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COMPARATIVE STUDY OF ELONGATED CHROMOSOMES  
IN SHEEP AND GOATS AND A PROPOSED STANDARD

by

Stephen H. Mensher

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal, Dairy and Veterinary Sciences

Approved:

UTAH STATE UNIVERSITY  
Logan, Utah

1987

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The attainment of this graduate degree would not have been possible without the complete confidence and encouragement given me by my father, Morton J. Mensher, to whom I send my love and appreciation.

This thesis is dedicated to the loving memory of my mother, Harriet B. Mensher.

Stephen H. Mensher

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## ABSTRACT

Comparative Study of Elongated Chromosomes  
in Sheep and Goats and a Proposed Standard

by

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Utah State University, 1987

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Department: Animal, Dairy and Veterinary Sciences

A cell synchronization technique was used that consistently yielded numerous elongated chromosomes, which are necessary for producing high-resolution G-banding. The prometaphase stage of contraction was determined to have the greatest utility for detailed comparative analysis and, therefore, was used in the preparation of the proposed standard high-resolution karyotypes and idiogram.

A proposed standard high-resolution G-band karyotype of the domestic sheep and goat is presented, along with a high-resolution G-band idiogram of the goat. The karyotypes presented contain considerably more bands than contemporary karyotypes and provide much greater resolution for linear differentiation. The X chromosome of the sheep is acrocentric, rather than having a minute p arm as recently reported.

A side-by-side comparison of high-resolution G-band sheep and goat chromosomes indicates identical equivalence of banding patterns. Of special note is the homology of banding patterns for the six acrocentric autosomes of the goat and the respective three

submetacentric autosomes of the sheep, reinforcing the evidence that the goat is representative of the ancestral karyotype.

C-bands and NORs of the sheep and goat are also presented. When C-banded, the centromeric regions of the sheep and goat acrocentric autosomes were found to be densely stained. The centromeric regions of the X and Y, however, were not stained differently from the chromosome arms. In the sheep submetacentrics, chromosomes 1 and 3 showed weak C-bands while chromosome 2 showed stronger bands.

In the sheep and goat, 10 NORs located terminally on five chromosomes pairs were found. Sheep NORs occurred on the three pairs of submetacentric autosomes and on one large and one small pair of acrocentric autosomes. Goat NORs occurred on four large and one small pair of acrocentric autosomes. Since the banding patterns obtained when staining for NORs do not permit individual identification of each chromosome, duplicate G-banding and silver staining of elongated chromosomes of both sheep and goat would need to be conducted to identify specific NOR-bearing chromosomes.

(56 pages)

## INTRODUCTION

The significance of the regular transmission of chromosomes and the inheritance of biological characters led to the formation of the chromosome theory of inheritance and the founding of cytogenetics at the beginning of the 20th century. However, it was not until the mid-1950s that its potential was first demonstrated for animals. Animal cytogenetics, for the most part, had to await either the application or development of three basic innovations.

The first of these innovations was the addition of colchicine, a plant alkaloid, to growing tissue prior to fixation. When a cell enters mitosis under the influence of colchicine, the spindle fails to form, and the cell, therefore, is temporarily arrested at metaphase. Thus, colchicine treatment increases the number of mitotic cells available for observation.

The second innovation involved the use of a hypotonic pretreatment for chromosome preparations. When a cell is subjected to a hypotonic solution, some of the fluid flows through its semi-permeable membrane from the dilute solution outside the cell to the concentrated solution inside the cell, tending to equalize the concentrations. This movement of fluid into a cell spreads the chromosomes, thereby enabling them to be observed individually.

Cytogeneticists must be able to make detailed analysis of chromosomes and segments of chromosomes. The third innovation was the establishment of methods that linearly differentiated nonhomologous chromosomes. This gave rise to a number of different banding techniques. One such technique is G-banding, so called



because the bands are produced by staining with Giemsa stain, which is preceded by a variety of alternative pretreatments such as with the proteolytic enzyme trypsin.

The development of the G-banding technique showed that each chromosome has a different and reproducible ultrastructure. Giemsa stains untreated chromosomes more or less uniformly, so the generation of G-bands is dependent upon treatment of the preparations after fixation to remove some component of the chromosome. The production of G-bands, therefore, lies in some variation of the DNA-protein (non-histone) interaction between large blocks of the chromosome which may be coordinately organized in some way as yet unknown, possibly as units of replicon organization or of transcription.

Chromatin refers to the interphase form of a eukaryote cell's nuclear genetic material, of which there are two major types: euchromatin, which is genetically active and becomes condensed during cell division and diffused during interphase; and heterochromatin, which is genetically inactive and remains condensed all the time. Heterochromatin is subclassified into two types: facultative and constitutive.

Facultative heterochromatin represents inactivated and condensed segments of euchromatin, an example of which is the Barr body or inactivated X chromosome. The process by which euchromatin becomes inactivated into facultative heterochromatin is one-directional, and once in this state, facultative heterochromatin remains as such.

Constitutive heterochromatin contains most of the highly repetitive DNA sequences of the genome and is preferentially located

near centromeres, telemers, and the nucleolus organizers, which suggests that constitutive heterochromatin has a largely structural role in chromosome organization. A second banding technique produces C-bands, which demonstrates areas of constitutive heterochromatin. Henceforth, all reference to heterochromatin will be of the constitutive type.

A third type of banding technique is called silver staining, as it involves the use of silver nitrate. This procedure shows the nucleolus organizer regions (NORs), the chromosomal locations of most ribosomal DNA.

Structural rearrangements in chromosomes are the result of transverse breakage in one or more chromosomes with the subsequent reunion of the broken ends in such a way that the linear order of the genetic material is disturbed. A translocation occurs when breakage in a chromosome is followed by the transfer of a chromosome segment from its usual position to a new position, usually on a different chromosome.

The most frequent type of translocation that occurs in the family Bovidae, and many other taxa as well, is the Robertsonian translocation (centric fusion) which occurs between two acrocentric chromosomes. The break points occur very close to the centromere in the region of the heterochromatin, and the broken ends rejoin to form one large biarmed chromosome. The result is a reduction in the diploid number, but the basic number of chromosome arms remains the same. As a result of Robertsonian translocations, the numerical and structural alteration of chromosome complements during the course of evolution has undoubtedly played an important role in the process of speciation.

The working tool of the cytogeneticist is the karyotype--the physical grouping of chromosomes cut out of a photographic print and arranged in pairs in a systematic order based on their size and morphology. Over the past years, staining techniques for producing banded karyotypes have had application in cytogenetic studies of domestic animals. In recent years, modified techniques have been developed that have enabled the cytogeneticist to use chromosomes at an earlier and more elongated state, resulting in high-resolution G-bands.

Therefore, it is important in animal cytogenetics that a more elongated karyotype with an associated idiogram be established. High-resolution chromosome analysis would allow the cytogeneticist to identify small but discrete chromosome changes that otherwise may go undetected. Since sheep and goats are considered near relatives and since prior banding patterns have not shown any differences in chromosome homologies, reexamining the chromosome complements using high-resolution elongated chromosomes to verify this similarity would be most useful.

