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Identification and Patterns of Synapsis of the Autosomally Translocated Y-chromosome of the Indian Mongoose, *Herpestes auropunctatus* (Hodgson)*

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Abstract. The multiple sex chromosome system, X_1X_2Y ♂/$X_1X_1X_2X_2$ ♀, in the small Indian mongoose, *Herpestes auropunctatus,* results from a translocation of a part of Y chromosome to an autosome. It is not possible to distinguish the autosome which harbours the Y chromosome element in the somatic complement. By employing the surface-spreading technique to prophase I meiocytes we have identified the region to which the Y chromosome has been translocated as the short arm of chromosome 9 which is a subtelocentric chromosome. This Y chromosome component lacks heterochromatin and no sex vesicle is organised during meiotic prophase. This suggests to us that Y heterochromatin in mammals may be required for the production of a sex vesicle.

Introduction

In mammals the Y chromosome is specific for maleness and exerts a decisive influence on the ontogeny of spermatocytes. Its association, either side-by-side (parasynapsis) or end-to-end (telosynapsis), with the X chromosome during the first meiotic prophase is normally a prerequisite to ensure its proper disjunction during anaphase-I. In mouse and man, male carriers of an X- or a Y-autosome interchanges are associated with spermatogenic arrest. This occurs at the pachytene stage in X–A and at the spermatid stage in Y–A heterozygotes (Chandley, 1981). This contrasts with the situation where such translocations form an integral part of the sex chromosome system as they do in a number of mammals (Fredga, 1970).

The Indian mongoose, *Herpestes auropunctatus* (Order Carnivora), was the first Eutherian species in which the Y chromosome could not be identified in somatic cells and which showed an odd diploid number (2n = 35)

* We take great pleasure in dedicating this paper to our revered teacher Prof. S.P. Ray-Chaudhuri, who initiated us to the field of Cytogenetics, on the occasion of his 75th birth day

in males as compared to females (2n=36) (Fredga, 1964). Studies on meiosis in this species revealed an X chromosome-associated heterotrivalent at metaphase I implying the translocation of the sex determining region of the Y chromosome to an autosome. Such a sex chromosome system is not unique to *H. auropunctatus* (see Fredga, 1970) but, whereas in other species it is easy to identify the respective sex chromosomes, in *H. auropunctatus* investigations with Giemsa banding, autoradiography and C-banding techniques have all failed to reveal the Y chromosomal material in the mitotic complement; nor has it been possible to unambiguously identify the autosome to which it has been translocated (Cohen and Chandra, 1970; Sen and Sharma, 1979).

Failure to detect the Y chromosomal material by routine cytochemical methods clearly indicates that in *H. auropunctatus* the heterochromatin of the progenitor Y has been almost totally eliminated. This implies that the translocated portion of the Y chromosome must consist predominantly of the sex-determining segment. It would be of interest, therefore, to identify unambiguously the Y bearing autosome as well as to evaluate the extent of synapsis this chromosome would have with the two X chromosomes during meiotic association. This would give a clear idea about the amount of Y chromosomal material retained in *H. auropunctatus*.

The adaptation of the surface spreading technique for light microscopy to identify individual synaptonemal complexes (SCs) as well as the axial element of sex chromosomes in primary spermatocytes has provided a relatively simple method for both meiotic karyotyping and for the visualization of various modes of sex chromosome association during meiotic prophase (Fletcher, 1979). By applying this technique to meiocytes of the mongoose, it should be possible to identify both the Y bearing autosome and the nature of its association with the X_1 chromosome. In the present paper we have employed this technique with meiocytes to achieve this end and by extrapolating our observations to the somatic complement we find that a subtelocentric chromosome, the 9th pair of the complement, carries the Y chromosomal material.

Materials and Methods

Three individuals were used for the present study. Mitotic chromosomes were prepared from colchicinised bone marrow cells employing techniques routinely used in our laboratory (Sharma and Raman, 1973). C-banding was performed using method of Sumner (1972). For fluorescence studies Actinomycin D-Hoechst technique was followed (see Nanda and Raman, 1981).

