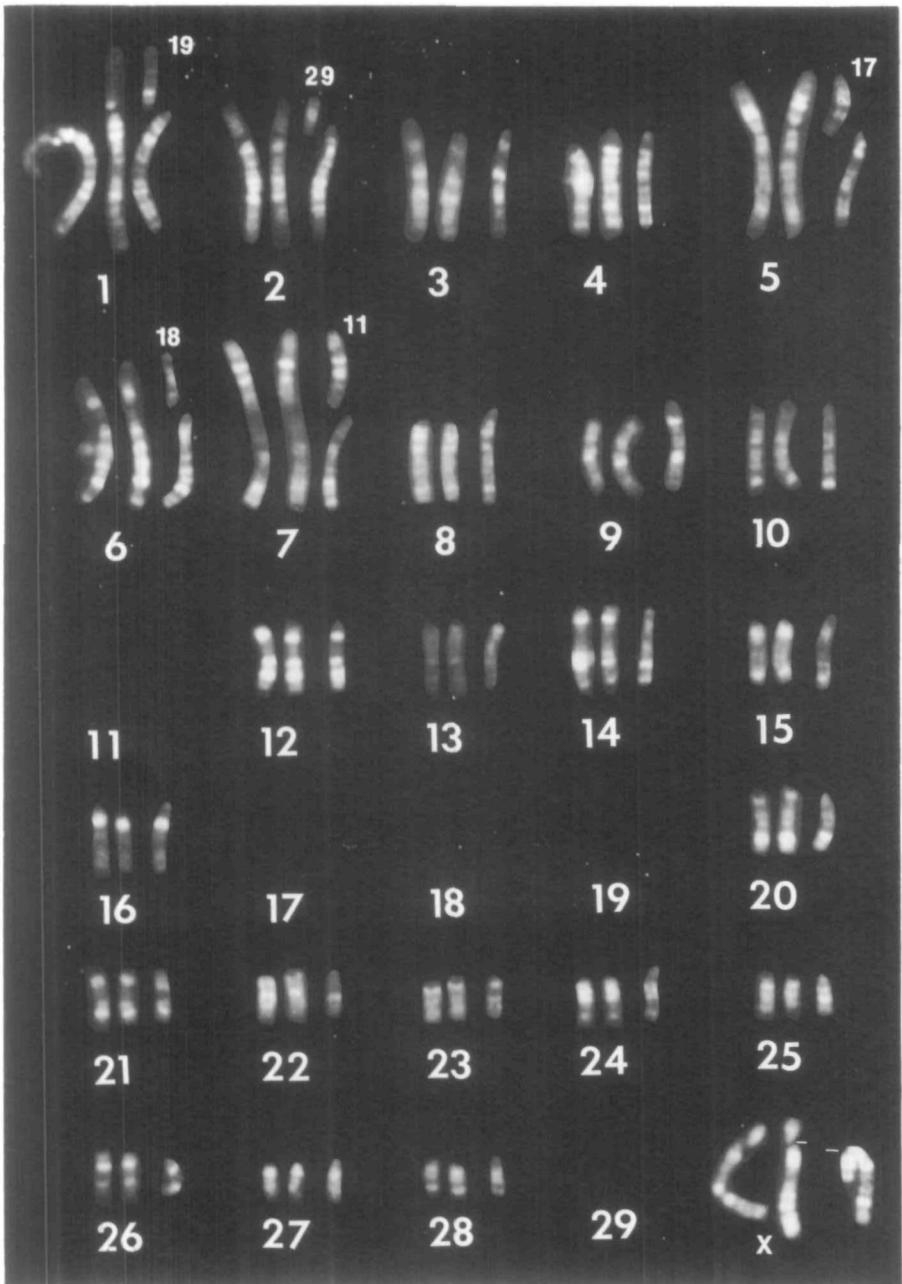


Figure 8. A female QFH-band *Kobus ellipsiprymnus* (waterbuck) karyotype ($2n = 50$) consisting of 38 acrocentric autosomes, 10 biarmed autosomes, and two biarmed X chromosomes, numbered according to the Reading Conference (1980) cattle standard. The equivalent cattle chromosomes are positioned to the right of each waterbuck chromosome pair. Cattle chromosomes 11, 17, 18, 19, and 29 are inverted. White lines mark the centromere position of the X chromosomes. Waterbuck 9 and 14 are banded as those of the chamois (Figure 5).

