Meiotic Chromosomes in the Male Slender Loris, *Loris tardigradus lydekkarianus* (Cabrera)

Application of cytogenetics to taxonomic problems has greatly helped in the classification of various groups of animals. Chromosome studies in primates, though meagre, have provided valuable taxonomic information. The lorises are considered as ancestors of the monkeys[1] and it was hoped that studies on lorises would be rewarding. The present study relates to meotic chromosomes of 5 male lorises procured from the forests of Madras, South India. The seminiferous tubules exposed to 0.7% sodium citrate at room temperature for 20 min, were stained with Feulgen and lacto-aceto-orcein. Photographs of the desired stages were taken from temporary squash preparations which were later made permanent.

Observations. Spermatogonial metaphases show 62 chromosomes and consist of both meta and acrocentric chromosomes. One of the large submetacentric chromosomes, the *X* chromosome, shows a secondary constriction (Figure 1). A similar constriction was also observed in the *X* chromosome of the sex bivalent complex (Figures 6, 7d, e, f).

Autosomal bivalents at pachytene show a characteristic chromomere appearance (Figure 2). The sex chromosomes are enclosed in the sex vesicle which stains pink in Feulgen preparations. Association between the sex chromosomes within the vesicle is not clear. However, during diplotene they can easily be identified. There are 30 autosomal bivalents showing 2–3 chiasmata (Figure 3). In certain preparations, the sex chromosomes show a lightly staining euchromatic and a darkly staining heterochromatic segments (Figure 5). In both *X* and *Y* chromosomes the centromere lies in the heterochromatic region. A secondary constriction lies towards the distal end of the heterochromatic segment of the *X* (Figures 6, 7d, e, f). In the short euchromatic region, which is at the distal end of one arm, chiasma formation is noticed (Figure 5a). In the following stages, a typical linear configuration of the sex bivalent held together by terminal chiasma (Figure 4) is seen. In metaphase I, most of the chiasmata are terminalized except in longer bivalents. The sex bivalent always occupies a peripheral position (Figure 6) and consists of a large submetacentric *X* (Figure 7a–f) and small metacentric *Y*. Sex chromosomes segregate in the first division (Figures 8, 9).

Discussion. The diploid number in this loris is 62, as also reported by EGOZCUE et al.[2] from the kidney cells cultured in vitro. According to EGOZCUE there are 18 pairs of meta or submetacentrics and 13 pairs of acrocentric chromosomes. However, in the other Asian loris, *Nycticebus coucang* ($2n = 50$), the karyotype consists of only metacentric chromosomes[3]. It is interesting that in these 2 ecologically close species the morphology and the number of autosomes differ, while the sex chromosomes appear to be similar, i.e. the *X* is a long submetacentric and the *Y* is a small metacentric. Thus changes in the chromosome complex in the 2 species have been confined only to the autosomes. The morphological similarities of the sex chromosomes probably reflect their conservative nature[4].

In the primates, the chromosome number tends to be reduced by the decrease in the number of acrocentrics through structural rearrangements. On the basis of this, BENDER and CHU[5] regard species with more metacentric chromosomes as highly 'specialized' whereas those with more acrocentrics as 'generalized' The assumption is that the species with a lower chromosome number have departed farther from the ancestral type. Following this argument, the slow loris, *Nycticebus coucang*, with 50