Thus, the first objective of this study was to establish an elongated karyotype for the sheep and goat and to prepare an idiogram of the goat karyotype. The second objective was to compare G-band, C-band, and NOR homologies in the sheep and goat and to determine specific chromosomes involved in the evolution of the sheep karyotype.

The animals used in this study were the St. Croix and Rambouillet sheep and the Spanish goat. The two breeds of sheep were selected because they represent two unique genotypes that were

developed during the process of domestication. The St. Croix is a hair sheep and was developed for meat production, whereas the Rambouillet is a major wool breed developed for both its wool and meat characteristics.

## LITERATURE REVIEW

Historical Aspects of the Chromosomes  
of Domestic Sheep (*Ovis aries*) and  
Domestic Goat (*Capra hircus*)

In early research on the chromosomes of sheep, Shiwago (1931) and Berry (1938) were the first to report the correct diploid number of 54. Berry (1941) identified six U-shaped chromosomes in the sheep, which correspond to what are now known as six large submetacentric chromosomes.

Makino (1943), in a complete historical review of early chromosome studies on goats as well as sheep, indicated that Sokolov (1930) was the earliest author to report on goat chromosomes, and he reported the correct diploid number of 60. Makino (1943) compared the chromosomes of the sheep with the goat and noted the marked similarities. He postulated that the two species may have descended from a common ancestor and that the six chromosomes of the sheep that were V-shaped at anaphase could have arisen by a fusion of goat-type, rod-shaped chromosomes at their ends.

The ability to study chromosomes increased greatly as techniques were developed that produced banding patterns that were constant and specific for each chromosome pair. A band is defined here as a part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or lighter with specific staining methods. Bands that stain darkly with one method may stain lightly with other methods. The chromosomes are visualized as consisting of a continuous series of light and dark bands (Paris Conference (1971), 1972).

The Reading Conference in 1976 was organized for and resulted in the standardization of the banded karyotypes of domestic animals, including both sheep and goat (Ford et al., 1980). Since then, high-resolution banding techniques have increased the number of bands which can be perceived on a chromosome, allowing identification of small structural changes that previously would have gone unnoticed.

In their study of banded sheep and goat chromosomes, Evans et al. (1973) concluded the two species were similar enough that each of the two arms of the submetacentric sheep chromosomes was identified as specific goat chromosomes. They reported that the close homology in G-banding patterns between these related species indicates that the banding patterns are evolutionarily conservative and may be a useful guide in assessing interspecific relationships. Zartman and Bruere (1974) published a more detailed idiogram of the banding patterns of sheep from karyotypes that show greater detail than those of Evans et al. (1973).

#### Robertsonian Translocation

In their review of the superfamily Bovoidae, sheep (*Ovis*) and goat (*Capra*) being subgroups, Wurster and Benirschke (1968) indicate an almost exclusive use of the Robertsonian fusion mechanism of karyotype evolution. The centric fusion of acrocentric chromosomes results in an increase in meta or submetacentric chromosomes and a decrease in the diploid number. The fundamental number (NF) remains the same, however, for where there is a decrease in acrocentric autosomes, there is a proportional increase in the number of banded elements.

Domestic sheep (Ovis aries) have a diploid number of 54, including three pairs of submetacentric autosomes. With the use of G-banding, these submetacentrics have been determined to be centric fusions of six pairs of acrocentric autosomes found in the goat, diploid number of 60, the species considered most representative of the ancestral karyotype (Evans et al., 1973; Bunch et al., 1976). Evidence is presented suggesting that the chromosome evolution of domestic sheep came about by prezygotic selection toward lower chromosome numbers of  $2N = 58$  to 54 (Bunch and Foote, 1977).

Using refinements in chromosome identification techniques, a G-banded idiogram of *Capra* was presented by Bunch (1978) as the basis for identifying all reported biarmed formations of *Ovis*. The acrocentric autosomes involved in the evolution of  $2N = 58, 56, 54,$  and 52 *Ovis* karyotypes are: 1 and 5, 3 and 10, 4 and 9, and 11 and 17, lower (q) and upper (p) arms, respectively (Bunch, 1978).

Although domestic sheep typically display a  $2N$  of 54, three Robertsonian-like translocations have been reported to reduce their  $2N$  number from 54 to 49, depending upon the particular translocation involved (Bruere et al., 1974). These three translocations have been reported by Bunch (1978) to involve the acrocentric autosomes: 8 and 30, 12 and 15, and 11 and 29, lower and upper arms, respectively.

#### C-Bands

When chromosomes are subjected to procedures which are believed to denature and then reanneal DNA, heterochromatin associated with the centromeric region is darkly stained, contrasting with the pale staining of the chromosome arms (Arrighi and Hsu, 1971).

As reported by Evans et al. (1973), centromeric regions of the sheep and goat acrocentric autosomes when C-banded are very well marked and stain densely. However, in the sheep submetacentric autosomes, the centromeric heterochromatin comprises very small blocks, which are scarcely detectable in some preparations. In the sex chromosomes of these species, they found the centromeric regions of the X and Y were not stained differently from the chromosome arms.

Schnedl and Czaker (1974) found all acrocentric autosomes of sheep and goats to contain large regions of heterochromatin at the centromeres. The three pairs of sheep submetacentric autosomes were found to contain low amounts of heterochromatin, while the centromeres of the X and Y contained none or only small amounts.

Eldridge (1985) reported an absence of C-bands in the centromeric region of the sex chromosomes of sheep, but indicated a weak C-band in the long arm of the X chromosome. He also found weak C-bands in the number 1 and 3 submetacentrics, with a stronger C-band in the number 2 submetacentric.

#### Nucleolus Organizer Regions

Nucleolus organizer regions (NORs) are areas on certain chromosomes which contain genes that code for rRNA. Henderson and Bruere (1977) reported the NORs of domestic sheep, as shown by silver staining, to be located terminally on chromosome pairs 1, 2, 3, 4, and 25. Significant differences between individuals in the number of NORs per cell were found. The frequency of involvement of individual chromosome pairs in nucleolar organization was found to be a characteristic of individual animals.



Henderson and Bruere (1979) confirmed by duplicate G-banding and silver staining that the NORs of domestic sheep occur terminally on chromosome pairs 1p, 2q, 3q, 4, and 25. In goats, they found four large and one small acrocentric autosome pairs to contain NORs; however, an NOR was never found on the longest acrocentric autosome pair.

There are 10 NORs in domestic sheep, goat, cattle, and aoudad, and these are located terminally on chromosomes with homologous G-banding patterns. It appears that there has been a conservation of the number and location of NORs during the evolution of these members of the family Bovidae (Henderson and Bruere, 1979).

## MATERIALS AND METHODS

The animals utilized in this study were three St. Croix male sheep, three Rambouillet male sheep, and three Spanish male goats. The method used to culture leukocytes from each of these animals was a modification of the standard 72-hr whole blood culture technique of Moorhead et al. (1960).

