KARYOTYPE POLYMORPHISM IN INDIGENOUS GOATS FROM TWO ZAMBIAN PROVINCES

By

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DEDICATION

To my parents, wife and children

STATEMENT

The experiments reported in this thesis are my own work.

Signature: _____

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Date: 30th AUGUST 1990

ABSTRACT

Blood samples drawn from 20 goats in Southern and Eastern Provinces (Choma, Gwembe, Mazabuka, Petauke, Katete and Chipata) were analysed by the leucocyte culture method and the findings presented in idiograms. Goats from these two regions had 60 diploid chromosome number (58 autosomes and 2 sex-chromosomes). Mazabuka had the lowest mean relative chromosome length of 3.21 (with a 95 percent confidence interval of 2.71 to 3.71) while Gwembe and Katete had highest mean realative chromosome length, being 3.33 (2.89 to 3.77, 95 percent confidence interval). Between males and females, males had a lower mean relative chromosome length, (males 3.22 with a 95 percent confidence interval of 2.76 to 3.68; while females 3.33, with 2.89 to 3.77 percent at a 95 percent confidence interval). All autosomes were acrocentric. For the sex-chromosome pair, females had medium-sized acrocentric X-chromosomes while males had a large acrocentric X-chromosome and a small dot-like Y-chromosome.

The results from goats in these two provinces revealed similar Karyotypes, indicating that they are likely to be related. There were no aberrations observed. Due to small sample size, this study is not a definitive study; therefore, further research should be conducted to verify the chromatin band resemblances and differences through other staining procedures. Similar work should also be

undertaken in the remaining provinces. There were two main objectives associated with this study:

a) To determine Karyotype differences in species of some Zambian indigenous goats; and

b) To evaluate the occurances of Karyotype abnormalities in Zambian indigenous goats.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES

CHAPTER ONE:

INTRODUCTION

The goat is a ruminant belonging to the family Bovidae; sub-order Ruminantia; order Artiodactyla; tribe Caprini; genus Capra (Devendra and McLeroy, 1982). Available evidence from comparative morphology indicates that the bezoar of Western Asia is the main ancestor of most domestic goats (French, 1970;; Devendra and McLeroy, 1982).

The domestic goat is likely to have developed from the following 5 wild species: a) Capra hircus, the true goat including the bezoar, C.h. aegagrus; b) Capra ibex, the ibexes; c) Capra pyrenaica, the Spanish ibex; d) Capra caucasica, the caucasica tur; and e) Capra falconeri, the markhor (French, 1970; Devendra and McLeroy, 1982).

As for the goats of Africa, in general, their origin is from the C. ibex, C. hircus groups and their crosses (French, 1970) and they have a characteristic convex chanfrin (Roman nose) and long lop ears. These features occur in almost all the breeds found in Africa, e. g. Nubian, Sudan desert breed (or Sahel), Red Sokoto (or Maladi), the Cameroon Dwarf, Nigerian Dwarf, Tuareg, Small East African breed (Devendra and McLeroy, 1982). Goats are widely distributerd in Africa ranging from arid semi-desert humid rain forest regions. Due to their large numbers, classification of goats has been performed in the past to produce orderly identification.

Classification is generally based on systematic naming

and grouping of individual animals with similar characteristics; this process has resulted in the recognition of various breeds of goats. Classification is useful in that it serves as a convenient filing device or catalogue. Phylogenetic relationships between groups can also be expressed and studied carefully by breeders whose ultimate aim is to exploit various groups' production potential (Grove, Newell, Carthy and Mercer, 1966). The available methods of classification have in the past been on morphological appearance and these are ear shape and length, body size and height at withers, and function as well as origin. However, Karyotyping as a method of classification, resulting from advances in cytogenetics, has provided more accurate and precise information on breed resemblances and differences which have lead to perfection of classification.

The objectives of this study were

a) To determine Karyotype differences within the species of some Zambian indigenous goats and

b) To determine the occurences of karyotype abnormalities in the population of some Zambian indigenous goats.

CHAPTER TWO:

LITERATURE REVIEW

2.1 Chromosome Polymorphism

Chromosome polymorphism refers to variations in chromosomal length, thickness or size, shape and morphology. Goat variants can be considered to be polymorphic as in drosophilla and other species (Lubbs and Ruddle, 1971):

1) they occur at relatively high frequencies;
2) they are usually associated with normal phenotypes; and
3) they are frequently inherited from generation to generation.

Likely alterations to chromosomal polymorphism could arise through major chromosomal aberrations which occur in natural populations such as inversions (paracentric and pericentric); translocations (mutual interchanges, centric fusion and dissociation); and duplications and deletions (terminal, interstitial deletions) (White, 1973; Avers, 1976; and Dyer, 1979).

Goats having similar chromosomal lengths, thickness, shape and morphology have been classified named and grouped as a breed, for example, Boar, Nubian, and Angora. Grove et. al. (1966) stated that classification is useful since it serves as a convenient filing device or catalogue; in addition phylogenetic relationships between groups could be expressed. Also animal breeders can study the different

traits of goats, select and combine the desirable characteristics through cross-breeding in order to achieve heterozygosity thereby contributing to the genetic improvement in productive efficiency (Cundiff and Gregory, 1977).

Devendra and McLeroy (1982) explained the available methods of classification based on phenotypic morphological appearance such as ear shape and length, body size and height at withers, function and origin. The other more precise method used in classification is karyotyping.

2.2 Methods of Breed Classification

2.2.1 Origin

Devendra and McLeroy (1982) explained that goats can be classified according to origin as European, Oriental, Asian and African. The advantages of this method are that it is easy, and that more precise classification can be made by continents where definite geographical boundaries are well defined. There are three disadvantages: 1) not only are these geographical territories vast but it is also difficult in defining the boundaries especially where goats are kept on range, for example Middle-east breeds and European breeds; 2) considerable over-lapping occured earlier as goats were moved between territories hence some breeds have been found to be common on more than one territory, e. g. *C. ibex* group is found in Africa and Asia; and 3) origin may also vary in relation to history and changing patterns in

agriculture, for example introduction of exotic breeds into Africa from Europe.

2.2.2 Ear Shape and Length

This method of classification has been based on visual examination of the ear shape and length (Devendra and McLeroy, 1982). It is easy to classify the goats using this method but the major disadvantage with this method is that there is wide variation in ear shape ranging from small prick ears (e. g. temperate breeds) to long pendulous ears (e. g. tropical breeds).

2.2.3 Function

Goats have been classified in terms of their major function: meat, milk, fibre or skin production (e. g. Angora breed for hair, Sahel breed for meat; Maabite breed for milk and Sudan desert breed for meat and skin production). The only advantage with this method is that it is easy, but the disadvantage of this method is that it assumes all goats have been adequately bred and all their production potential has been exploited to its fullest extent (Devendra and McLeroy, 1982).

2.2.4 Body Size and Height at Withers

This method takes account of body size dividing the goats into 3 groups: 1) Large breeds over 65 cm tall, weighing between 20-65 Kg, e. g. Angora of South Africa; 2) Small breeds, 51-65 cm tall and weighing 19-37 Kg, e. g. Somali breed of Somalia; and 3) Dwarf breeds of under 50 cm tall and weighing 18-25 Kg, e. g. Congo dwarf of upper Nile,

Uganda and Zaire. The advantage of this method, as Devendra and McLeroy (1982) pointed out, is that it is more precise than the other methods (ear shape and length, function and origin) since it considers height and weight. Height at withers appears to be correlated to body size.

2.2.5 The Karyotype Method

Karyotyping is the analysis of chromosome complement in metaphase stage arranged according to morphological appearance (Hafez, 1974). All detectable structural features of the chromosome including number, size and morphology, as seen at mitotic metaphase (Dyer, 1979), have been classified into three main types based on the position of the centromere and relative lengths of the long and short arms such as matacentric, sub-metacentric, acrocentric and telocentric (Hafez, 1974). Dyer (1979) added that a karyotype is usually recorded after an appropriate pretreatment to make the individual chromosomes clearer and is presented in diagrammatic form known as an idiagram. In the karyotype method, chromosome examination of blood leucocytes is involved.

There are three main advantages with this method: 1) a conclusion, based on karyotype characters, of the chromosome numbers, size and morphology would be readily available; 2) any disorders in the structural appearance of the chromosomes would be detected easily; and 3) information on karyotype would serve as added knowledge to animal breeders in genetic exchange information. The major disadvantage

with this method is that it is expensive, since expensive chemicals and equipment are required.

2.3 Goat Karyotypes

Goats have a diploid chromosome number of 60, 58 autosomal chromosomes and 2 sex chromosomes (Hansen, 1973; Khavary, 1973; White, 1973; Hafez, 1974; Prakash, 1981; Hansanbasic, Kljajic, Milosevic and Horsic, 1984; and Pattnanayak and Patro, 1986).

Khavary (1973) found that the autosomal size ranged from 1 micron to 14 microns in most domesticated livestock. The Y-chromosome has been found to be the smallest (Hansen, 1973; Hafez, 1974; Prakash, 1981; Yeo, 1984 and Pattanayak and Patro, 1986).

With respect to structure, Hansen (1973) and Prakash (1981, 1986) working with Jamunapani and Barbari goats; Yeo (1984) working with Korean native goats and Hansanbasic et. al. (1984) working with local goats from Herzegoria, Yugoslavia, all reported that autosomes were acrocentric or telocentric.