To study the sex chromosomal association in meiocytes slides were prepared in the following way:

1. Cells exposed to prolonged hypotonic treatment were dropped on to 0.4% formvar-precoated slides.

2. A few drops of fixative (0.4% paraformaldehyde, pH 8.5) were added to the hypotonic on the slides, to fix the cells.

3. After 15–20 min, the fixed cells were dipped into 0.4% photoflo and air dried.

4. Aqueous silver nitrate staining was carried out at 50° C in a moist chamber for 3–4 hr. The silver impregnation was monitored by repeated observation under light microscope.

Certain slides were stained with Coomassie brilliant blue in place of silver nitrate for 15 min to 1 hr at room temperature. The staining solution was prepared according to Wang and Juurlink (1979). The CBB-stained slides were rinsed in distilled water, air dried and mounted in DPX. The CBB staining was comparable to silver nitrate and some times preferred due to its simplicity and rapidity (Nanda, 1981).

Giemsa stained meiocyte preparation for observing metaphase-I cells were made by the usual technique of hypotonic treatment and acetic acid-methanol fixation.

Results

The somatic count in *H. auropunctatus* (2n=35 ♂/36 ♀) agrees with that reported by Fredga (1964). This author subsequently claimed that whereas the four large t chromosomes of the diploid set all have small but distinct short arms in the female, only 3 of them did so in the male. He, therefore, identified the chromosome lacking the short arm as the Y (Fredga, 1970). We have failed to identify such a heteromorphism and are of the opinion that while the X_1 is a medium-sized metacentric both the X_2 and Y are medium-sized subtelocentrics (Fig. 1). Bone marrow cells were also grown in vitro in the presence of Hoechst 33258 for 8 and 6 hr prior to harvesting to inhibit the overall condensation of metaphase chromosomes in order to determine whether heteromorphic regions could be discerned in less condensed chromosomes but with no success. Hence the identification of the Y chromosome was not possible in the mitotic complement. C-banding, as well as Hoechst fluorescence analysis (Fig. 2) also fail to reveal any characteristic sex chromosome related heterochromatin, though the C-band pattern does corroborate the earlier report of Sen and Sharma (1979) that only 2 pairs of autosomes display prominent blocks of pericentric heterochromatin in *H. auropunctatus* with only faint indication of C-banding in other chromosomes.

Meiosis

Meiosis has been observed both in acetic acid-methanol fixed and Giemsa-stained, as well as in surface-spread, silver-stained, meiocytes. In the former no sex vesicle like structure is found in pachytene cells (Fig. 3). The apparent lack of the sex vesicle is further confirmed by fixing the cells directly without any hypotonic treatment. These cells, stained with Giemsa, also fail to display a characteristic sex vesicle. Only rarely it is possible to identify the X_1 chromosome at the zygotone/pachytene stage (Fig. 3). During metaphase I, as reported by Fredga, the X_1 chromosome, which is a diffused, negatively heteropycnotic element, forms an end-to-end association with one member of a bivalent thus implying the presence of a Y chromosome element on that chromosome (Fig. 5g–i).

The surface-spread, silver-stained zygotene/pachytene sparmatocytes, on the other hand, reveal a clear association between the X_1 chromosome and a part of one of the autosomal bivalents (Fig. 4). The X_1 chromosome is easily marked by its conspicuous, thickened axis in the X_1-autosome