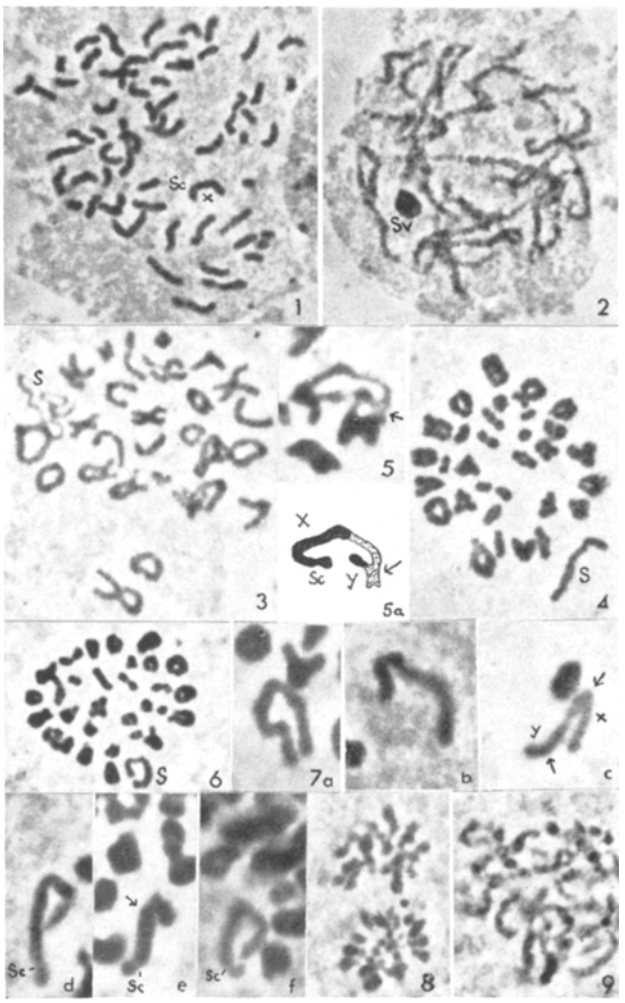

Fig. 1. Spermatogonial metaphase showing 62 chromosomes. The X chromosome shows a secondary constriction (Sc). × 1200. Fig. 2. Pachytene nucleus with a sex vesicle (Sv). × 1200. Fig. 3. Diplotene nucleus. Note the sex bivalent (S) highly distended. × 1200. Fig. 4. Diakinesis. The sex bivalent (S) shows a typical end-to-end association. × 1200. Fig. 5. Diakinesis. Portion of the nucleus with the sex chromosomes. Note a darkly staining (heterochromatic) and a lightly staining (euchromatic) region in the sex chromosomes. Chiasma (arrow) is seen in the euchromatic region. × 1800. Fig. 5a. Line drawing of Figure 5. Fig. 6. Metaphase I. 31 bivalents with the sex bivalent at the periphery. A secondary contriction is seen at the distal end of the *X* chromosomes. × 1200. Fig. 7a–f. Various configurations of the sex bivalent. Arrow indicates the region of the centromere. Secondary constriction (Sc) is seen at the distal end of the *X* chromosome (d, e, f). × 1800. Fig. 8. Anaphase I. × 1200. Fig. 9. Secondary spermatocyte showing a highly condensed *X* chromosome. × 1200.

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metacentric chromosomes is to be considered more 'specialized' and diverged from the ancestral type, while the slender loris, *L. t. lydekkarianus* with 62 chromosomes comprising both acro- and metacentrics, is regarded as 'generalized' and nearer to the ancestral type.

The sex chromosomes in pachytene are confined to the sex vesicle as in man, mouse, and field vole. The exact nature of association within the vesicle is not clear. At later stages, however, the 2 segments, the euchromatic and heterochromatic, are recognisable. Homology between the sex chromosomes can be accounted for only in this short euchromatic segment. Chiasma formed in this segment, rapidly terminalises to form typical end-to-end type of association. Similar association has also been observed in the hamster sex chromosomes[6] and is considered to be due to the crossing over in a minute euchromatic segment which terminalizes before they become visible as separate units.

Failure to demonstrate a side-to-side paring between *X* and *Y* in many mammals has raised doubts regarding chiasma formation between them[7-9]. However, recent electron microscope studies[10] have clearly demonstrated a side-to-side pairing in the sex vesicle of hamster and mouse[11].

Zusammenfassung. Die diploide Chromosomenzahl bei *Loris tardigradus lydekkarianus* beträgt 62, und der Karyotyp besteht sowohl aus akro- wie aus metazentrischen Chromosomen. Beobachtungen über die meiotischen Chromosomen des Männchens ergeben, dass sich die Chiasmaformation zwischen *X* und *Y* befindet.

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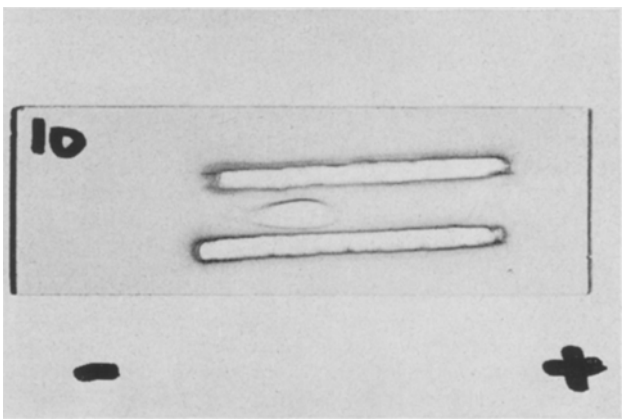
A Serum Antigen Detected in Cattle by Micro-Immuno-Diffusion

Interest in inherited differences in serum antigens (allotypes) has been increasing in recent years in view of their importance in genetics and in plasma protein chemistry. Intraspecific genetic differences in serum proteins have been described for a number of mammalian species[1-5]. Very recently they have been detected in cattle[6] also. This communication reports a serum antigen detected in cattle by micro-immuno-diffusion. The antiserum used for these studies was obtained by iso-immunization (one injection s.c. and two i.m., each at an interval of 10 days; and 6 months later, as a booster dose, one more injection s.c.) with pooled bovine sera diluted 1:1 with hemagglutination buffer (Difco Laboratories, Detroit, USA) and then emulsified with an equal volume of Freund's complete adjuvant. Before being diluted, the pool of bovine sera was frozen and thawed 8 times to produce some slight denaturation or alteration of the proteins and in this way to increase their antigenicity. Serum samples were tested on 26×76 mm microscope slides using 1% Special agar-Noble (Difco Laboratories, Detroit, USA) as supporting medium. The diameter of the wells was 1.7 mm, the distance between the central well and the 4 peripheral wells was 2 mm.

Each serum sample was tested undiluted as well as diluted by two-fold dilutions up to 1/8. The antiserum was used undiluted. The slides were read after 12 h. At this time all the precipitation lines had developed and further incubation did not reveal additional lines.

The mobility of the Ci(a+) antigen (Figure) was studied by immuno-electrophoresis using the micromethod described by HIRSCHFELD[7] (barbiturate buffer, pH 8.6, 7 V/cm for 90 min).

The data of the Table correspond well with the results expected if the antigen follows mendelian segregation with negative subjects homozygous for a recessive gene Ci and positive subjects homozygous or heterozygous for the allelic gene Ci^A (named provisionally using a two letter abbreviation derived from the name of the animal that produced the antiserum). As expected, matings of negative parents gave only negative offspring. Matings of positive parents gave at least 75% positive offspring



Immuno-electrophoresis mobility of the Ci(a+) antigen. Direction of migration: from left (cathode) to right (anode). Amidoschwartz staining. Upper trough: antiserum collected after the booster dose (same antiserum used for the immuno-diffusion studies). Lower trough: antiserum collected after the third injection.

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