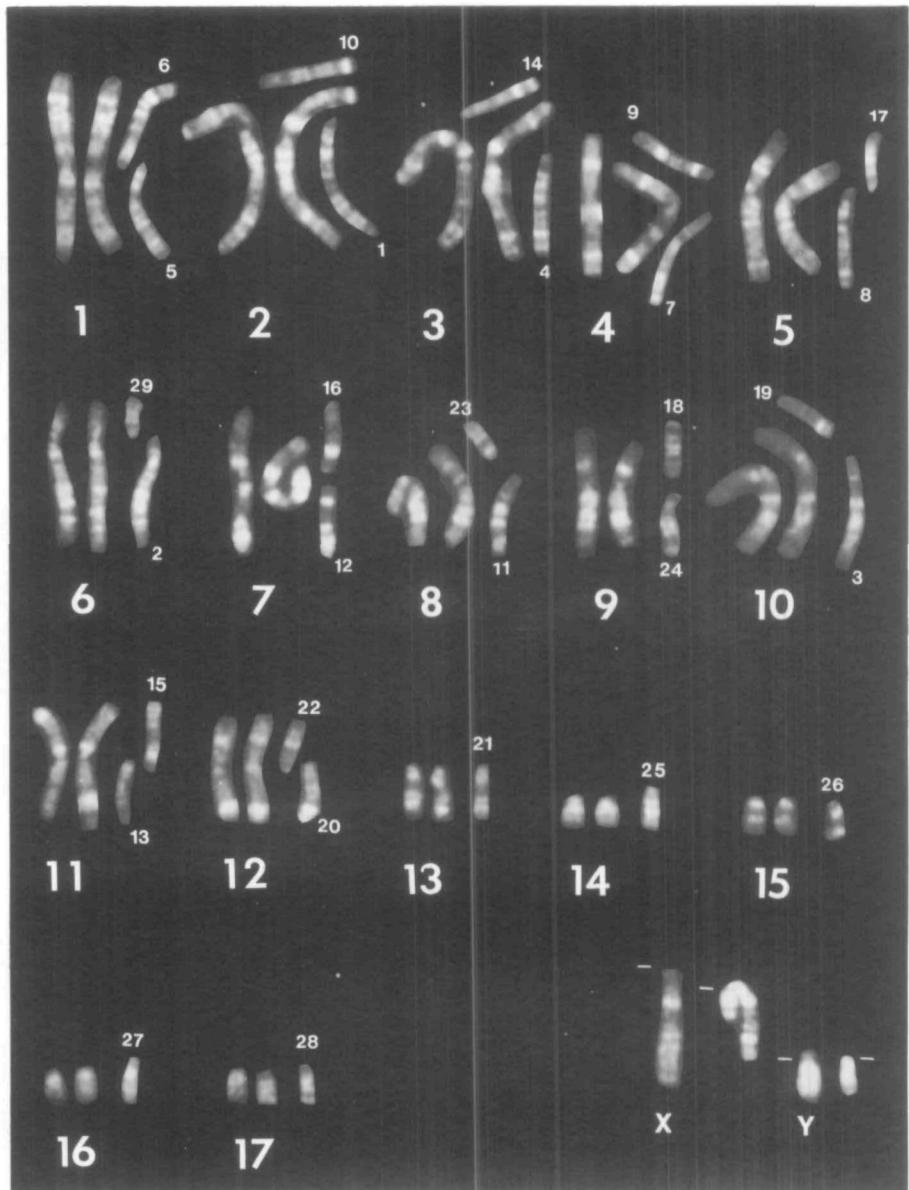


Figure 9. A male QFH-band *Damaliscus lunatus jimela* (topi) karyotype ($2n = 36$) consisting of 10 acrocentric autosomes, 24 biarmed autosomes, an acrocentric X, and an acrocentric Y. The topi autosomes are arranged by arm number (biarmed before acrocentric autosomes) and relative size (by inspection). The topi autosomal pairs are positioned above the large numbers. The equivalent cattle autosomes are positioned next to the corresponding topi pair, and cattle autosomes are inverted when associated with the p arm of a topi biarmed autosome. Each cattle autosome is numbered (small numbers) according to the Reading Conference (1980) standard. The sex chromosomes arranged from left to right are topi X, cattle X, topi Y, and cattle Y. White lines mark the centromere position of the sex chromosomes. The p arms of topi autosomes 3 and 4, cattle 14 and 9, respectively, are banded as those of the chamois (Figure 5).