In the case of X-chromosomes, Hansen (1973) and Pattnanayak and Patro (1986), working with preparations made from bone marrow of Ganjam and Black Bengal and F1; Prakash (1981) studying chromosomal complements of the Jamunapari and Barbari goats and Yeo (1984) working with the Korean native goats all found that the X-chromosome was telocentric while Hansanbasic et. al. (1984) found that the X-chromosome

was medium-sized acrocentric.

Basrur and Courbrough (1964) found that the X-chromosome was the largest acrocentric chromosome. The Y-chromosome which is the smallest of the chromosomes was originally described in the acrocentric series (Basrur and Courbrough, 1964; Hancock and Jacobs, 1966; Makino, 1943; Padeh Wysoki and Soller, 1971); and then later as metacentric (Basrur and Stolts, 1967; Chandra, Hungerfood, Wagner and Snyder, 1967; Ilbery and Williams, 1967; Makino, Shimba, Sofuni and Ikeuchi, 1967). However, Hansen (1973), Khavary (1973) and Prakash (1981, 1986) have found that Y-chromosome was sub-metacentric while Yeo (1984) reported it to be metacentric. Pattnanayak and Patro (1986) reported it as a small, round, dot-like structure.

The techniques of band marking as originally used for man have been used for the goat; bands C, G, N, and Q (Evans, Buckland and Summer, 1973; Nadler, Hoffman and Woolf, 1974; Schnedl and Czaker, 1974; Hansen, 1973; Henderson and Bruere, 1979).

2.4 Aberrations

Aberrations that are present in natural populations have a very low frequency of occurence in domestic livestock (Avers, 1976); for example, Cribiu and Lherm (1986) found few chromosomal abnormalities in goats. The chromosomal abnormalities that have been described (Padeh et. al., 1971; Sohrab and Phil, 1972; Cribiu and Lherm, 1986) have been

categorised into 3 classes: firstly, abnormalities of structure, for example Robertsonian translocation; secondly, abnormalities of number, for example anueploid or polyploid; and thirdly, abnormalities of Karyotype which lead to an ambiguous phenotype with associated intersexuality.

2.4.1 Structural Abnormalities

Several abnormalities that have been observed result from Robertsonian translocations - also called centric fusion (Cribiu and Lherm, 1986). They are achieved by the rejoining of 2 acrocentric chromosomes; for example meta-submetacentric monocentric chromosome and meta-submetacentric neochromosome which is dicentric. These abnormalities (Cribiu and Lherm, 1986) do not appear to produce any remarkable effect on the structure and conformation of the carrier animals but result in the lowering of fertility in female heterozygotes (Padeh et. al., 1965, 1971; Soller, Wysoki and Padeh, 1966).

2.4.2 Abnormalities of Number

These are caused by the non-separation (non-disjunction) of chromosomes during mitosis or meiosis of the germinal cells during the first division of the zygote (Nicholas, 1987). The only abnormality of this sort reported to occur in the goat is a mixoploid complex 60, XY/60, X, X composed of a line of normal male cells 60, XY, the Y gonosome being of variable length and a monosonic line 59 (Rieck, Hohn, Loeffer, Marx and Bohm, 1975). This was thought to come about by the non-separation of the Y-

gonosome during the first mitotic division of the embryo, following the fusion of a normal zygote 60, XY with an abnormal zygote 59, X; the absence of the Y-chromosome occuring later (Rieck et. al., 1975).

2.4.3 Abnormalities of Karyotype

These comprise mosaics XX/XY and intersexes XY and XX (Cribiu and Lherm, 1986).

2.4.3.1 Intersex XY

Only one case of this type has been described in goats. The animal had a female appearance but at the same time had a long hypertrophied clitoris and a narrow vagina, presenting a very masculine internal genital tract with testicles found near the inguinal region. This was considered a case of a feminised testicle caused by a recessive mutant gene, which prevented the sexual organs from responding fully to the hormone androgen (Cribiu and Lherm, 1986).

2.4.3.2 Intersex XX

Basrur and Coubrough (1964) found that intersex XX occurs frequently in the Swiss milk breeds, Saanen and Toggenburg, and 15% are characterized by the abscence of horns (polled). Absence of horns and intersex are transmitted by the same allele or two closely allied alleles or a loci situated on an autosome. The first is transmitted as a simple dominant character with complete effect (Biggers and McFeely, 1966; Harmerton, Oickson, Pollard, Grieves and Short, 1969; McFeely,

Hare and Biggers, 1967 and Short, Hamerton, Grieves and Pollard, 1968), while the second behaves like a limited recessive sexual characteristic with variable expressive. In fact the intersexes P/P vary from the almost normal male to the almost perfect female (McFeely, 1967).

Pseudohermaphrodite males have scrotal or inguinal testicles with the derivatives of the wolfian canal well-developed and the derivatives of the Mullerian canal, rudimentary; in contrast to the second group of pseudehermophrodite males. The true hermaphrodite has a hypertrophied clitoris, intraabdominal testicals or ovotestis, a well-developed uterus and vagina and small seminal vesicles, (Hamerton et. al., 1969; Hancock and Jacobs, 1966; McGeady and Fitzpatrick, 1967; Short et. al., 1968; Soller, OPodeh, Wysoki and Ayalon, 1969).

2.4.3.3 The Intersexes XX/XY

Karyotype 60 XX/60 XY of goat intersexes result from fertilization by sperm with a series of non-separations during the first post-zygote division, or the exchange of haemopoetic tissue between 2 foetus of different sex in utero (Cribiu and Lherm, 1986). Two true hermaphrodites and an ovotestis on 1 side and a testicle on the other; all goats XX/XY are freemartins with a hypertrophic clitoris and a female structure derived from Mullerian canals and hypoplastic inguinal testicles.

Freemartinism is thought to be caused by the vascular anastomosis between early placentation of 2 foetus of opposite sex (Bongso, Thavalingam and Mukherjee, 1982; Padeh, Wisoki, Ayalon and Soller, 1965; Smith and Dunn, 1981; Soller et. al., 1969).

CHAPTER THREE:

MATERIALS AND METHODS

3.1 Experimental Animals

The total number of goats sampled, from both Southern and Eastern Provinces, was 20 (5 adult females and 5 adult males from each of the provinces). All were indigenous goats.

3.2 Blood Collection Procedures

Blood was drawn from the jugular vein using a disposable 10 ml syringe and needle, and immediately transferred into 10 ml tubes impregnated with lithium heparin. The tubes were shaken gently for 3 to 5 minutes to allow thorough mixing of the blood with the lithium heparin and then were kept in a cooler box prior to laboratory examination. The equipment is indicated in Appendix 1.

3.3 Chemicals, Solutions and Cultivation Media

3.3.1 Colchicine

One gram of freeze-dried colchicine powder was dissolved into 100 ml of Double Distilled Water (DDW). The solution was then sterilized by filtration through a 0.22 uM pore-size filter and stored in aliquots of 0.1 ml in a refrigerator between 2 degrees C and 4 degrees C (Appendix 1).

3.3.2 Phytohemagglutinin - M (PHA - M)

Five millilitres of DDW was added to freeze-dried phytohemagglutinin-M. The stock solution of 0.1 ml was stored at -20 degrees C (Appendix 1).

3.3.3 Hypotonic Solution

A 0.075 Molar potassium chloride (KCl) solution was made by dissolving 100.59 g of KCl into a litre of DDW. The solution was stored in the refrigerator at a temperature of between 2 degrees C and 4 degrees C (Appendix 1).

3.3.4 Eagles Minimum Essential Medium (MEM)

MEM powder (9.4 g) was dissolved in 800 ml DDW and autoclaved for 15 minutes. Then 100 ml of the MEM solution was mixed with a pre-prepared solution of L-glutamine (0.3 g of L-glutamine dissolved in 200 ml of DDW sterilized by passage through a 0.22 uM filter). Then the total 300 ml of solution was mixed with the remaining, to make a total solution of one litre MEM. Sodium bicarbonate (NaHCO3) was then used to adjust the pH to 7. The medium was stored in the refrigerator at a temperature between 2 degrees C and 4 degrees C.

3.4 Fixative and Stain

3.4.1 Carnoy's Solution

A ratio of 1 part acetic acid to 3 parts of methylalcohol was used. Fifty millilitres of acetic acid was added to 150 ml of methanol. It was stored at room temperature.

3.4.2 Giemsa Solution

Twenty millilitres of 0.067 M Potassium hydrogen phosphate, KH2PO4, was mixed with 20 ml of 0.067 M Sodium hydrogen phosphate , Na2HPO4. The final solution was stored at room temperature.

3.5 Leucocyte Culture Analysis

One mililitre of whole blood was mixed with 3 ml of tissue culture medium (Eagle's MEM) pH 7, which was supplemented with 20% Calf serum. PHA-M (0.1 ml) was added and then the resulting suspension incubated at 37 degrees C for 72 hours. All the additions were done under clean bench conditions. Following incubation, 1 to 2 drops of colchicine were added to the culture using a 5 ml pipette, and the culture was allowed to settle for 90 to 120 minutes. The blood sample was then transferred to 'V'-bottom centrifuge tubes and centrifuged at 0.165 g Relative Centrifugal Force (RCF) for 5 minutes. The supernatant was aspirated. To the deposit at the bottom of the centrifuge tube was added 5 ml of the hypotonic solution and the tubes allowed to settle for 10 minutes. The supernatant was aspirated, 5 ml of Carnoy's solution was added to the leucocyte deposit for fixation (1 volume acetic acid : 3 volumes methanol) and allowed to fix for 20 minutes. This was repeated 3 times in order to wash the cells thoroughly.