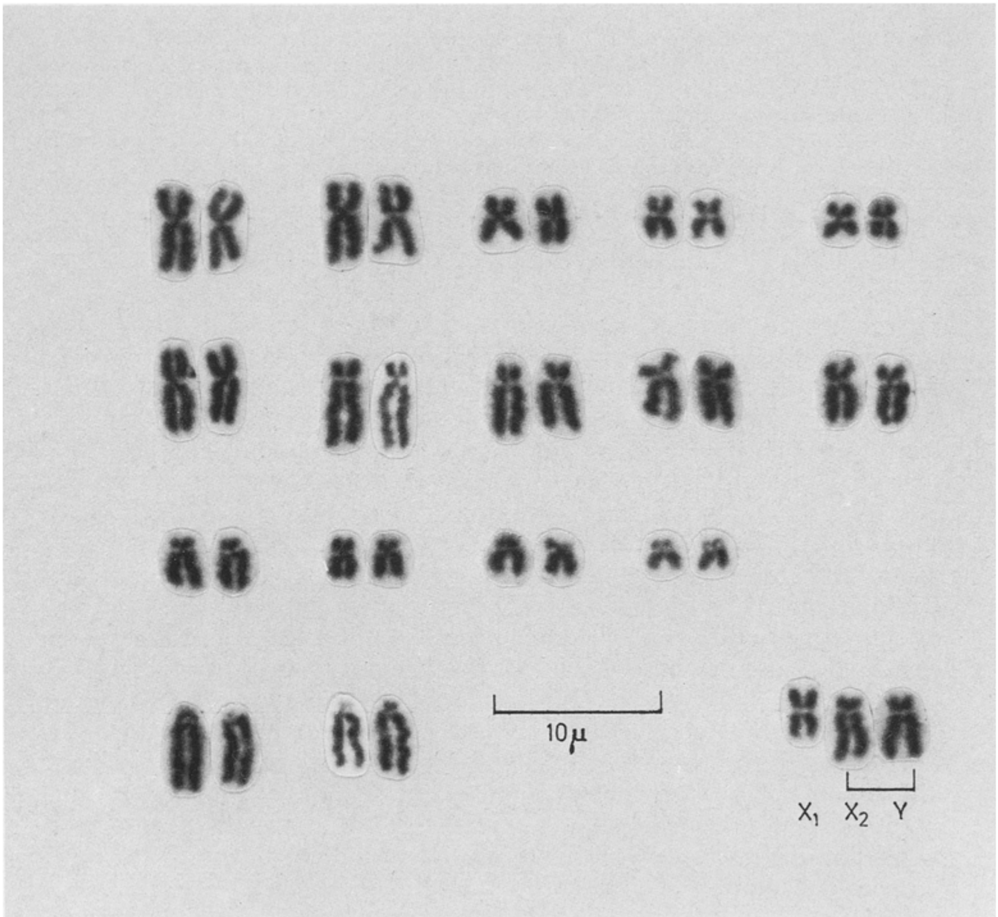


Fig. 1. Karyotype of a male *H. auropunctatus*. Since it is not possible to individually identify the X_2 and Y chromosomes they are grouped by a common bracket (⎵)

heterotrivalent and is either smooth, without any bulges and swellings, or, in certain cells has a serrated appearance. However, unlike the observations of Pathak and Lin (1981) autologous pairing within either the X_1 or any other bivalent was seldom observed. Out of 148 pachytene cells analysed, association of the X_1 with an autosomal bivalent was clearly observed in 115 cases. Of these, pairing of a small, terminal portion of the autosome with the X_1-chromosome was found in 50 cells (Fig. 5a, b) while in 65 cells this association is strictly end-to-end (Fig. 5c). In most other cells the X_1 chromosome was present as a univalent though in a few the precise association was indistinct. The side-by-side or end-to-end types of associations observed are, in all probability, an expression of respective stages of pachytene progression. The linear, end-to-end association holds the heterotrivalent together until metaphase-I as evidenced by the metaphase-I