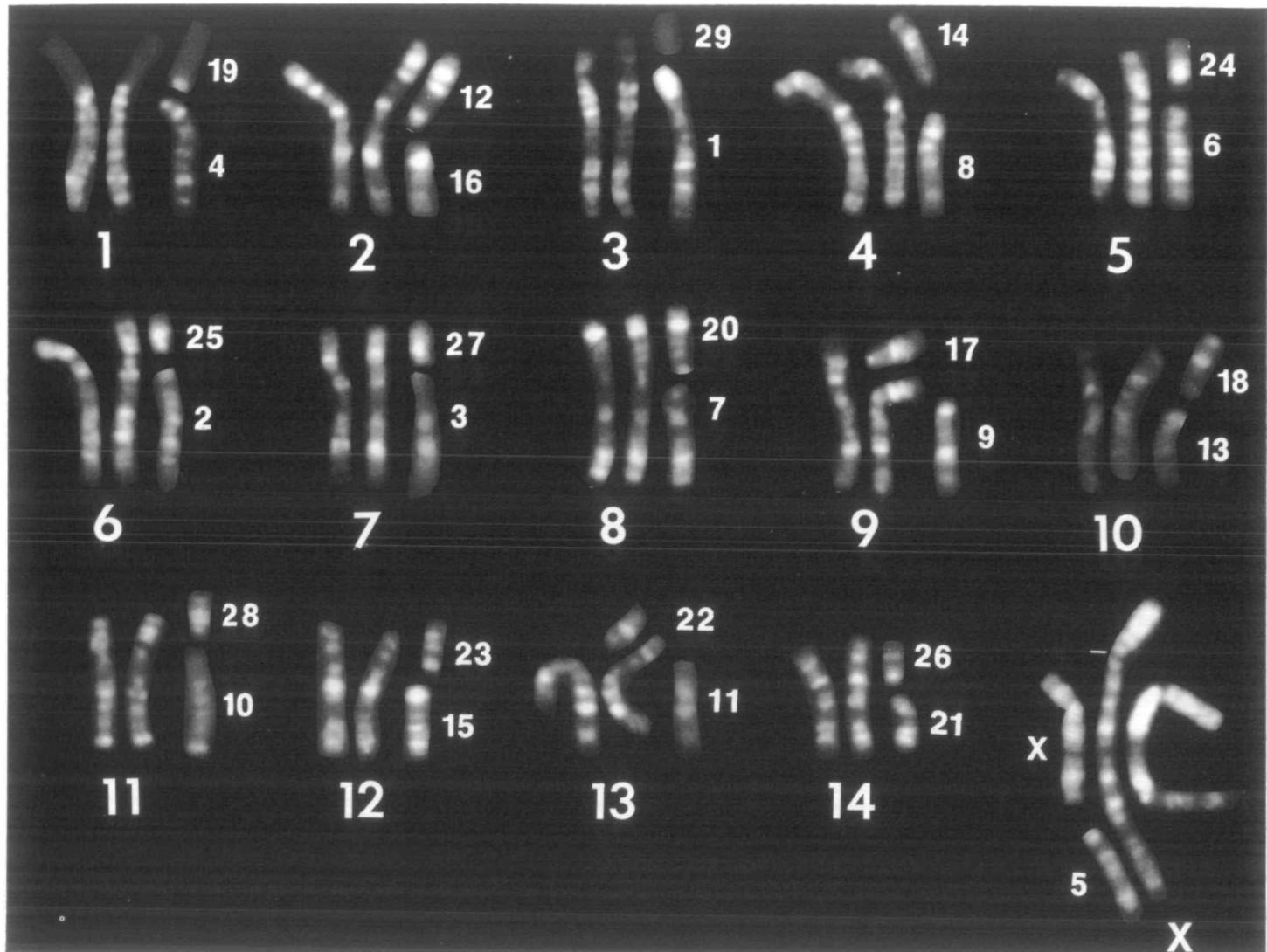


Figure 10. A female *Antilope cervicapra* (blackbuck) QFH-band karyotype ($2n = 30$) consisting of 28 biarmed autosomes and two biarmed X chromosomes. The blackbuck autosomal pairs are arranged and numbered (large numbers) according to relative size (estimated). The equivalent cattle acrocentric autosomes forming the arms of the blackbuck biarmed autosomes are positioned to the right or above each pair, and are numbered according to the Reading Conference (1980) standard (small numbers). Cattle autosomes associated with p arms are inverted. Cattle X and 5 are positioned to the left of the blackbuck early replicating X, and white lines mark the centromere position of X chromosomes. The p arm of blackbuck 4 (cattle 14) and the q arm of blackbuck 9 (cattle 9) are banded as chamois 14 and 9 (Figure 5).

pericentromeric region of the X chromosome as negative staining. The similarity in X chromosome appearance for representatives of the Hippotraginae and Alcelaphinae suggested a phyletic closeness for these bovid subfamilies. Although X chromosome band differences are extensive, the region of the X chromosome corresponding to cattle Xq tends to be conserved (e.g., Cape buffalo, gaur, greater kudu, chamois, blackbuck, Roosevelt's gazelle). Chromosome size and centromere position differences were also noted for the Y chromosome (e.g., relatively large size of the greater kudu Y chromosome; acrocentric indicus Y chromosome and submetacentric taurus Y).

In addition to size and intrachromosomal-

al rearrangements, autosome to sex chromosome translocations were noted. The blackbuck and Roosevelt's gazelle, as well as other *Gazella*, share an autosome (cattle equivalent 5) to X translocation. Efron et al. (1976) suggested that this shared chromosomal condition indicated that *Antilope* and *Gazella* are congeneric. Also, male Roosevelt's gazelle that we karyotyped had what is believed to be an autosome (cattle equivalent 16) to Y chromosome translocation. Greater kudu also had an autosome to Y translocation [t(Y; 13); Buckland and Evans 1978], and cattle equivalent 13 of the greater kudu (X2) had additional chromatin in the pericentromeric region. In situ hybridization experiments with cattle male-specific probes are

planned to establish if the additional chromatin was derived from the greater kudu ancestral Y.

The non-Robertsonian chromosomal differences that we and others have reported for bovids are not extensive. This is especially obvious for the autosomes. Since bovids are chromosomally conservative, noted differences will prove valuable in assessing Bovidae taxonomy, because variability will not be so great that chromosomal homologies cannot be established.