After the third washing, a few drops of the leucocyte-cells suspension were smeared onto a clean glass slide and

Stained with Giemsa solution. The slides were examined microscopically and those cells which exhibited well-spread metaphase chromosomes were selected for photography. The chromosomes were cut out from the photographic paper and arranged according to length, size and structure to form the idiograms (Miyake, Inove, Kanagawa, Ishikawa and Mogi, 1982).

3.6 Statistical Analysis

Mean length was estimated and it's 95% confidence interval determined for each pair of chromosomes. For each pair of chromosomes the relative chromosome length was calculated as a ratio of chromosome length to the total length of all the chromosomes.

CHAPTER FOUR:

RESULTS

Blood samples from Northern, Luapula and Central Provinces were not analysed because the blood cells haemolysed. Thus, only blood samples from Southern and Eastern Provinces were analysed.

4.1 Chromosome Number and Length

The 6 indigenous goats for which idiogram were constructed had 60 diploid chromosome number (58 autosomes and 2 sex chromosomes). The chromosome measurements were made after identification and pairing to form idiograms. The chromosome length in microns and relative chromosome length (in percentage) are given in Tables 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6. Estimated relative chromosome length (in percentage), for Choma, Gwembe, Mazabuka, Petauke, Katete and Chipata are 3.22, 3.33, 3.21, 3.23, 3.33 and 3.22, respectively (Tables 4.7 and 4.8).

Table 4.1: Chromosome length of a goat sampled from Choma

Chromosome pair	Chromosome length (microns)	Relative Chromosome length (%)
1	10.5	5.37
2	10.3	5.27
3	9.7	4.96
4	9.4	4.81
5	9.0	4.60
6	8.7	4.45
7	8.5	4.35
8	8.2	4.20
9	7.8	3.99
10	7.6	3.88
11	7.4	3.78
12	7.25	3.70
13	6.9	3.53
14	6.7	3.43
15	6.4	3.27
16	6.0	3.07
17	5.8	2.96
18	5.5	2.81
19	5.3	2.71
20	5.0	2.56
21	4.8	2.45
22	4.7	2.40
23	4.3	2.20
24	4.1	2.09
25	3.9	1.99
26	3.8	1.94
27	3.5	1.79
28	3.0	1.53
29	2.5	1.28
X	8.4	4.30
Y	.5	0.25

Table 4.2: Chromosome length of a goat sampled from Gwembe

Chromosome pair	Chromosome length (microns)	Relative Chromosome length (%)
1	10.2	5.35
2	9.9	5.19
3	9.6	5.04
4	9.4	4.93
5	9.0	4.72
6	8.8	4.62
7	8.6	4.51
8	8.2	4.30
9	7.9	4.14
10	7.8	4.09
11	7.3	3.83
12	6.9	3.62
13	6.6	3.46
14	6.0	3.15
15	6.0	3.15
16	5.8	3.04
17	5.7	2.99
18	5.3	2.78
19	5.1	2.67
20	4.9	2.57
21	4.5	2.36
22	4.0	2.09
23	3.95	2.07
24	3.9	2.05
25	3.85	2.02
26	3.7	1.94
27	3.5	1.84
28	3.1	1.63
29	2.9	1.52
X	8.2	4.30

Table 4.3: Chromosome length of a goat sampled from Mazabuka

Chromosome pair	Chromosome length (microns)	Relative chromosome length (%)
1	12.3	6.18
2	11.0	5.55
3	9.9	5.00
4	9.6	4.85
5	9.0	4.54
6	8.8	4.42
7	8.3	4.16
8	8.1	4.09
9	8.0	4.04
10	8.0	4.01
11	7.5	3.79
12	7.1	3.58
13	6.8	3.41
14	6.3	3.15
15	6.0	3.03
16	5.7	2.88
17	5.3	2.65
18	5.1	2.55
19	4.9	2.45
20	4.7	2.35
21	4.5	2.25
22	4.4	2.19
23	4.2	2.09
24	4.0	2.02
25	4.0	1.99
26	3.7	1.84
27	3.4	1.69
28	2.3	1.64
29	3.1	1.54
X	10.0	5.05
Y	1.0	.50

Table 4.4: Chromosome length of a goat sampled from Petauke

Chromosome pair	Chromosome length (microns)	Relative chromosome length (%)
1	12.1	5.29
2	11.8	5.17
3	11.6	5.09
4	11.1	4.86
5	10.5	4.59
6	10.2	4.46
7	9.9	4.33
8	9.6	4.20
9	9.3	4.07
10	8.9	3.89
11	8.5	3.72
12	8.0	3.50
13	7.7	3.79
14	7.3	3.19
15	7.1	3.10
16	6.9	3.02
17	6.7	2.93
18	6.3	2.76
19	6.2	2.71
20	5.9	2.58
21	5.7	2.49
22	5.5	2.41
23	5.3	2.32
24	4.9	2.14
25	4.6	2.01
26	4.2	1.84
27	3.9	1.70
28	3.7	1.63
29	3.5	1.53
X	11.0	4.82
Y	.5	0.22

Table 4.5: Chromosome length of a goat from Katete

Chromosome pair	Chromosome length (microns)	Relative chromosome length (%)
1	12.0	5.09
2	11.9	5.05
3	11.8	5.00
4	11.6	4.92
5	11.4	4.83
6	10.9	4.62
7	10.5	4.45
8	10.2	4.32
9	9.8	4.16
10	9.6	4.07
11	9.4	3.98
12	8.9	3.77
13	8.5	3.60
14	8.2	3.48
15	7.9	3.25
16	7.6	3.22
17	7.0	2.97
18	6.6	2.79
19	6.2	2.63
20	6.0	2.54
21	5.6	2.37
22	5.5	2.33
23	5.1	2.16
24	4.8	2.03
25	4.7	1.99
26	4.3	1.82
27	3.9	1.65
28	3.5	1.48
29	3.0	1.27
X	9.4	3.98

Table 4.6: Chromosome length of a goat sampled from Chipata

Chromosome pair	Chromosome length (microns)	Relative chromosome length (%)
1	11.5	5.53
2	11.0	5.29
3	10.8	5.20
4	10.4	5.00
5	9.9	4.76
6	9.4	4.52
7	9.3	4.47
8	8.9	4.28
9	8.5	4.09
10	8.1	3.89
11	7.8	3.75
12	7.5	3.60
13	7.2	3.46
14	6.9	3.32
15	6.5	3.13
16	6.2	2.98
17	5.8	2.79
18	5.6	2.69
19	5.2	2.50
20	4.7	2.26
21	4.5	2.16
22	4.3	2.06
23	4.1	1.97
24	3.9	1.87
25	3.8	1.82
26	3.6	1.73
27	3.6	1.73
28	3.5	1.68
29	3.4	1.64
X	10.9	5.24
Y	1.0	0.48

Table 4.7: Summary of relative chromosome lengths based on male goat karyotype from both Southern and Eastern Provinces.

Place	Total chromosome pair	Mean	SEM	d. of f.	to .95	95% confidence interval
Choma	29 XY	3.22	0.227	30	2.042	2.76-3.68
Mazabuka	29 XY	3.21	0.244	30	2.042	2.71-3.71
Petauke	29 XY	3.23	0.228	30	2.042	2.77-3.70
Chipata	29 XY	3.22	0.245	30	2.042	2.72-3.72

Table 4.8 Summary of relative chromosome lengths based on female goat karyotype from both Southern and Eastern Provinces.

Place	Total chromosome pair	Mean	SEM	d. of f.	to .95	95% confidence interval
Gwembe	29 XX	3.33	0.217	29	2.045	2.89-3.77
Katete	29 XX	3.33	0.217	29	2.045	2.89-3.77

4.2 Chromosome Structure

Chromosome structure is given in Table 4.9 and plates 1 to 12. All the autosomes were acrocentric. Goats from Southern Province had smaller, thinner arms whereas those from Eastern Province had slightly larger and thicker arms. In all cases, the x-chromosomes in females were medium-sized and acrocentric (Fig 4.2 and 4.5). In the case of the males, the X - Chromosome was found to be large and acrocentric while the Y - Chromosome was found to be small and dot-like in structure (Fig 4.1, 4.3, 4.4 and 4.6).

4.3 Aberrations

Four general types of chromosome modification which lead to a change in the linear order of the genes on the aberrant - namely, deletions (also called deficiences), duplications, inversions and translocations (i. e. Robertsonian translocation) or even cases of centric fusion - were absent in all the samples that were examined.