Under sterile procedures, a syringe containing 0.25 ml sodium heparin (20,000 IU/ml) was used to obtain 10 ml of peripheral blood. One-half ml of this blood was placed in a polystyrene centrifuge tube with 7 ml supplemented culture medium RPMI 1640 (10% FCS, 2% L-glutamine, 1% Pen-Strep) and 1.5% PHA mitogen. The tube was then placed in an incubator for 72 hrs at 37° C.

Figure 1 outlines the following technique used for obtaining high-resolution elongated sheep and goat chromosomes:

After 72 hrs incubation, the cells were centrifuged and then blocked at the G<sub>1</sub>-S border (Figure 2) by resuspending them in supplemented culture medium containing 10<sup>-9</sup> M methotrexate (amethopterin) for 17-21 hrs. Following blocking, the cells were centrifuged, resuspended in, and washed once with Hanks Balanced Salt Solution (HBSS without Ca<sup>++</sup> or Mg<sup>++</sup>), recentrifuged and released from the block by resuspending in supplemented culture medium containing 10<sup>-9</sup> M thymidine for 4.5 hrs. Colcemid (0.05 mcg/ml) was then added for 7 min, after which the cells were harvested.

The cell synchronization technique described by Yunis et al. (1978) for use with human cells and modified by Maciulis et al. (1984) for use with horse cells likewise proved necessary for use

with sheep and goat cells in this study. This modified technique decreased the concentrations of methotrexate and thymidine from  $10^{-7}$  M to  $10^{-9}$  M, since a concentration of  $10^{-7}$  M methotrexate resulted in extremely few mitoses and  $10^{-7}$  M thymidine blocked the leukocytes instead of releasing them from the  $G_1$ -S border. Also, this technique shortened the time of cell release from 5 hr 5-10 min to 4 hr 30 min, and the time in colcemid from 10 min to 7 min, in order to obtain less contracted chromosomes. Use of this modified technique greatly increased the number of cells in prophase, prometaphase, and early metaphase.

To obtain G-bands, 48-hr heat-pretreated slide preparations were placed in a trypsin solution (0.25% trypsin, 0.1% EDTA, HBSS) for approximately 2 min. The slides were then rinsed in HBSS, 70% ethanol, 95% ethanol, and air-dried. Lastly, the slides were stained (3.5 min) with 2% Giemsa- $PO_4$  buffer (pH 7.2) (Wang and Federoff, 1972).

To obtain C-bands, room-temperature-aged (7 days) slide preparations were pre-treated in 0.2 M HCl for 20 min. The slides were then placed in a 37° C saturated barium hydroxide solution for approximately 4 min and rinsed first with 0.2 M HCl and then 2XSSC (standard sodium citrate). The slides were then flooded with 2XSSC, overlaid with a coverslip, and placed overnight in a moist chamber at 37° C. This was followed by rinsing the slides in 2XSSC, 70% ethanol, and 95% ethanol. Lastly, the slides were counterstained (6-8 min) with 2% Giemsa- $PO_4$  buffer (pH 7.2) (Sumner, 1972).

To obtain NORs, room-temperature-aged (7 days) slide preparations were flooded with a 1% silver nitrate solution (1g

AgNO<sub>3</sub>, 2 ml H<sub>2</sub>O, 1 drop 1% formalin), overlaid with a coverslip, and placed in a moist chamber at 37° C. After the area under the silver nitrate solution started turning a rust color (4-5 hrs), the slides were rinsed off with water and then counterstained (15 sec) with 2% Giemsa-PO<sub>4</sub> buffer (pH 7.2) (Bloom and Goodpasture, 1976).

Individual chromosome spreads were photographed at 1000X using high-contrast Kodak film Pan 2415 with an Olympus OM-2 camera mounted on a Zeiss microscope. The film was developed using Kodak D-19 developer and fixer according to the manufacturer's specifications for high-contrast film. The negatives were enlarged 8.5X and prints made using Kodak Dektol developer and fixer, with a water-0.05% glacial acetic acid stop bath solution used between the developer and fixer steps. The prints were then washed for 0.5 hr and placed in a print flattening solution (Pakasol) for 10 min, after which the prints were dried.

Karyotypes were prepared from high-resolution G-banded, C-banded, and NOR spreads of all sheep and goats studied. A high-resolution G-band idiogram of the goat was prepared from several G-banded karyotypes.

## HIGH-RESOLUTION ELONGATED CHROMOSOMES

LEUKOCYTES (density gradient - isolated white blood cells, PHA)

or

WHOLE BLOOD (PHA mitogen)

↓ 72 hrs

CELLS ARRESTED ( $10^{-9}M$  amethopterin)

↓ 17-21 hrs

CELLS RELEASED ( $10^{-9}M$  thymidine)

↓ 4.5 hrs

COLCEMID TREATMENT

↓ 7 min

HARVEST

Figure 1. Technique used for obtaining high-resolution elongated sheep and goat chromosomes.

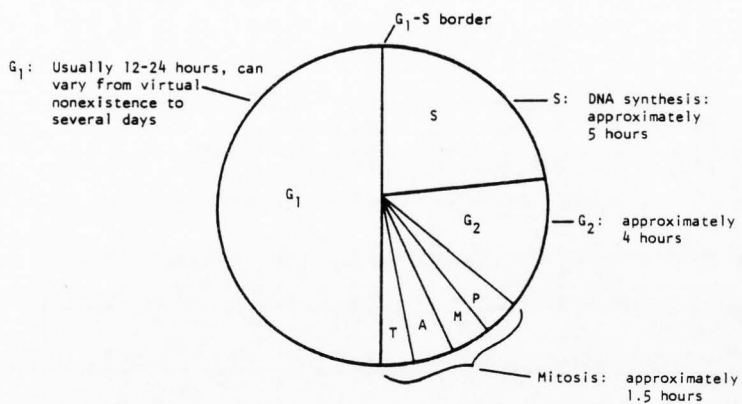


Figure 2. Mitotic cycle. P = prophase, M = metaphase, A = anaphase, T = telophase, (P + M + A + T = mitosis; G<sub>1</sub> + S + G<sub>2</sub> = interphase).

## RESULTS

Figure 3 shows a diagrammatic representation of sheep chromosome 1 in different degrees of contraction. The earliest and most elongated chromosome stage presented is late prophase. Late prophase chromosomes contained the greatest number of bands but were the most difficult to work with because only 2% of the chromosome spreads per slide were at this stage of contraction. Also, late prophase spreads had many chromosomes that overlapped, which resulted in distorted banding patterns.

The next stage of contraction shown is prometaphase. Six percent of the chromosome spreads were at prometaphase, which had relatively few overlaps. It was at this stage of contraction that the chromosome had the greatest utility for detailed comparative analysis, and thus the prometaphase chromosome was selected for use in this study.

The last three stages presented are early metaphase, mid-metaphase, and late metaphase. The mid-metaphase chromosome is at the stage of contraction that has been reported in most publications. These later and more contracted stages provided considerably fewer bands and less detail than prometaphase.