Chromosomes and Speciation

On examination of available bovid cytogenetic data, it is probable that the ancestral diploid number for the Bovidae was

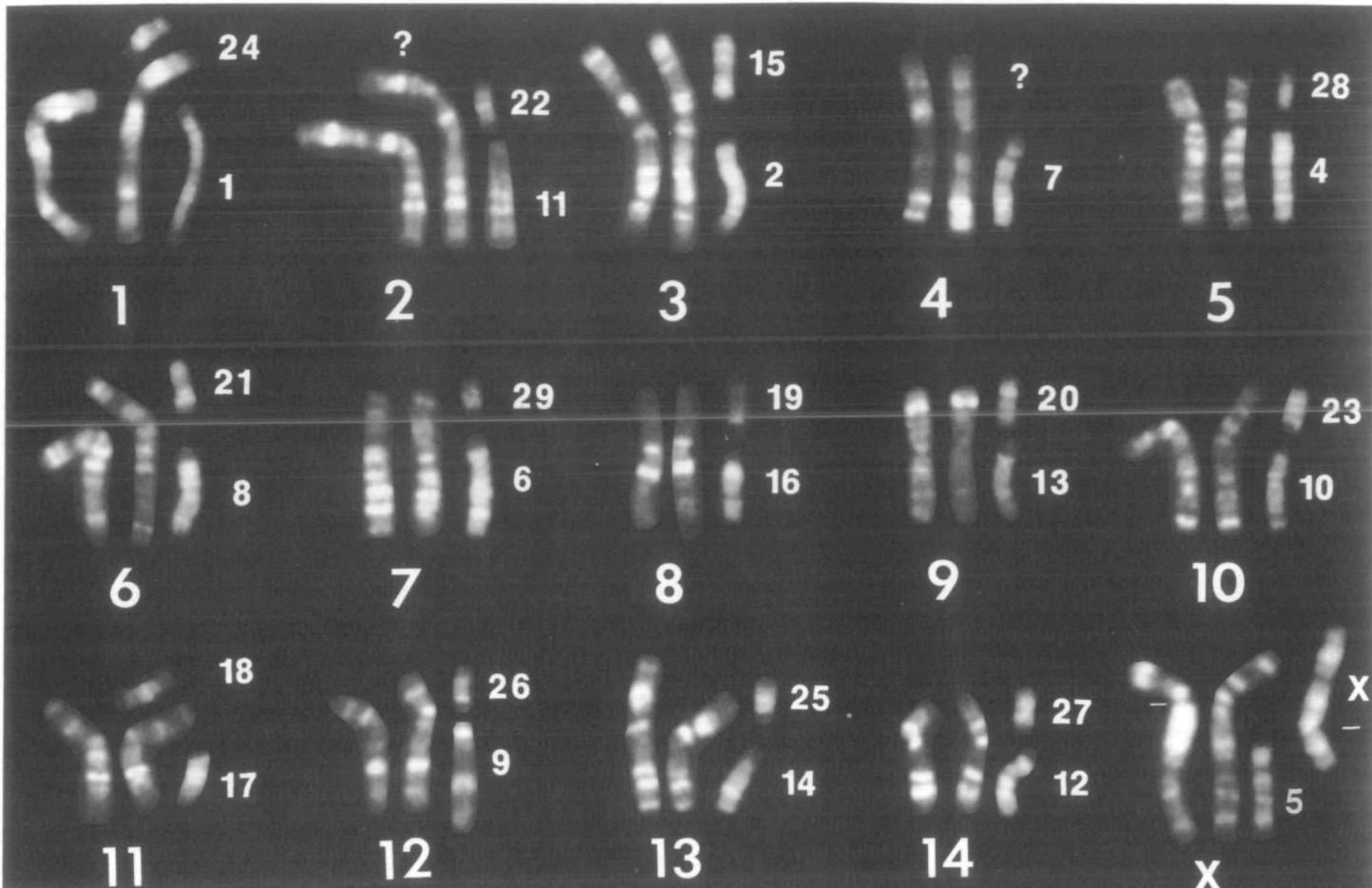


Figure 11. A female *Gazella granti* (Roosevelt's gazelle) QFH-band karyotype ($2n = 30$) consisting of 28 biarmed autosomes and two biaxed X chromosomes. Roosevelt's gazelle autosomal pairs are arranged and numbered according to relative size (estimated). Equivalent cattle acrocentric autosomes are positioned to the right or above the blackbuck autosomal arm to which they correspond, and are numbered (small numbers) according to the Reading Conference (1980) standard. Domestic cow X and 5 are positioned to the right of the Roosevelt's gazelle early replicating X, and white lines mark the centromere position of the X chromosomes. The q arms of gazelle chromosomes 12 and 13 (cattle 9 and 14) are banded as chamois 9 and 14 (Figure 5). Question marks are positioned next to autosomal material believed to have been derived from cattle equivalent chromosome 3.

60 with 58 acrocentric autosomes. Species representing different subfamilies have been shown to possess this basic karyotype, which means that it is common and that speciation within the Bovidae has taken place in some instances without a correlated change in the diploid number. In other instances, such as among the Antilopinae, diploid number is quite variable, resulting from Robertsonian translocations and, in some instances, from tandem fusions and autosome to sex chromosome translocations (Koulischer et al. 1972).

The role of chromosomes in speciation is controversial, and, as indicated above, bovid speciation has taken place in some instances without gross karyotypic changes. But when centric fusions have resulted in monobrachial homologies, especially among taxonomically close species, it is tempting to consider the Baker and Bickham (1986) model of speciation by monobrachial centric fusion. In this mod-