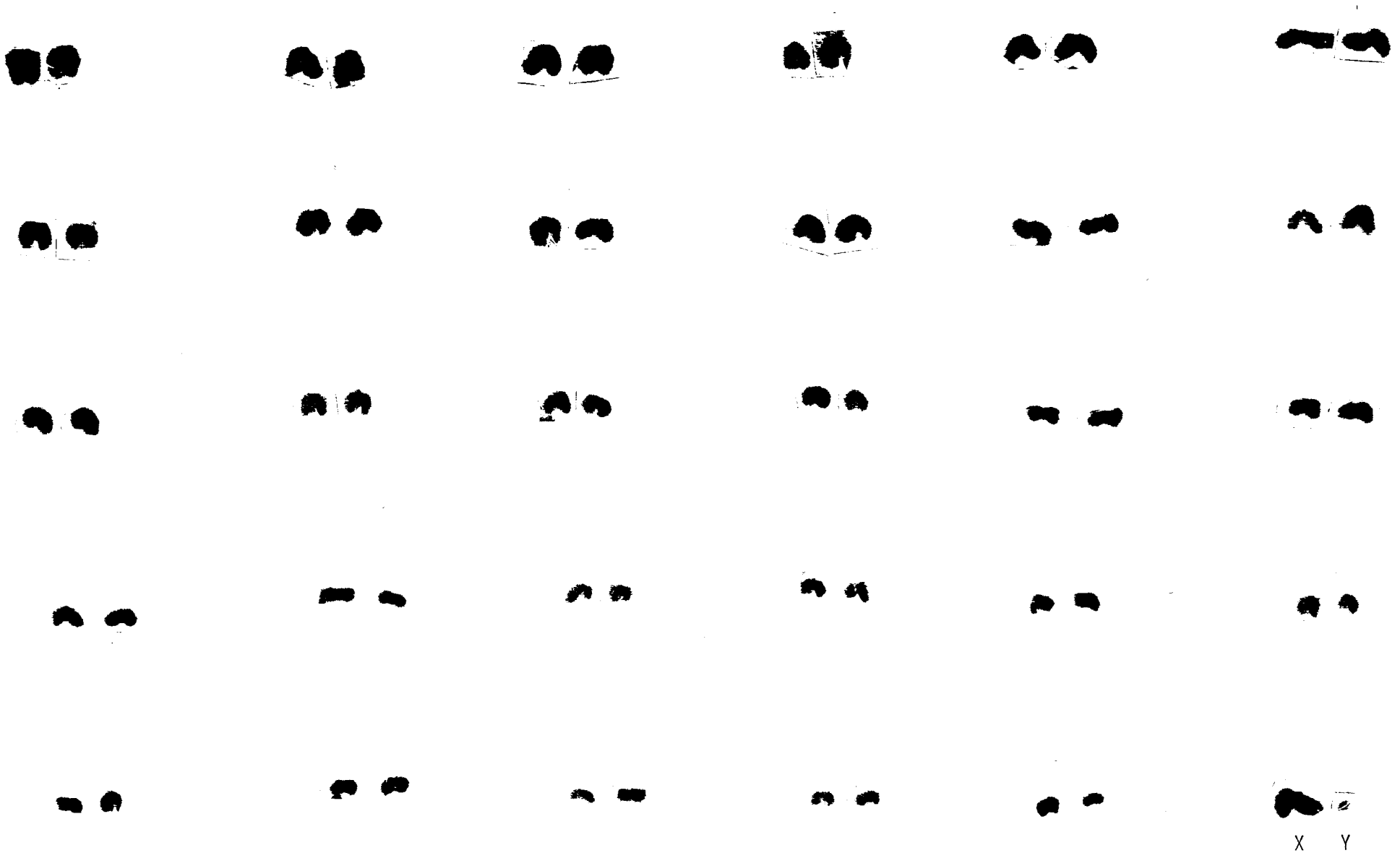


Figure 4.1: Goat karyotype (male), sample obtained in Choma.

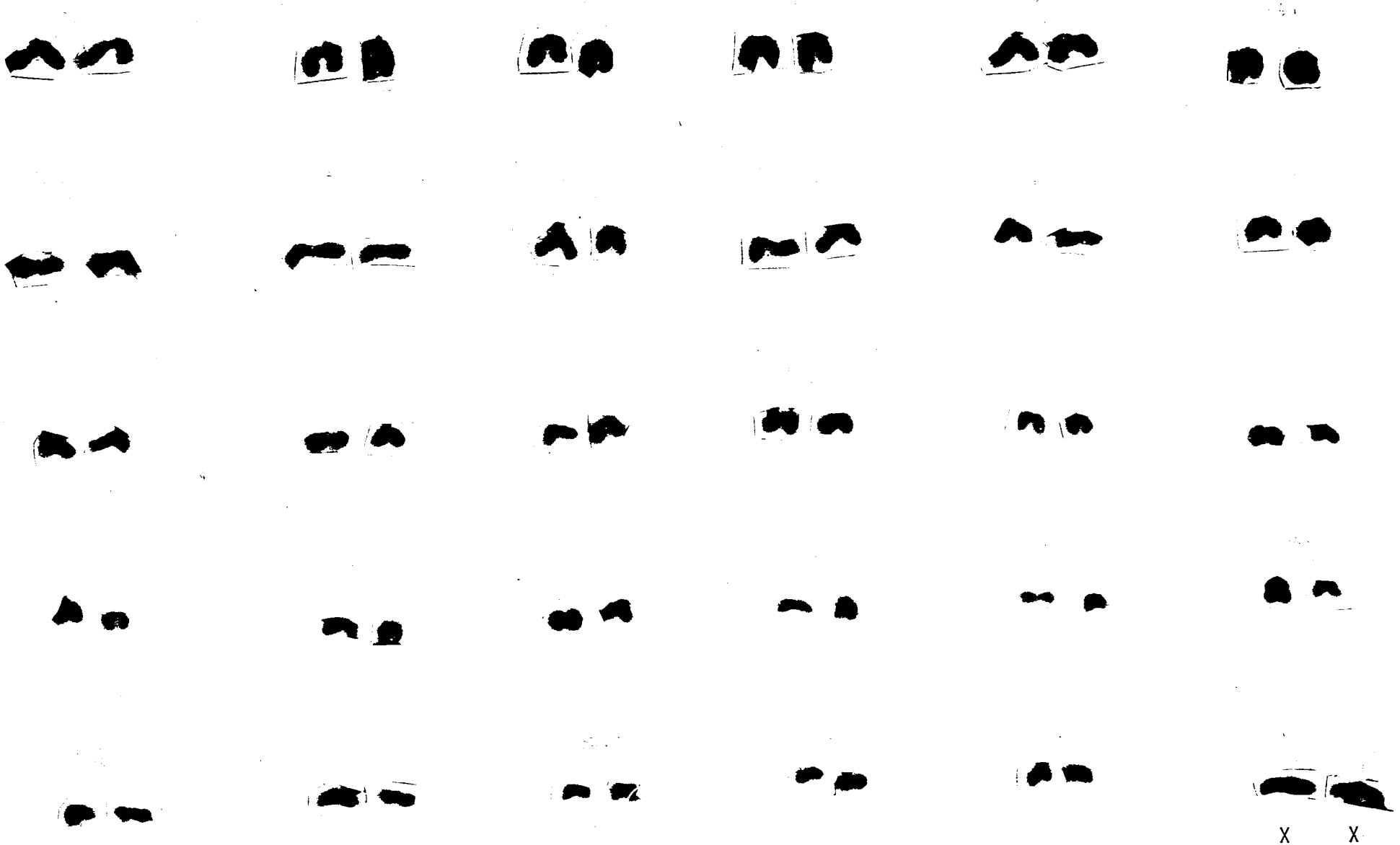


Figure 4.2:: Goat karyotype (female), sample otained in Gwembe.

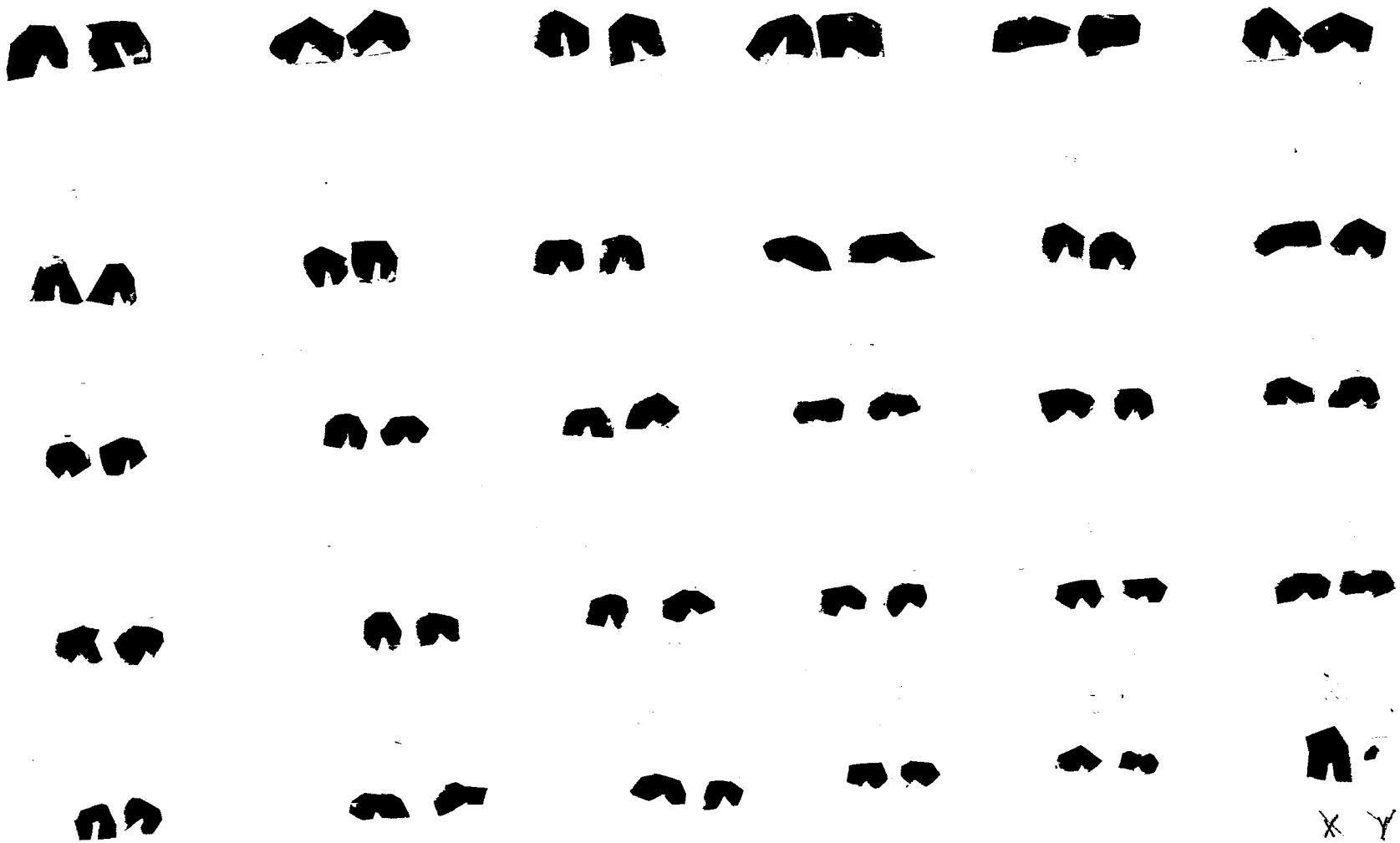


Figure 4.3: Goat karyotype (male), sample obtained in Mazabuka.