Both sheep and goat have Y chromosomes that are minute in size. Slides prepared using the elongated chromosome technique were treated only with Giemsa stain, in order that the composition of the chromosome be intact so as to effectively determine centromeric position. Figure 4 presents the Giemsa-stained Y chromosome of sheep and goat, which are both shown to be banded. Shown alongside the

goat biarm are two other configurations of the Y chromosome that were also observed. These manifestations are evidently the result of the staining technique.

Figure 5 presents a proposed standard domestic sheep high-resolution G-band karyotype, prepared by using prometaphase chromosomes from three different karyotypes. The karyotype of the sheep has 54 chromosomes, which comprises 26 pairs of autosomes and 1 pair of sex chromosomes. The autosomes are arranged into two major groups: three pairs of biarmed submetacentrics, and 23 pairs of single-armed acrocentrics. The X chromosome is the longest acrocentric, while the Y is a minute biarm.

Figure 6 presents a proposed standard domestic goat high-resolution G-band karyotype, prepared by using prometaphase chromosomes from three different karyotypes. The karyotype of the goat has 60 chromosomes, which comprises 29 pairs of autosomes and 1 pair of sex chromosomes. The autosomes are arranged into one group of 29 pairs of single-armed acrocentrics. The X chromosome is the fourth largest acrocentric, while the Y is a minute biarm.

Figure 7 shows a high-resolution G-band idiogram of the domestic goat, prepared using the karyotypes utilized in the prometaphase karyotype of Figure 6. The idiogram presented indicates that the high-resolution G-band karyotype of the domestic goat contains approximately 367 bands.



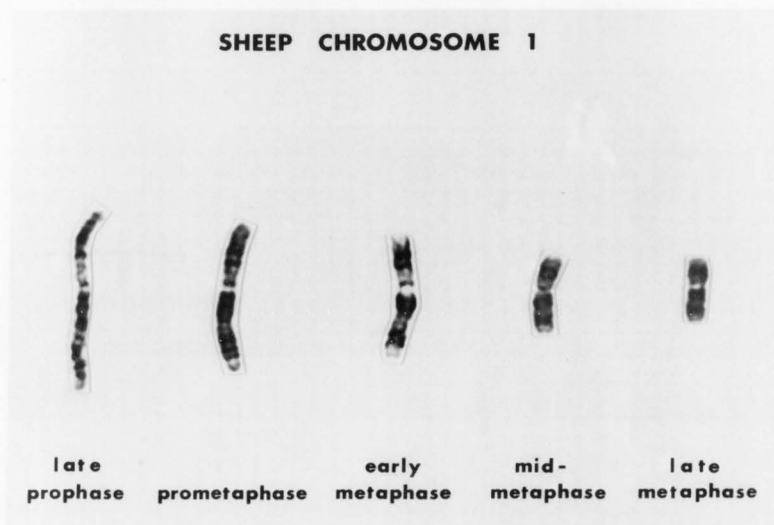


Figure 3. Diagrammatic representation of sheep chromosome 1 at different stages of mitosis.

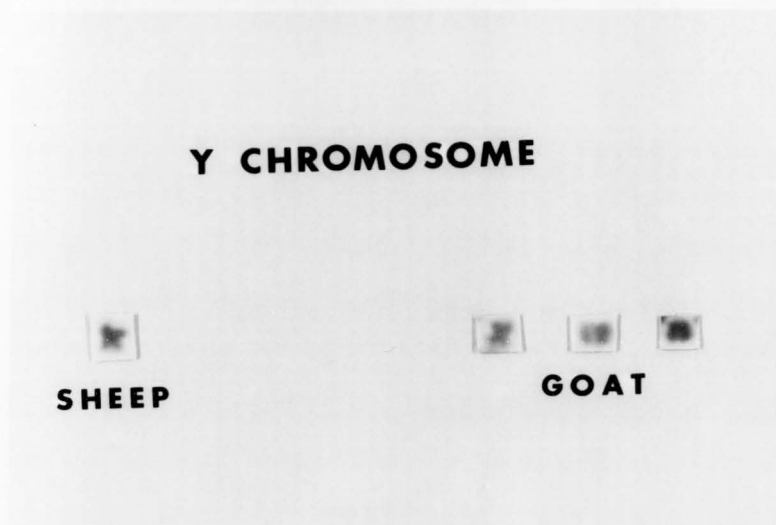


Figure 4. Blarm Y chromosome of sheep and goat. Shown alongside the goat blarm are two other Y chromosome configurations frequently observed.

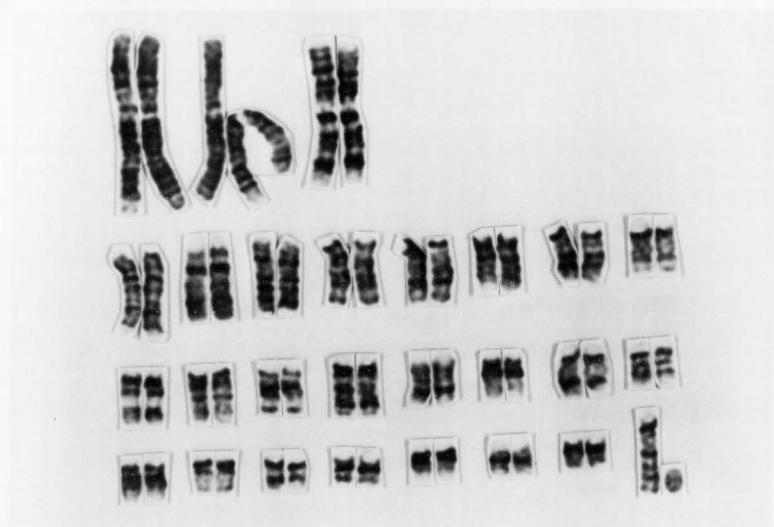


Figure 5. Proposed standard domestic sheep high-resolution G-band karyotype.



Figure 6. Proposed standard domestic goat high-resolution G-band karyotype.