el, the authors proposed that different populations of the same species fixed for monobrachially homologous biarmed chromosomes would immediately be reproductively isolated as a result of meiotic problems. Centric fusion is known to be common among the Bovidae, and numerous monobrachially homologous biarmed chromosomes are found among closely related species. This is obviously true for the Antilopinae (Effron et al. 1976), Tragelaphini (Buckland and Evans 1978), and Caprini (Bunch and Nadler 1980). But, because most bovid cytogenetic data are derived from captive animals (Benirschke and Kumamoto 1987), the population dynamics of the Baker and Bickham model remain untested for the Bovidae.

Conclusions

This cytogenetic treatment was conducted to evaluate the extent of chromosome conservation in the Bovidae by evaluating

species representing several subfamilies, and our findings confirmed earlier reports that chromosome band homologies are extensive. We did not attempt a phyletic reconstruction, because too few species were analyzed to make such a treatment meaningful. It is evident that a more extensive cytogenetic survey of the Bovidae and related families will shed further light on bovid phylogeny and will allow an evaluation of bovid taxonomy.

References

- Baker RJ and Bickham JW, 1986. Speciation by monobrachial centric fusions. Proc Natl Acad Sci USA 83: 8245-8248.
- Basrur PK and Gilman JPW, 1964. Blood culture method for the study of bovine chromosomes. Nature 204: 1335-1337.
- Benirschke K and Kumamoto AT, 1987. Challenges of artiodactyl cytogenetics. Kromosomo 2(45):1468-1478.
- Bruere AN, Chapman HM, Jaine PM, and Morris RM, 1976. Origin and significance of centric fusions in domestic sheep. J Hered 67:149-154.