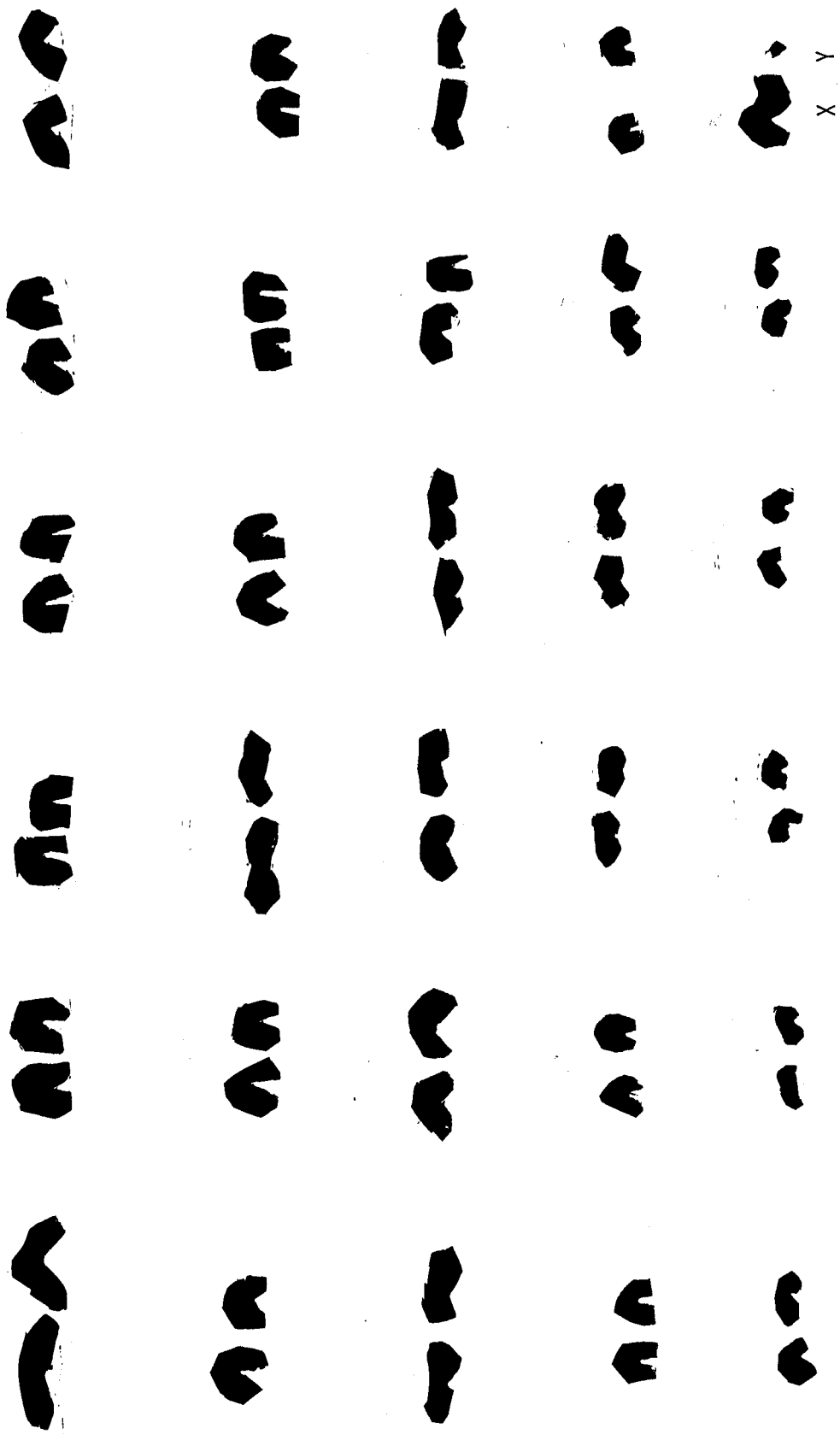


Figure 4.4: Goat karyotype (male), sample obtained in Petauke.

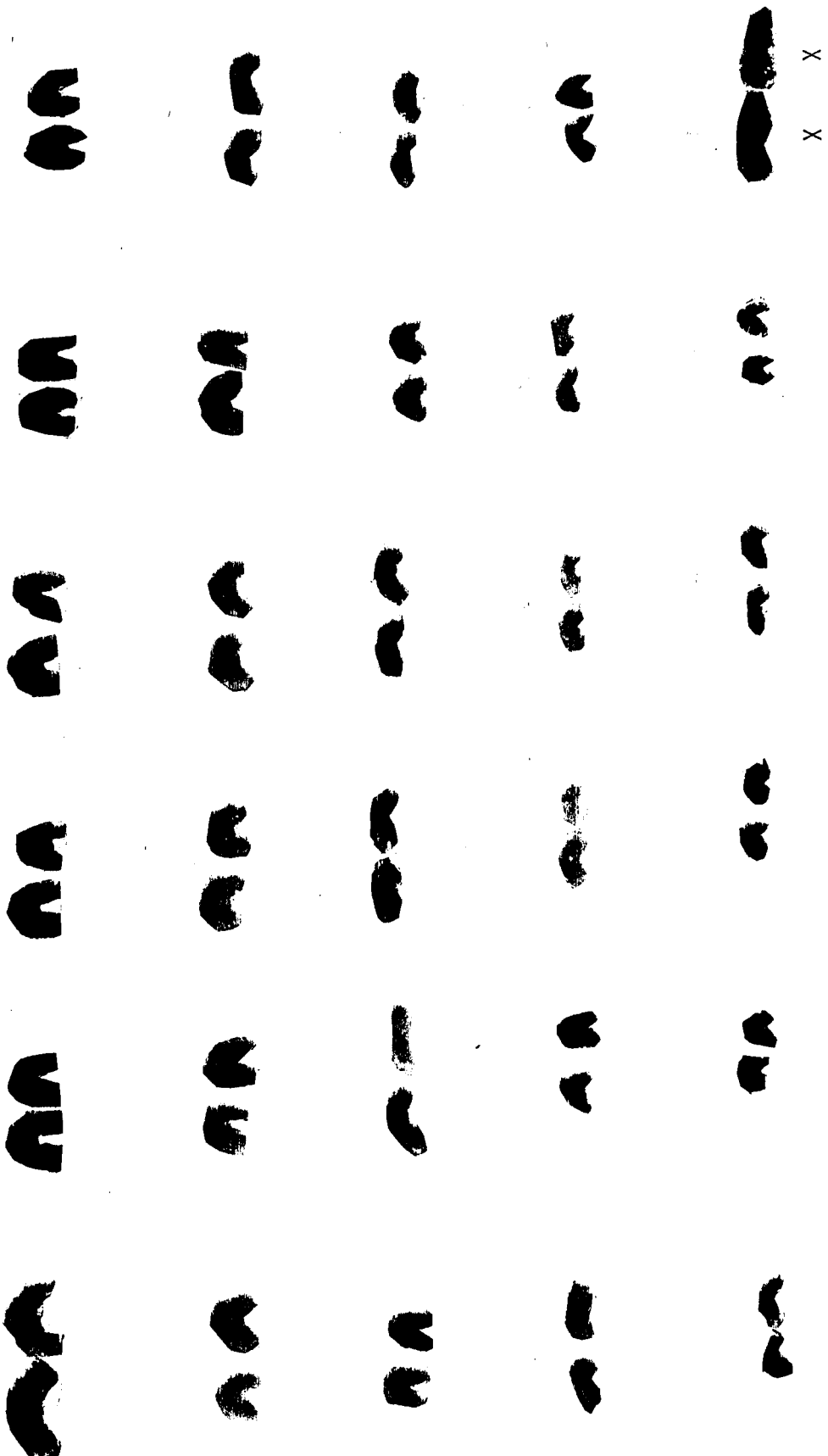


Figure 5.4: Goat karyotype (female), sample obtained in Katete.