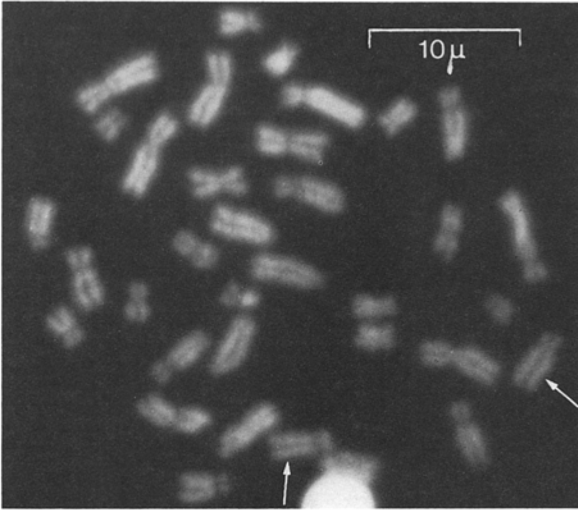


Fig. 2. A somatic metaphase from a male mongoose stained with H-33258

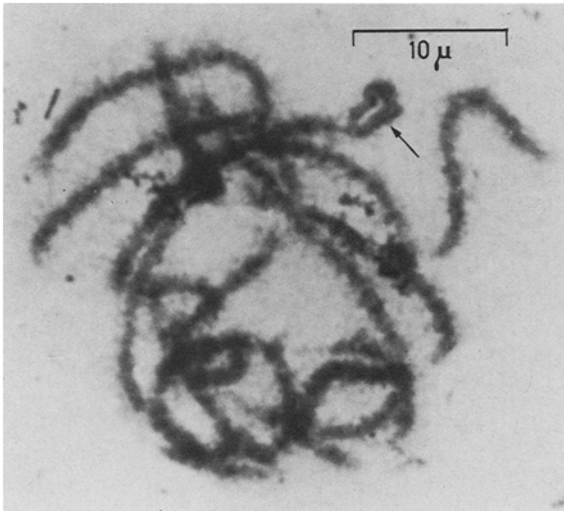


Fig. 3. Giemsa-stained pachytene plate which fails to show a sex vesicle. The X_1 chromosome is marked by an *arrow*

configurations (Fig. 5j–l). The X_1-autosome association thus makes it clear that a substantial, cytologically resolvable, segment of the Y chromosome is retained on an autosome. Synapsis is particularly clear in certain favourable plates where a small segment of the autosome, thinner than the bivalent, can be seen extending towards, and pairing with, the X_1-chromosome

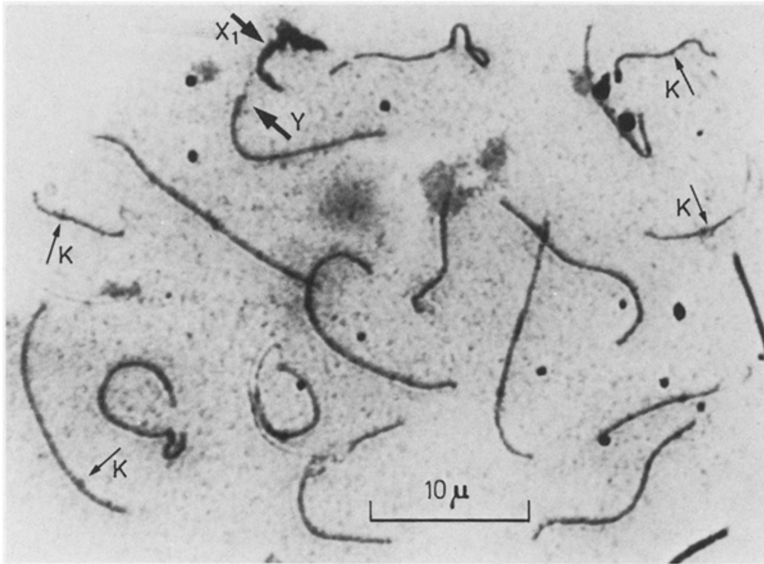
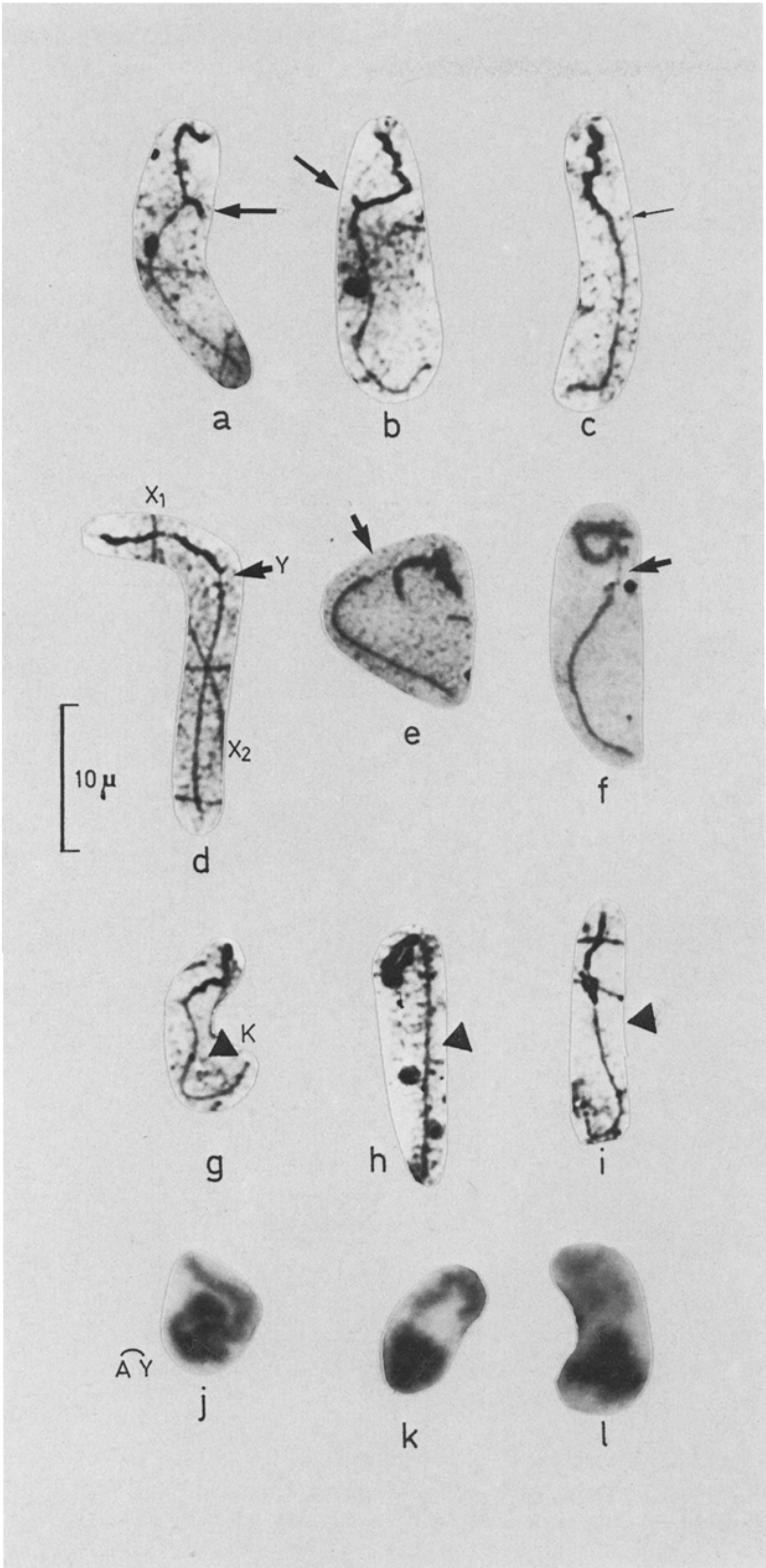