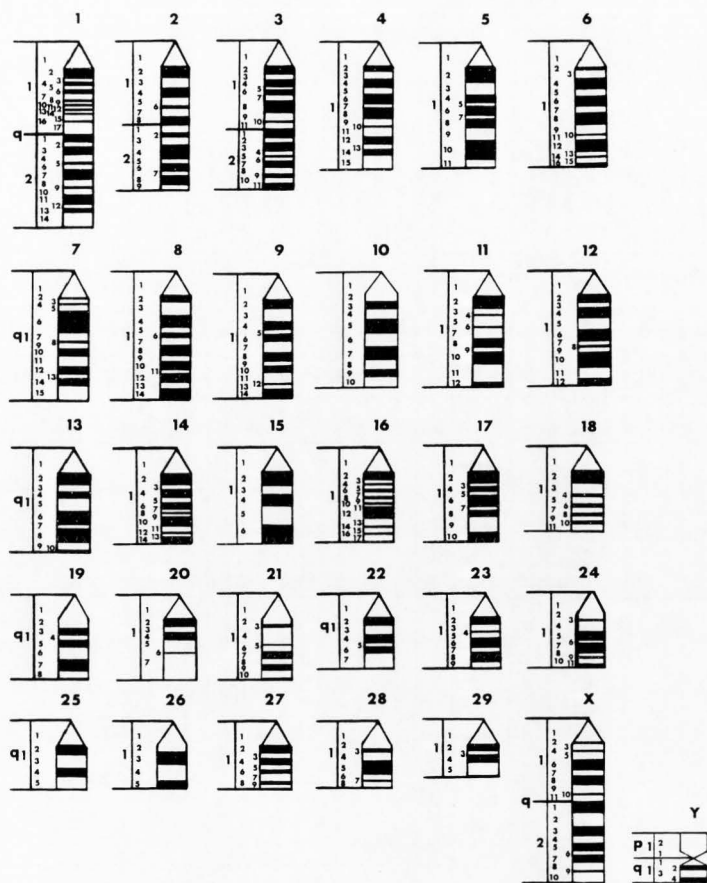


Figure 7. High-resolution G-band idiogram of the domestic goat.

Descriptions of high-resolution G-banded prometaphase goat chromosomes comprising the standard goat karyotype are as follows:

1q - 2 regions

1 1 centromere  
1 2 dark band  
1 3 light band  
1 4 dark band  
1 5 light band  
1 6 dark band  
1 7 light band  
1 8 narrow dark band--often merging with 1 10  
1 9 narrow light band--often not visible  
1 10 narrow dark band--often merging with 1 8  
1 11 narrow light band  
1 12 narrow dark band--often merging with 1 14  
1 13 narrow light band--often not visible  
1 14 narrow dark band--often merging with 1 12  
1 15 light band  
1 16 narrow dark band  
1 17 broad light band

2 1 dark band  
2 2 light band  
2 3 dark band--often merging with 2 5  
2 4 light band--often not visible  
2 5 narrow dark band--often merging with 2 3  
2 6 light band  
2 7 dark band  
2 8 light band  
2 9 narrow dark band  
2 10 light band  
2 11 dark band  
2 12 light band  
2 13 dark band  
2 14 terminal broad light band

2q - 2 regions

1 1 centromere  
1 2 prominent broad dark band  
1 3 light band  
1 4 dark band  
1 5 light band  
1 6 narrow dark band  
1 7 light band  
1 8 prominent dark band

2 1 light band  
2 2 dark band  
2 3 light band  
2 4 prominent broad dark band  
2 5 light band  
2 6 prominent dark band  
2 7 light band  
2 8 prominent dark band  
2 9 terminal light band

3q - 2 regions

1 1 centromere  
1 2 prominent dark band  
1 3 light band  
1 4 dark band--often merging with 1 6  
1 5 narrow light band--often not visible  
1 6 dark band--often merging with 1 4  
1 7 light band  
1 8 prominent dark band  
1 9 light band  
1 10 pale dark band  
1 11 light band

2 1 dark band  
2 2 light band  
2 3 dark band  
2 4 light band  
2 5 pale dark band  
2 6 light band  
2 7 dark band  
2 8 light band  
2 9 pale dark band  
2 10 light band  
2 11 terminal pale dark band

4q - 1 region

- 1 1 centromere
- 1 2 narrow dark band
- 1 3 light band
- 1 4 prominent dark band
- 1 5 light band
- 1 6 prominent dark band
- 1 7 light band
- 1 8 prominent dark band
- 1 9 light band
- 1 10 narrow dark band
- 1 11 light band
- 1 12 dark band
- 1 13 light band
- 1 14 dark band
- 1 15 terminal broad light band

5q - 1 region

- 1 1 centromere
- 1 2 prominent very broad dark band
- 1 3 prominent broad light band
- 1 4 dark band
- 1 5 narrow light band
- 1 6 dark band
- 1 7 narrow light band
- 1 8 dark band
- 1 9 prominent broad light band
- 1 10 prominent very broad dark band
- 1 11 terminal light band

With very broad dark bands on each end (1 2, 1 10) and three narrower dark bands in the middle (1 4, 1 6, 1 8), this chromosome has a distinctive symmetrical appearance.

6q - 1 region

- 1 1 centromere
- 1 2 narrow dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 dark band
- 1 9 light band
- 1 10 narrow dark band
- 1 11 light band
- 1 12 dark band
- 1 13 light band
- 1 14 narrow pale dark band--often merging with 1 16
- 1 15 light band--often not visible
- 1 16 terminal dark band--often merging with 1 14



7q - 1 region

- 1 1 centromere
- 1 2 pale dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 prominent very broad dark band
- 1 7 light band
- 1 8 pale narrow dark band--often merging with 1 10
- 1 9 light band--often not visible
- 1 10 dark band--often merging with 1 8
- 1 11 light band
- 1 12 dark band
- 1 13 light band
- 1 14 dark band
- 1 15 terminal broad light band

The prominent very broad dark band (1 6) is characteristic and the most easily identifiable feature.

8q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 broad light band
- 1 4 prominent broad dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 prominent broad dark band
- 1 9 light band
- 1 10 dark band--often merging with 1 12
- 1 11 light band--often not visible
- 1 12 dark band--often merging with 1 10
- 1 13 light band
- 1 14 terminal dark band

9q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 broad light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 dark band--often merging with 1 10
- 1 9 light band--often not visible
- 1 10 dark band--often merging with 1 8
- 1 11 light band
- 1 12 narrow pale dark band
- 1 13 light band
- 1 14 terminal dark band

10q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 dark band
- 1 4 light band
- 1 5 prominent broad dark band
- 1 6 prominent broad light band
- 1 7 prominent broad dark band
- 1 8 light band
- 1 9 dark band
- 1 10 terminal light band

The two prominent broad dark bands (1 5, 1 7) separated by a prominent broad light band (1 6) are characteristic and the most easily identifiable feature.

11q - 1 region

- 1 1 centromere
- 1 2 prominent broad dark band
- 1 3 light band
- 1 4 narrow pale dark band
- 1 5 light band
- 1 6 narrow pale dark band
- 1 7 light band
- 1 8 dark band
- 1 9 light band
- 1 10 prominent broad dark band
- 1 11 broad light band
- 1 12 terminal pale dark band

The prominent broad dark bands (1 2, 1 10) are characteristic and the most easily identifiable feature.

12q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 narrow dark band--often merging with 1 10
- 1 9 light band--often not visible
- 1 10 dark band--often merging with 1 8
- 1 11 light band
- 1 12 terminal dark band

13q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 dark band
- 1 5 prominent light band
- 1 6 prominent broad dark band
- 1 7 light band
- 1 8 prominent broad dark band
- 1 9 light band
- 1 10 terminal narrow dark band

14q - 1 region

- 1 1 centromere
- 1 2 prominent broad dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 pale dark band
- 1 9 light band
- 1 10 dark band
- 1 11 light band
- 1 12 pale dark band
- 1 13 light band
- 1 14 terminal dark band

15q - 1 region

- 1 1 centromere
- 1 2 prominent broad dark band
- 1 3 light band
- 1 4 prominent dark band
- 1 5 prominent broad light band
- 1 6 terminal broad dark band

The two prominent dark bands (1 2, 1 4) followed by the prominent light band (1 5) and terminal broad dark band (1 6) are characteristic and the most easily identifiable feature.