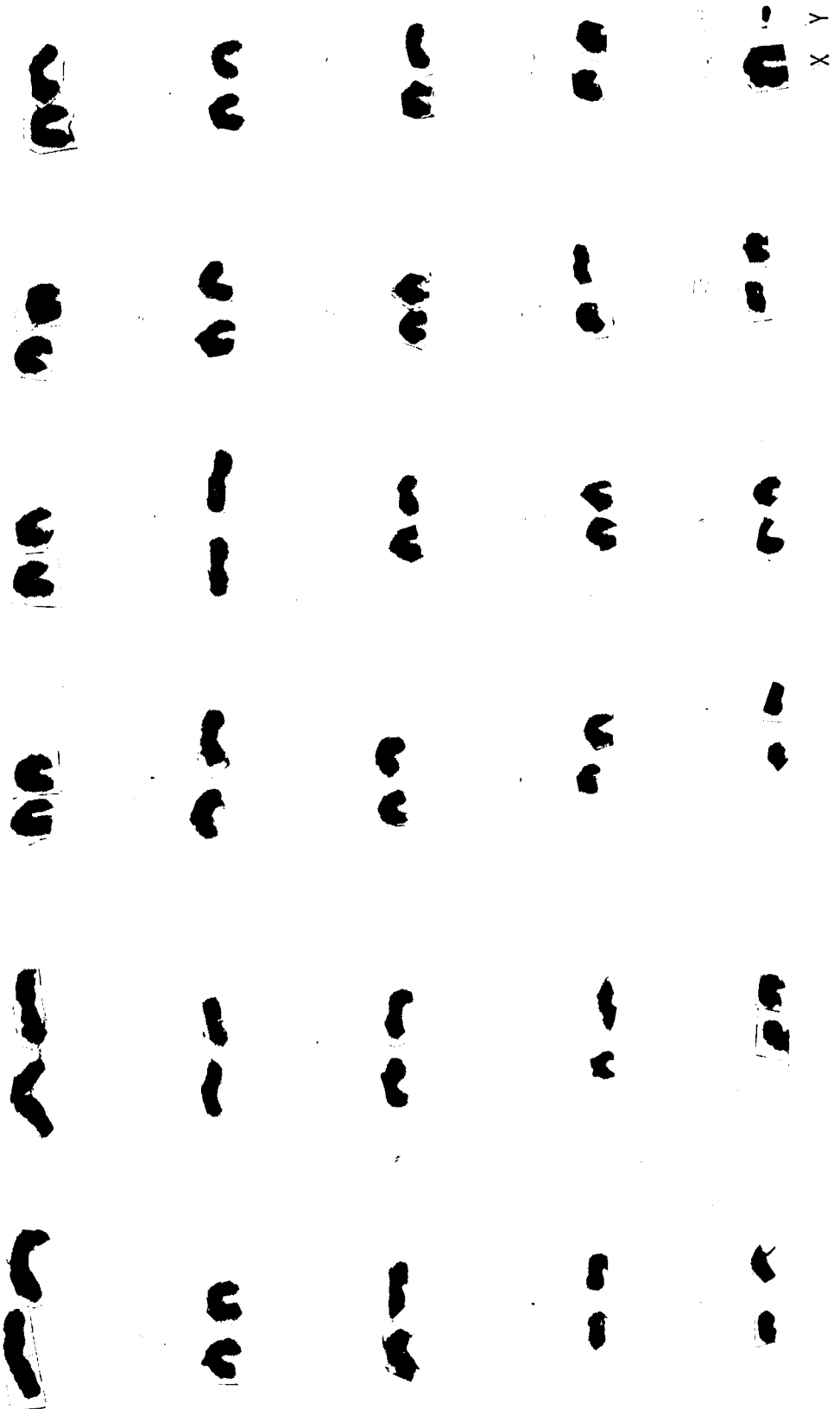
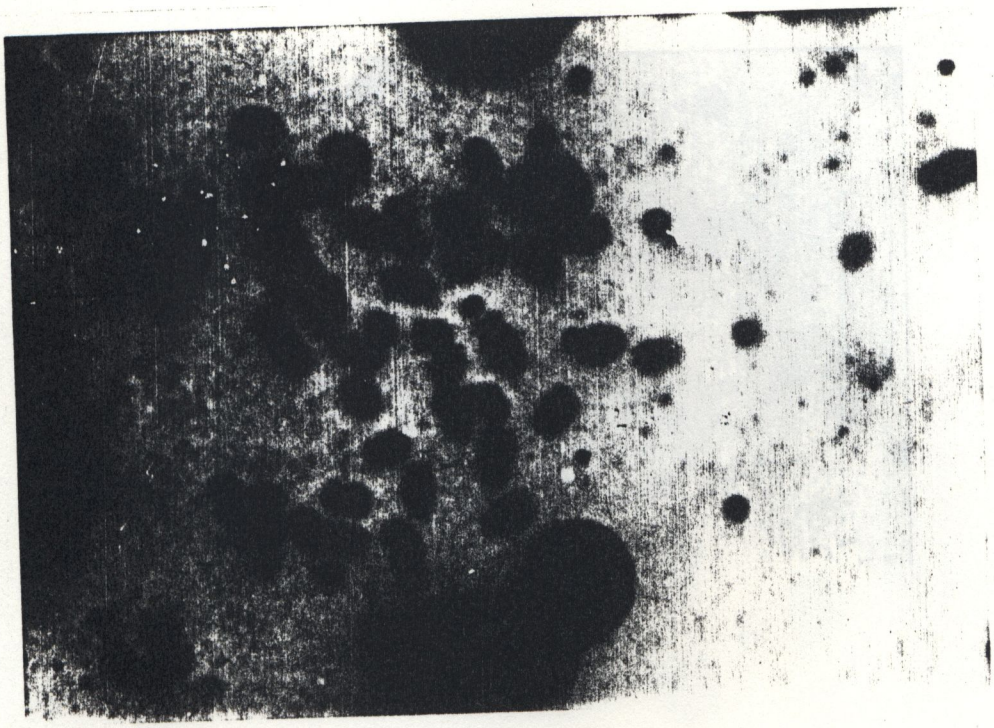


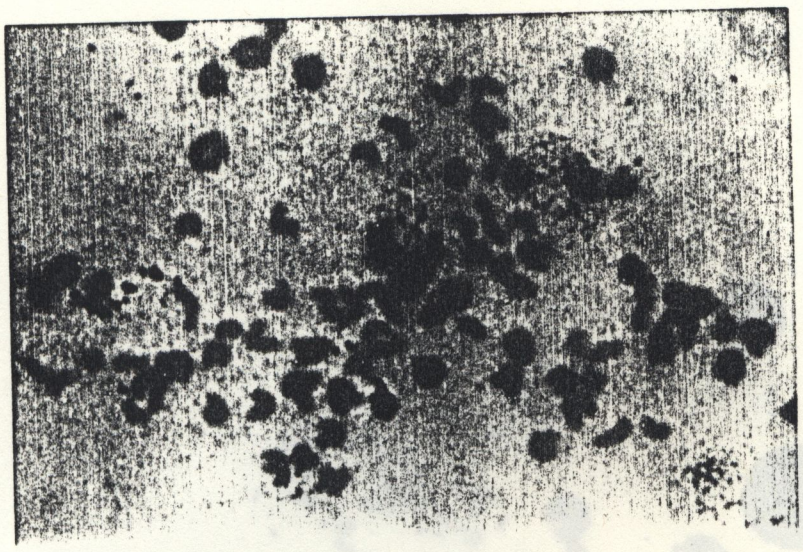
Figure 4.6: Goat karyotype (male), sample obtained in Chipata.



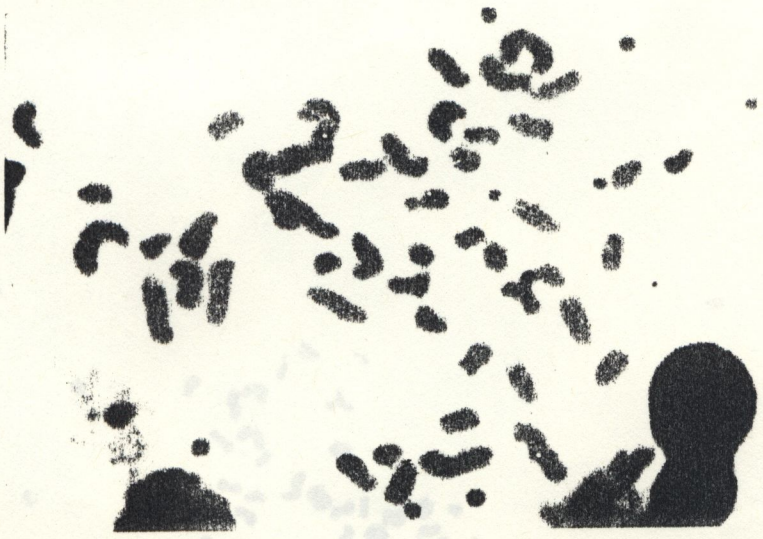
(1)

(2)

Plates (1) and (2) show chromosomes at metaphase stage (x1700). Prepared from samples collected at Choma.