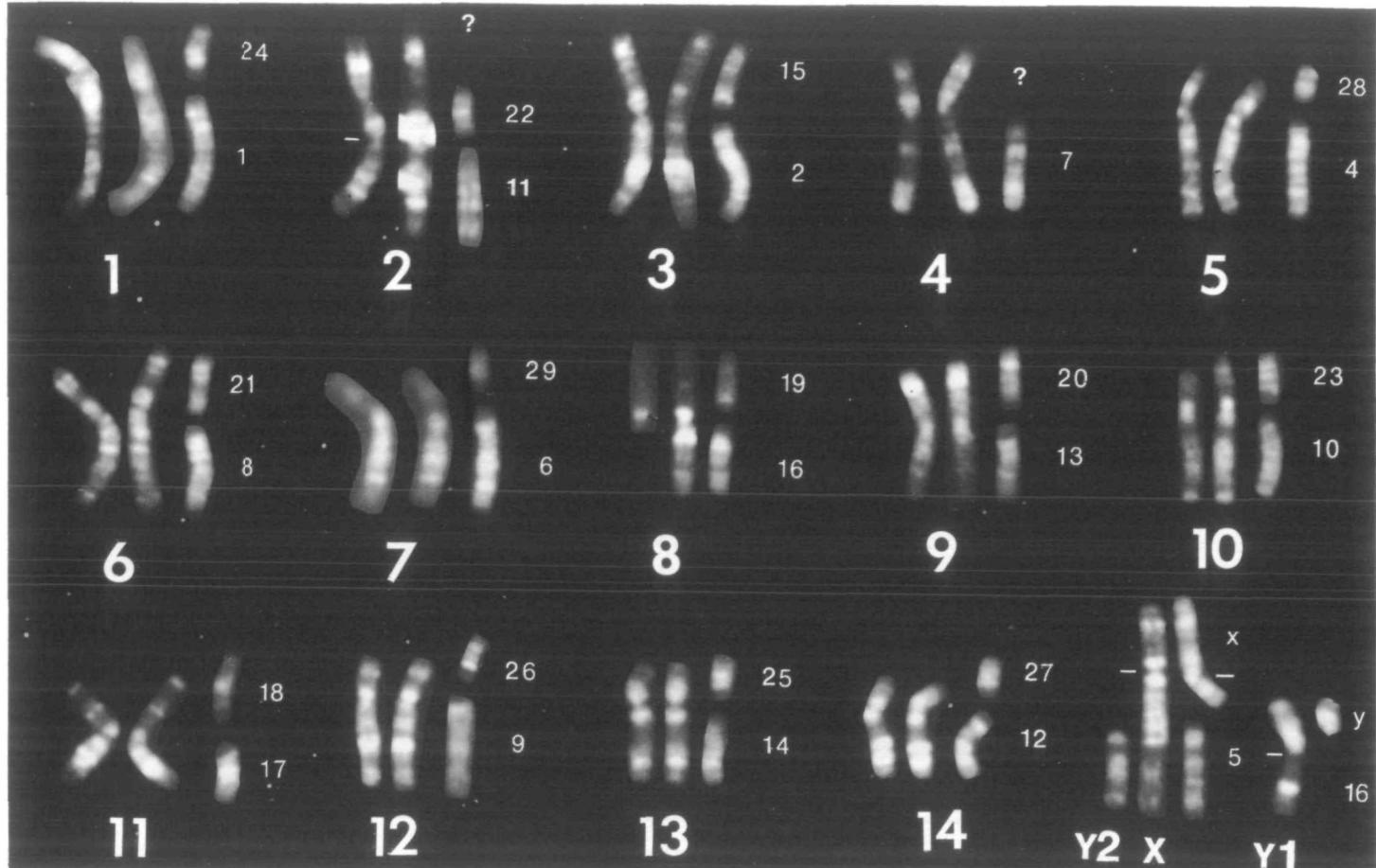


Figure 12. A male *Gazella granti* (Roosevelt's gazelle) QFH-band karyotype ($2n = 31$) consisting of 27 biarmed autosomes, two acrocentric autosomes (one labeled as Y2), a biarmed X, and a biarmed Y1. Equivalent domestic cow acrocentric autosomes are positioned to the right of the gazelle autosomal arms to which they correspond and are numbered according to the Reading Conference (1980) standard. Cattle X and 5 are positioned to the right of gazelle X, and cattle Y is positioned to the right of the p arm of gazelle Y. The number 16 positioned next to the q arm of gazelle Y marks that portion of gazelle Y that is believed to be autosomal material equivalent to cattle 16. The q arms of gazelle chromosomes 12 and 13 (cattle 9 and 14) are banded as chamois 9 and 14 (Figure 5). Question marks are positioned next to autosomal material believed to have been derived from cattle chromosome 3.