16q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 narrow light band
- 1 4 narrow dark band
- 1 5 narrow light band
- 1 6 narrow dark band
- 1 7 narrow light band
- 1 8 narrow dark band
- 1 9 narrow light band
- 1 10 narrow dark band
- 1 11 narrow light band
- 1 12 prominent broad dark band
- 1 13 light band
- 1 14 narrow dark band--often merging with 1 16
- 1 15 narrow light band--often not visible
- 1 16 narrow dark band--often merging with 1 14
- 1 17 terminal light band

Region 1 4 to 1 10 often hard to differentiate.

17q - 1 region

- 1 1 centromere
- 1 2 prominent dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 prominent dark band
- 1 7 light band
- 1 8 dark band
- 1 9 prominent broad light band
- 1 10 terminal dark band

The first four dark bands (1 2, 1 4, 1 6, 1 8) spaced evenly apart and followed by a prominent broad light band (1 9) are characteristic and the most easily identifiable feature.

18q - 1 region

- 1 1 centromere
- 1 2 prominent broad dark band
- 1 3 broad light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 light band
- 1 8 dark band
- 1 9 light band
- 1 10 dark band
- 1 11 terminal light band

19q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 prominent dark band
- 1 4 light band
- 1 5 prominent dark band
- 1 6 broad light band
- 1 7 prominent broad dark band
- 1 8 terminal light band

20q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 narrow dark band
- 1 7 terminal broad light band

21q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 dark band
- 1 4 broad light band
- 1 5 narrow pale dark band
- 1 6 light band
- 1 7 dark band
- 1 8 light band
- 1 9 dark band
- 1 10 terminal light band

22q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 broad light band
- 1 4 dark band
- 1 5 light band
- 1 6 dark band
- 1 7 terminal light band

23q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 narrow pale dark band
- 1 5 light band
- 1 6 broad dark band
- 1 7 light band
- 1 8 broad dark band
- 1 9 terminal light band

24q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 pale dark band
- 1 4 light band
- 1 5 broad dark band--often merging with 1 7
- 1 6 narrow light band--often not visible
- 1 7 broad dark band--often merging with 1 5
- 1 8 light band
- 1 9 narrow pale dark band
- 1 10 light band
- 1 11 terminal dark band

Depending upon the stage of contraction of chromosome and staining technique, band 1 3 can sometimes be seen as two separate pale bands.

25q - 1 region

- 1 1 centromere
- 1 2 prominent dark band
- 1 3 broad light band
- 1 4 prominent dark band
- 1 5 terminal broad light band

26q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 prominent broad dark band
- 1 4 broad light band
- 1 5 terminal dark band

27q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 dark band
- 1 5 light band
- 1 6 pale dark band
- 1 7 light band
- 1 8 pale dark band
- 1 9 terminal light band

28q - 1 region

- 1 1 centromere
- 1 2 light band
- 1 3 dark band
- 1 4 light band
- 1 5 prominent broad dark band
- 1 6 light band
- 1 7 dark band
- 1 8 terminal light band

29q - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 dark band
- 1 5 terminal broad light band

xq - 2 regions

- 1 1 centromere
- 1 2 narrow dark band
- 1 3 light band
- 1 4 narrow dark band
- 1 5 light band
- 1 6 prominent broad dark band
- 1 7 light band
- 1 8 broad dark band
- 1 9 light band
- 1 10 narrow dark band
- 1 11 light band

- 2 1 prominent broad dark band
- 2 2 broad light band
- 2 3 dark band
- 2 4 light band
- 2 5 dark band
- 2 6 light band
- 2 7 dark band
- 2 8 light band
- 2 9 narrow pale dark band
- 2 10 terminal light band

Yp - 1 region

- 1 1 centromere
- 1 2 light band

Yq - 1 region

- 1 1 centromere
- 1 2 dark band
- 1 3 light band
- 1 4 terminal dark band

Figure 8 shows the side-by-side comparison of high-resolution G-band sheep and goat chromosomes. This comparison was prepared by first selecting one representative chromosome from each pair in the sheep karyotype and then arranging them according to the karyotype in Figure 5. Representative chromosomes from each pair in the goat karyotype were likewise selected and placed next to those of the sheep according to their similar banding patterns. The sheep and goat chromosomes were selected from their respective karyotypes in Figures 5 and 6 and from the other karyotypes prepared from both animals.

Figure 9 presents in tabular form the numerical chromosome equivalents of the sheep-goat comparison presented in Figure 8. The left column lists the sheep chromosomes in numerical order, while the right column lists the goat chromosome numbers next to their banding equivalents.

Figure 10 displays the six acrocentric chromosomes of the goat that have been involved in centric fusions to form the three submetacentric chromosomes of the sheep. As a result of Robertsonian or centric fusion, the numbers of chromosomes have been sequentially reduced from 60 in the goat to 54 in the sheep.

Figures 11 and 12 present C-band karyotypes of the sheep and goat, respectively. The centromeric regions of the sheep and goat acrocentric autosomes were found to contain large blocks of heterochromatin. In sheep, weak C-bands were found in the first and third submetacentric autosomes, with a stronger C-band in the second submetacentric. In the X and Y chromosomes of both sheep and goat, the centromeric regions were not stained differently from the



chromosome arms, indicating an absence of heterochromatin in this area.

Figures 13 and 14 present NOR karyotypes of the sheep and goat, respectively. In each species a total of 10 NORs located terminally on five chromosome pairs were found. Sheep NORs occurred on the three pairs of submetacentric autosomes and on one large and one small pair of acrocentric autosomes. In the goat, four large and one small acrocentric autosome pair exhibited NORs. The total number of 10 NORs were not always found to be expressed in each individual animal of each species. As shown in Figure 15 of the sheep and Figure 16 of the goat, only 8 of the 10 NORs are expressed.



Figure 8. Sheep-goat high-resolution G-band comparison.

SHEEP	GOAT
1 P Q	3 1
2 P Q	4 2
3 P Q	10 5
4	6
5	7
6	8
7	9
8	11
9	12
10	13
11	14
12	15
13	16
14	17
15	18
16	19
17	20
18	21
19	22
20	23
21	24
22	25
23	26
24	27
25	28
26	29
X	X
Y	Y

Figure 9. Numerical chromosome equivalents of sheep-goat high-resolution G-band comparison.



Figure 10. Robertsonian translocations that led to the  $2N = 54$  karyotype of domestic sheep. Numerical ascription of goat acrocentrics was based on the chromosome numbering as presented in Figure 9.