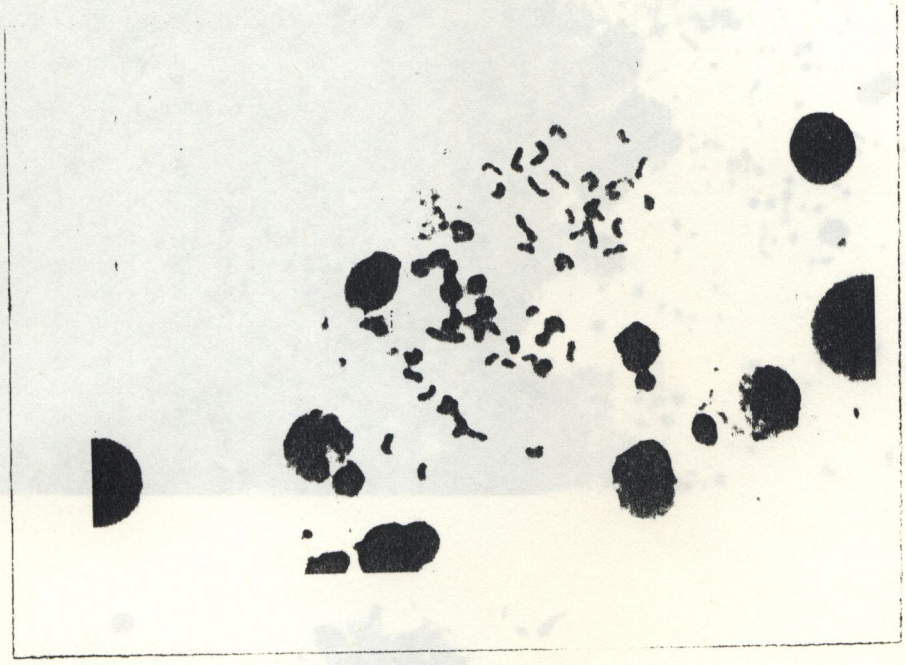


(3)

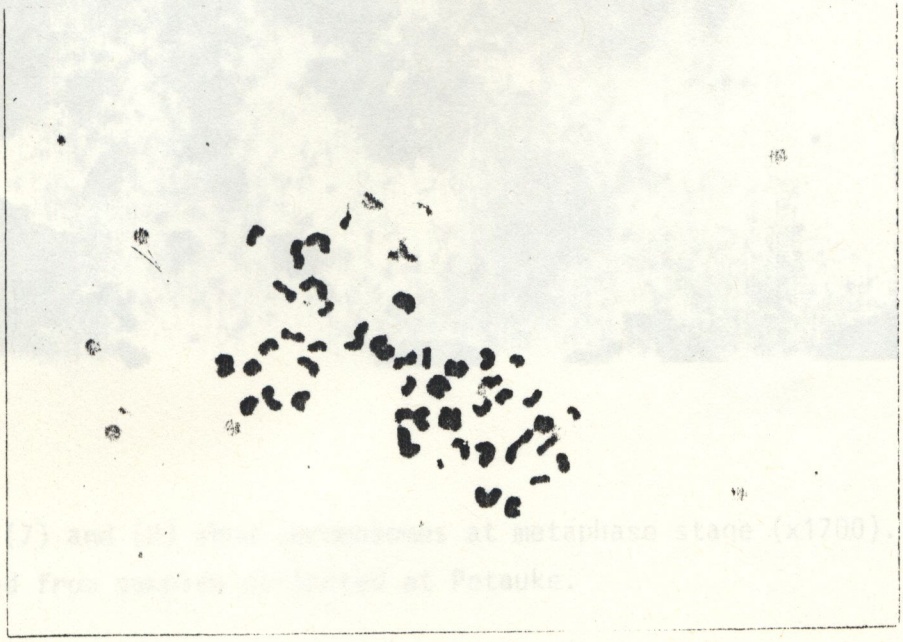


(4)

Plates (3) and (4) show chromosomes at metaphase stage (x1700). Prepared from samples collected at Gwembe.

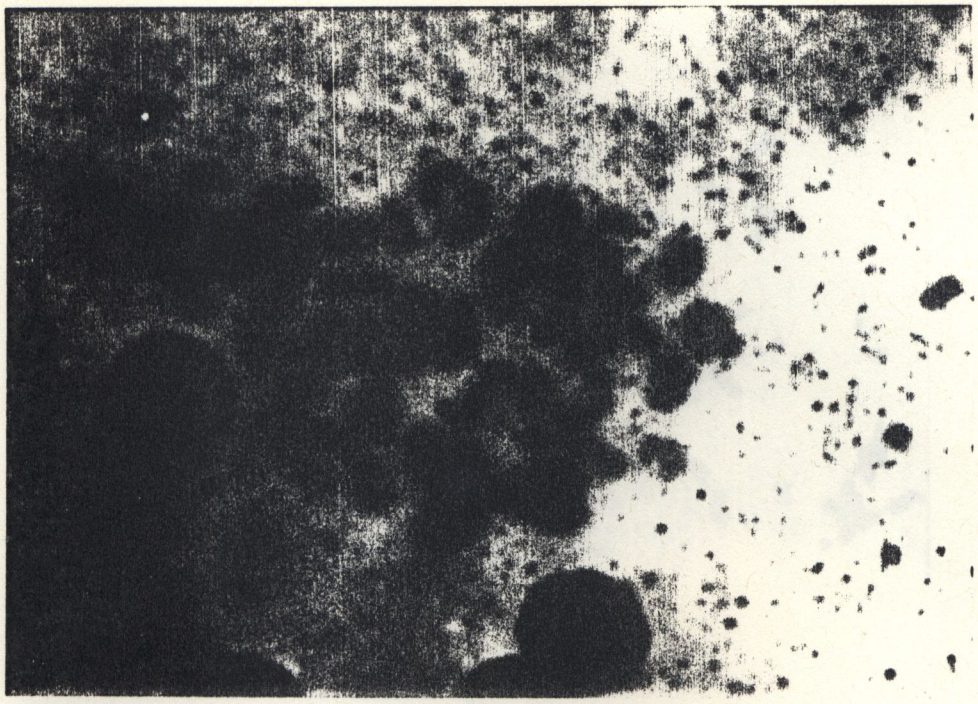


(5)

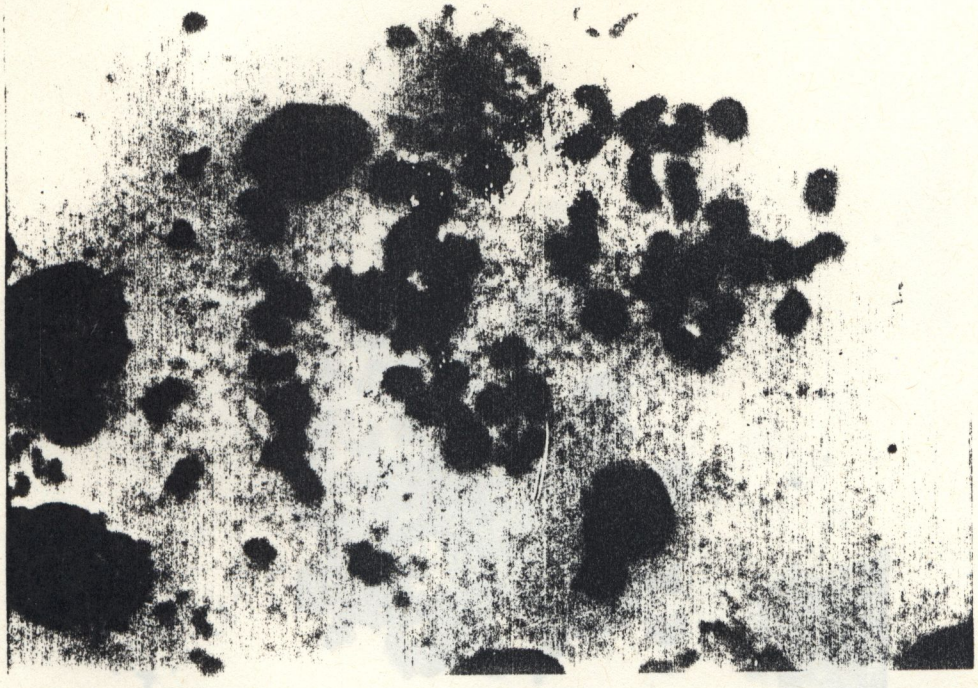


(6)

Plates (5) and (6) show chromosomes at metaphase stage (x1700).
Prepared from samples collected at Mazabuka.

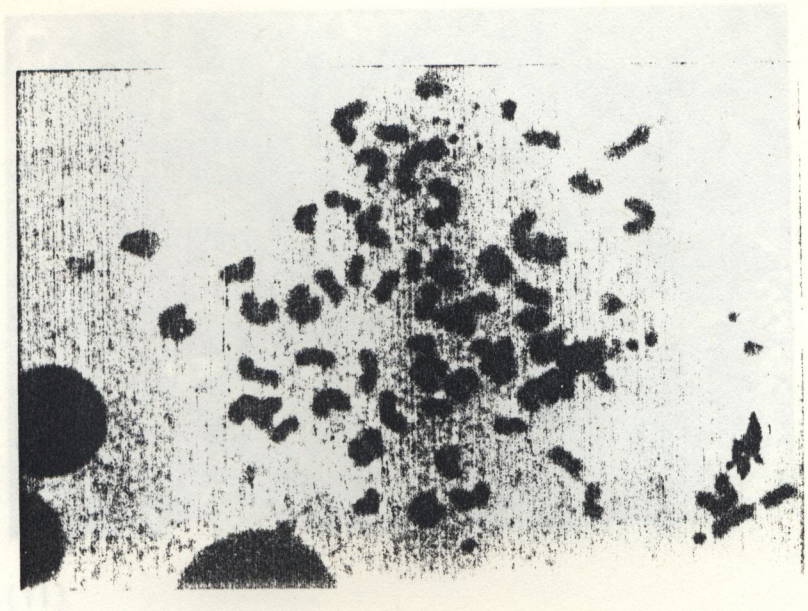


(7)