Buckland RA and Evans HJ, 1978. Cytogenetic aspects of phylogeny in the Bovidae, G-banding. *Cytogenet Cell Genet* 32:64–71.

Bunch TD and Nadler CF, 1980. Giemsa-band patterns of the tahr and chromosomal evolution of the tribe Caprini. *J Hered* 71:110–116.

Di Berardino D and Iannuzzi L, 1981. Chromosome banding homologies in swamp and murrah buffalo. *J Hered* 72:183–188.

Efron M, Bogart MH, Kumamoto AT, and Benirschke K, 1976. Chromosome studies in the mammalian subfamily Antilopinae. *Genetica* 46:419–444.

Evans HJ, Buckland RA, and Sumner AT, 1973. Chromosome homology of heterochromatin in goat, sheep and ox studied by banding techniques. *Chromosoma* 42:383–402.

Gentry AW, 1978. Bovidae. In: *Evolution of African mammals* (Maglio VJ and Cooke HBS, eds). Cambridge: Harvard University Press; 540–572.

Hayes H, Petit E, and Dutrillaux B, 1991. Comparisons of RBG-banded karyotypes of cattle, sheep, and goats. *Cytogenet Cell Genet* 57:51–55.

Hediger R, 1988. Die in situ Hybridisierung zur Gen-

kartierung beim Rind und Schaf (PhD dissertation). Zürich: Eidgenössischen Technischen Hochschule.

ISCNDA, 1989. International system for cytogenetic nomenclature of domestic animals. *Cytogenet Cell Genet* 53:65–79.

Koujischer L, Tijskens J, and Mortelmans J, 1972. Chromosomes and speciation in the superfamily Bovoidea. *Genen Phaenen* 15(2–3):65–72.

Lowenstein JM, 1986. Bovid relations based on serum immunology. *Suid-Afrikaanse Tydskrif vir Wetenskap* 82:77–78.

Märki U and Robinson TJ, 1984. Y chromosome dimorphism in Afrikaner bulls. Proceedings of the 6th European Colloquium on Cytogenetics Domestic Animals. Zurich: Institute for Animal Production; 87–95.

Mayr B, Tesarik E, Auer H, and Burger H, 1987. Nucleolus-organizer regions and heterochromatin in three species of Bovidae. *Genetica* 75:207–212.

Reading Conference, 1980. Proceedings of the first international conference for the standardization of banded karyotypes of domestic animals. *Hereditas* 92:145–162.

Ryder OA, Kumamoto AT, Aman RA, Kat P, and Jonyo

J, 1990. A genetic puzzle: chromosomes of the waterbuck *Kobus ellipsiprymnus* in Lake Nakuru National Park, Kenya. *Zoological Society of San Diego, Zoonooz* 63(9): 12–13.

Schweizer D, 1981. Counterstain-enhanced chromosome banding. *Human Genet* 57:1–14.

Todd NB, 1975. Chromosomal mechanisms in the evolution of artiodactyls. *Paleobiology* 1:175–188.

Vaughan TA, 1986. *Mammalogy*. New York: CBS College Publishing; 210 p.

Vrba ES, 1979. The significance of bovid remains as indicators of environment and predation patterns. In: *Fossils in the making* (Behrensmeyer AK and Hill AP, eds). Chicago: University of Chicago Press; 247–271.

Womack JE, 1990. Gene mapping in the cow. In: *Domestic animal cytogenetics: advances in veterinary sciences and comparative medicine*, vol. 34 (McFeely RA, ed). Boston: Academic Press; 251–271.

Wurster DH, 1972. Sex chromosome translocations and karyotypes in bovid tribes. *Cytogenetics* 11:197–207.

Wurster DH and Benirschke K, 1968. Chromosome studies in the superfamily Bovoidea. *Chromosoma* 25: 152–171.