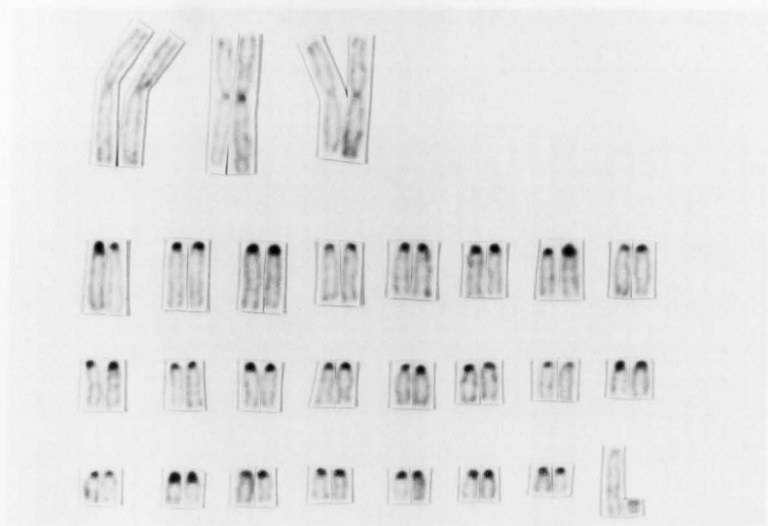


Figure 11. Sheep C-bands.

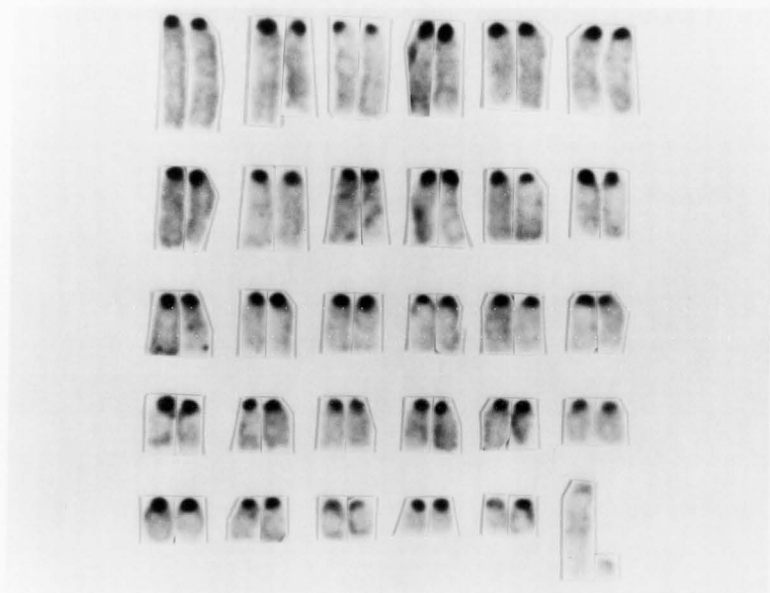


Figure 12. Goat C-bands

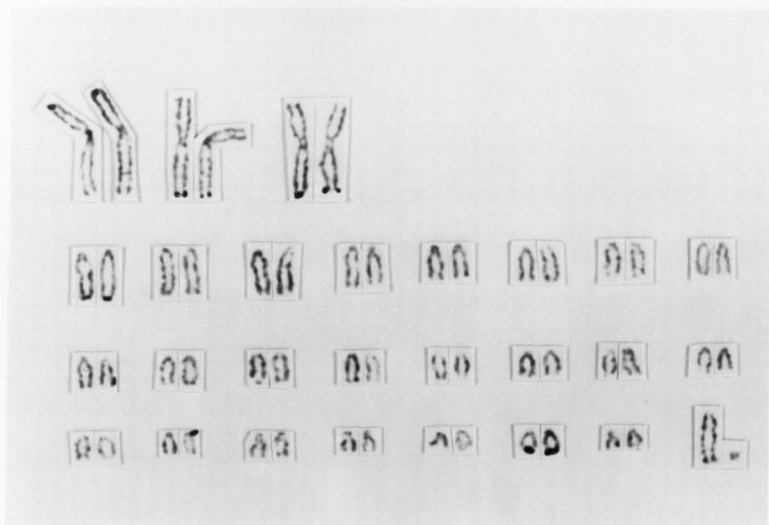


Figure 13. Sheep NORs expressed on 10 chromosomes.

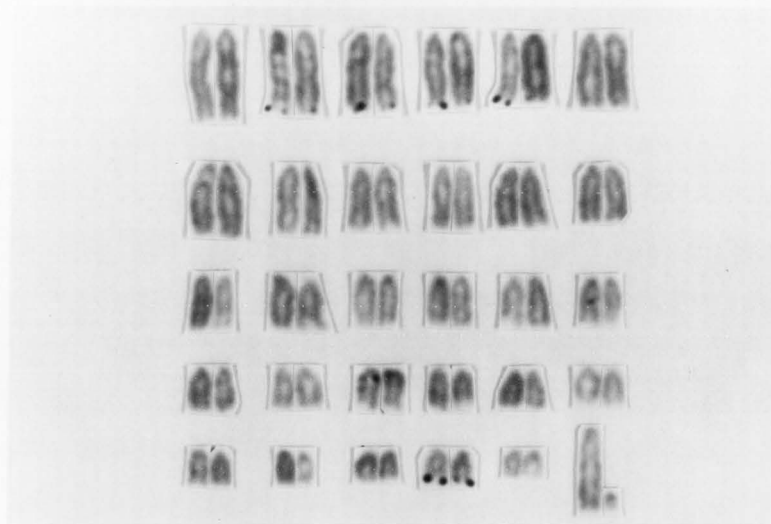


Figure 14. Goat NORs expressed on 10 chromosomes.



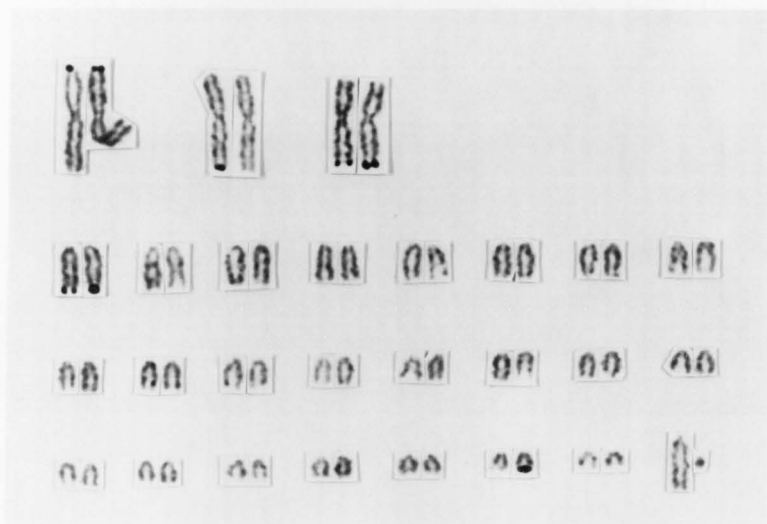


Figure 15. Sheep NORs expressed on 8 chromosomes.