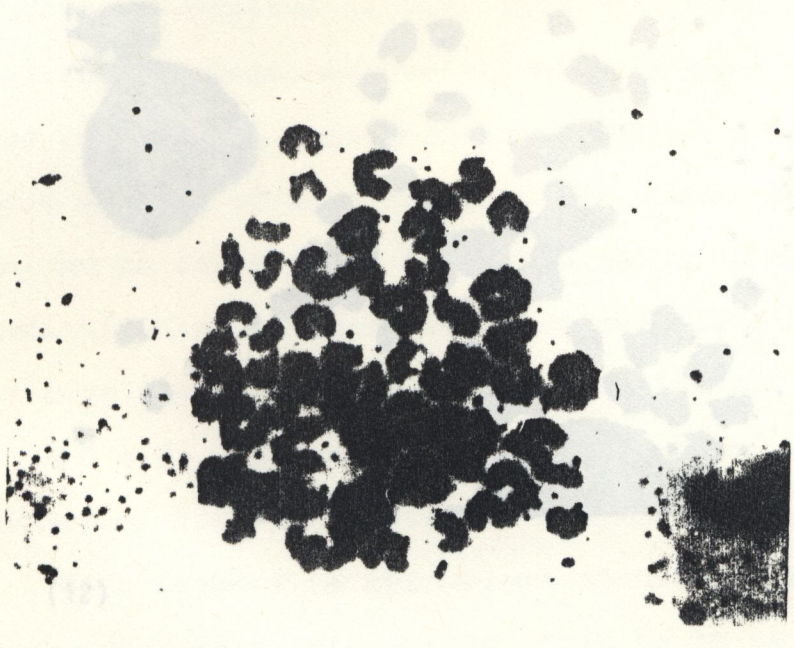


(8)

Plates (7) and (8) show chromosomes at metaphase stage (x1700). Prepared from samples collected at Petauke.

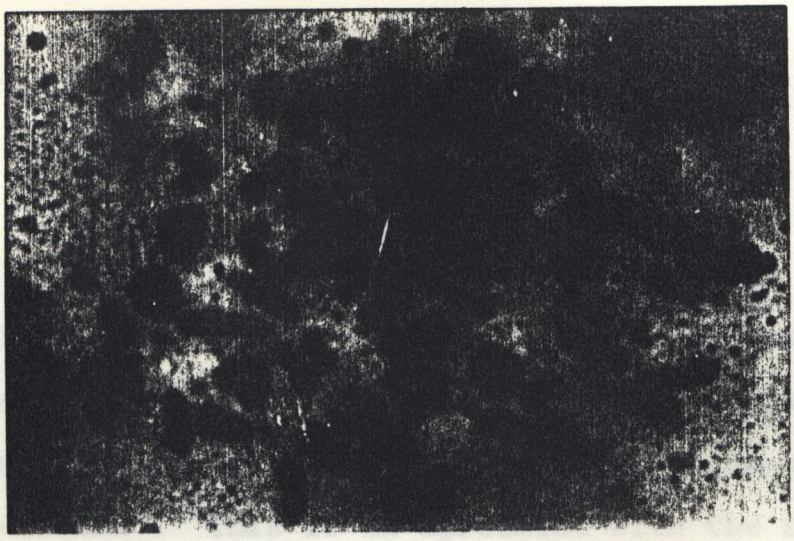


(9)

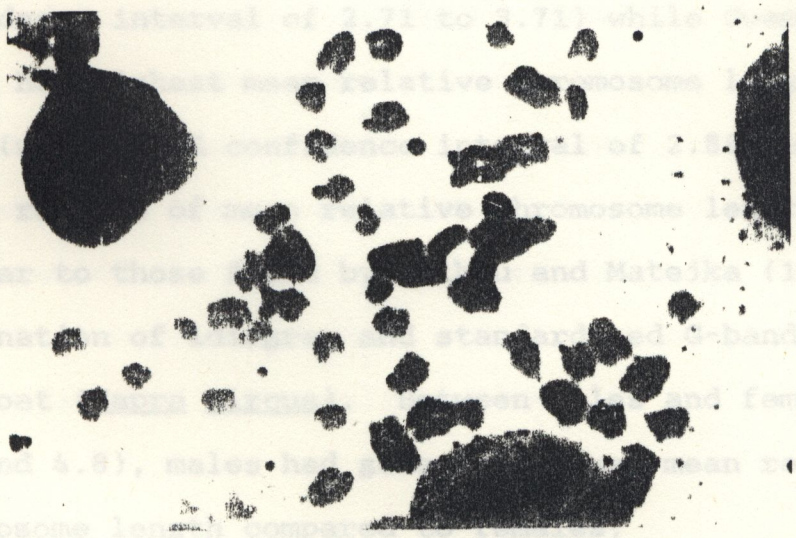


(10)

Plates (9) and (10) show chromosomes at metaphase stage (x1700). Prepared from samples collected at Katete.



(11)



(12)

Plates (11) and (12) show chromosomes at metaphase stage (x1700). Prepared from samples collected at Chipata.

CHAPTER FIVE:

DISCUSSION

With respect to chromosome number, goats in Southern Province (Choma, Gwembe, Mazabuka) and Eastern Province (Petauke, Katete and Chipata) were found to have diploid chromosome number of 60, (58 autosomal chromosomes and 2 sex chromosomes). This is in agreement with the findings of White (1973); Khavary (1973); Hansen (1973); Hafez (1974); Prakash (1981); Yeo (1984); Hansanbasic et al (1984) and Pattnanayak and Patro (1986).

In this study, goats from Mazabuka had the lowest mean relative chromosome length, being 3.21 (with a 95% confidence interval of 2.71 to 3.71) while Gwembe and Katete goats had highest mean relative chromosome length, being 3.33 (with a 95% confidence interval of 2.89 to 3.77). These results of mean relative chromosome lengths are similar to those found by Cribiu and Matejka (1987) in their examination of idiogram and standardized G-band karyotype of the goat (Capra hircus). Between males and females (Table 4.7 and 4.8), males had generally lower mean relative chromosome length compared to females.

With respect to chromosome structure, all autosome chromosomes were acrocentric, this is in agreement with the reports by Hansen (1973); Prakash (1981, 1986); Yeo (1984) and Hansanbasic et al (1984). In the case of sex chromosomes, females were found to have medium sized and

acrocentric X chromosome while the male X chromosome was large and thick acrocentric. This finding is in agreement with what Hansanbasic et al (1984) reported; however, it is in disagreement with Yeo (1984), who reported the X chromosome to be telocentric in some cases and acrocentric in other cases. As for the Y-chromosome, this study found that in both provinces, it was very small and dot-like in structure. Although this finding differed with those of Hansen (1973); Khavary (1973) and Prakash (1981; 1986), who reported that the Y-chromosome was submetacentric and Yeo (1984) who found it as metacentric; it is in agreement with the result of Pattnanayak and Patro (1986).

In the case of aberrations, there were no abnormalities revealed in the samples examined from both provinces. This does not mean that no aberrations exist as the sample size examined was relatively small. However, the findings of this study do correspond to that of Cribiu and Lherm (1986) who reported that the frequency of occurence of aberrations in natural populations were very low; hence, they cannot be easily detected with small samples.

CHAPTER 6:

CONCLUSION

Classification of Zambian indigenous goats has been based on origin, function, structural and morphological appearance (body size, ear shape and length, height at withers) (Devendra and McLeroy, 1982). However, accurate and precise information could be acquired through classification by karyotype method. In this study, chromosome analysis by the karyotype method was used to obtain information pertaining to genetic characterization and differentiation of Zambian indigenous goats.

The results from the two provinces showed that goats had a similar karyotype and are therefore likely to be related. Between provinces, there were no conclusive differences but between males and females, it was noted that females had higher mean relative chromosome length than males. There was no marked differences in the chromosome thickness suggesting that goats in these provinces might be of the same breed. However, due to the small sample size, the result might not be conclusive. Also, had Giemsa staining of the chromosomes been undertaken, more accurate measurements may have been obtained (Cribiu and Matejka, 1987).

One would therefore suggest that further research be conducted to compile more information on their resemblances and differentiation in the chromatin bands. This may be

undertaken using other staining procedures like G, R, and C - banding techniques, as these also make it possible to reveal chromosomal abnormalities (Cribiu and Matejka, 1987); such as translocations, deletions, duplications and inversions.

A similar study should be performed in the remaining provinces.

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APPENDIX 1

1 EQUIPMENT

- Biological cabinet (for tissue culture handling) made by Hitachi Japan
- Centrifuge
- Olympus microscope, model BH-2
- Yamato Incubator model IS-61
- Yamato Autoclave model SDA-30 for sterilizing glassware
- 10 ml syringes
- 10 ml Vacutainer tubes impregnated with lithium heparin
- 1 ml, 5ml and 10 ml sterilized pipettes
- 18 gauge needles
- 20 ml, 50 ml and 100 ml bottles for storing stock solutions
- Glass slides

2 CHEMICALS

- Mitogens (sterile): Phytohemagglutinin-M. Lyophilized product was stored between 2 degrees C and 8 degrees C (Supplier - Wako, Pure Chemicals Industries Ltd Japan)
- Medium: Eagle's Minimum Essential Medium (MEM) 1 litre, fortified with L-glutamine sterilized 20% calf serum and sodium bicarbonate (Supplier - Nissui Pharmaceutical Co Ltd Tokyo Japan)
- Colchicine (Wako Pure Chemicals Industries Tokyo Japan)
- Hypotonic solution: Potassium chloride KCL 0.075 M or Tri-sodium citrate)
- Carnoy's fixative solution: Acetic acid and methanol (1:3)
- Giemsa stain (Kanto Chemical Industries Tokyo Japan)