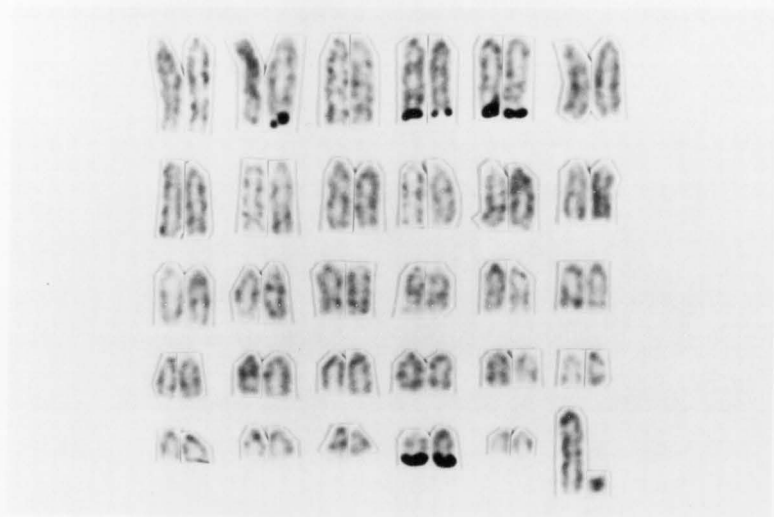


Figure 16. Goat NORs expressed on 8 chromosomes.

## DISCUSSION

The cell synchronization technique used consistently yielded numerous elongated chromosomes, which are necessary for studies involving high-resolution G-banding. The proposed standard high-resolution G-band karyotypes of the sheep and goat contain considerably more bands than have previously been depicted and described in the karyotypes of these animals. This increase in the number of bands provides much greater resolution for linear differentiation of specific regions within a chromosome and, therefore, should allow greater accuracy in comparative studies and in identifying specific chromosome changes associated with congenital disease and reproductive impairment.

As previously noted, two breeds of sheep, St. Croix and Rambouillet, were utilized in this study. The three different staining techniques for high-resolution G-banding, C-banding, and NORs were used with both breeds. No discernible differences were noted in banding patterns between breeds with any of the staining techniques.

Early in the study, excellent examples of elongated high-resolution G-band chromosomes of the domestic sheep were obtained. These were sent by my major professor, Dr. Thomas D. Bunch, to Dr. Susan E. Long at the University of Bristol, England, for her evaluation. Dr. Long was coordinator and Dr. Bunch a member of the Committee for Standardized Karyotype of Ovis aries. After material was compiled from many sources for study, the final preparation agreed upon at the 6th European Colloquium in Zurich,

1984, and subsequently published by the Committee for Standardized Karyotype of Ovis aries (1985) comprised many of the same chromosomes as presented in the prometaphase ovine karyotype of this study.

The grouping and numbering order of the chromosomes in the prometaphase sheep karyotype presented herein is in basic agreement with the karyotype published by the Committee for Standardized Karyotype of Ovis aries (1985). However, disagreement arises from their diagrammatic representation, which indicates that the X chromosome contains a minute p arm and is, therefore, submetacentric.

G-banded chromosomes cannot be used to accurately identify centromeric position, due to the altered physical appearance of the chromosome. Giemsa-stained slides, which were used for observing the Y chromosome of the sheep, were likewise utilized for studying the entire chromosome complement. No indication was found in any instance that the X chromosome of the sheep is anything but acrocentric. Eldridge (1985) presents a Giemsa-stained karyotype of a male sheep, clearly showing the X chromosome to be the largest acrocentric.

The numbering order of the proposed standard domestic goat high-resolution G-band karyotype presented herein follows that of the G-band karyotype of the domestic sheep proposed and published by the Committee for Standardized Karyotype of Ovis aries (1985).

The minute Y chromosome of both sheep and goat have been shown to be biarmed. However, even with the elongated chromosome technique and Giemsa staining, it is nevertheless difficult to ascertain exact arm proportions. With the added problems of working with chromosomes of such small size, along with the normal variations in slide

preparation and staining technique, defining the Y chromosome as metacentric or submetacentric can become quite subjective.

The definition of a metacentric chromosome is one in which the p/q arm ratio is equal to 1. Given this definition, it is unlikely many biarmed chromosomes can be categorized as such. Describing a chromosome as metacentric, whose arm ratio is not 1 but very close to it, then becomes an arbitrary decision. Until such time that a definition of a chromosome as metacentric or submetacentric is adhered to, and given the minute size of the sheep and goat Y chromosome, both terms can probably be applied with equal justification.

The similar banding patterns presented between the karyotypes of the domestic sheep and goat (Figure 8), utilizing high-resolution techniques, confirm the observation of researchers as far back as Makino in 1943 who noted the marked similarities and postulated the two species may have descended from a common ancestor. Of special note is the homology of banding patterns for the six acrocentric autosomes of the goat and the respective three submetacentric autosomes of the sheep. The findings by Bruere et al. (1974) and Bunch (1978) of Robertsonian-like translocations in wild species of sheep that have reduced the 2N number to as low as 49 reinforce the evidence that the goat, diploid number of 60, is the species most representative of the ancestral karyotype. Although it is not possible to actually reconstruct the evolution of these animals, it is believed that the karyotype of modern day species and breeds of sheep arose from stock with a goat-like karyotype.

With the results presented herein, an attempt has not been made

to compare the numbering of the chromosomes in both the sheep and goat karyotype and those involved in centric fusion to those published by Evans et al. (1973) and Bunch (1978). The conclusions of these researchers were based upon karyotypes assembled from chromosomes at a later and more contracted stage, making comparisons of numerical arrangements subjective and possibly misleading. With the technology now readily available to produce high-resolution G-banding, it would seem more useful for this work to be compared with future karyotypes of chromosomes displaying the same stage of contraction and resolution.

When C-banded, the centromeric regions of the sheep and goat acrocentric autosomes were found to be very well marked and densely stained, which is in agreement with that reported by Evans et al. (1973) and Schnedl and Czaker (1974). Also in agreement with these authors was the finding that the centromeres of the X and Y chromosomes of these species showed an absence of heterochromatin.

In the sheep submetacentric autosomes, chromosomes 1 and 3 showed weak C-bands, while chromosome 2 showed strong C-bands, a finding also reported by Eldridge (1985). However, a finding by Eldridge (1985) of a weak C-band in the arm of the X chromosome of the sheep could not be substantiated in this study.

In both sheep and goat, 10 NORs located terminally on five chromosome pairs were found. Sheep NORs occurred on the three pairs of submetacentric autosomes and on one large and one small pair of acrocentric autosomes, while goat NORs occurred on four large and one small pair of acrocentric autosomes. These findings are in agreement with that reported by Henderson and Bruere (1977, 1979).

Since the banding patterns obtained when staining for NORs do not permit individual identification of each chromosome, duplicate G-banding and silver staining of elongated chromosomes of both sheep and goat would need to be conducted to identify specific NOR-bearing chromosomes.

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