Fig. 4. A silver-stained pachytene plate showing an autosome attached with the X_1 chromosome (*thick arrows* at the point of association). The kinetochores in individual bivalents are denoted by *small arrows*

(Fig. 5d–f). This represents that portion of one autosome which has no homology with its homologue and pairs instead with the X_1.

In order to identify the autosome to which the Y chromosome material has been translocated 22 pachytene plates, 16 from one individual and 6 from another, have been karyotyped and measured (Fig. 6; Table 1). The centromeric position of the Y-bearing autosome has also been defined. As seen from the Table, in 16 plates the 9th largest chromosome is found to associate with the X_1 chromosome. In 2 plates it turns out to be the 10th largest while in one each it is the 2nd, 5th, 6th and 8th largest chromosome. We consider this discrepancy to be an artefact of differential contraction since in almost all the discrepant cells the size of chromosome was different. It is not always possible to locate the kinetochore on each bivalent, especially on the X_2-associated bivalent. Even in those cells where it was distinct the centromeric index of the Y bearing autosome varies between 10 and 30 in different cells. Nevertheless, there is no ambiguity about the biarmed nature of the chromosome concerned. When compared with the

Fig. 5a–l. Cut-outs of the sex heterotrivalents from (**a–i**) silver stained pachytene and (**j–l**) metaphase-I plates displaying different orientations of X-Y pairing. *Arrows* indicate the points of association between X_1 and Y. In **a–c** different degrees of synapsis, ranging from extensive pairing (**a**) to end-to-end attachment (**c**) are seen. Certain less frequently seen associations a thread like Y component stretching from X_2Y bivalent to pair with X_1, are illustrated in **d–f**. Kinetochore positions in X_2Y bivalents are seen in **g–i** (*arrowhead*). The retention of end-to-end association between sex chromosomes up to metaphase-I is depicted in **j–l**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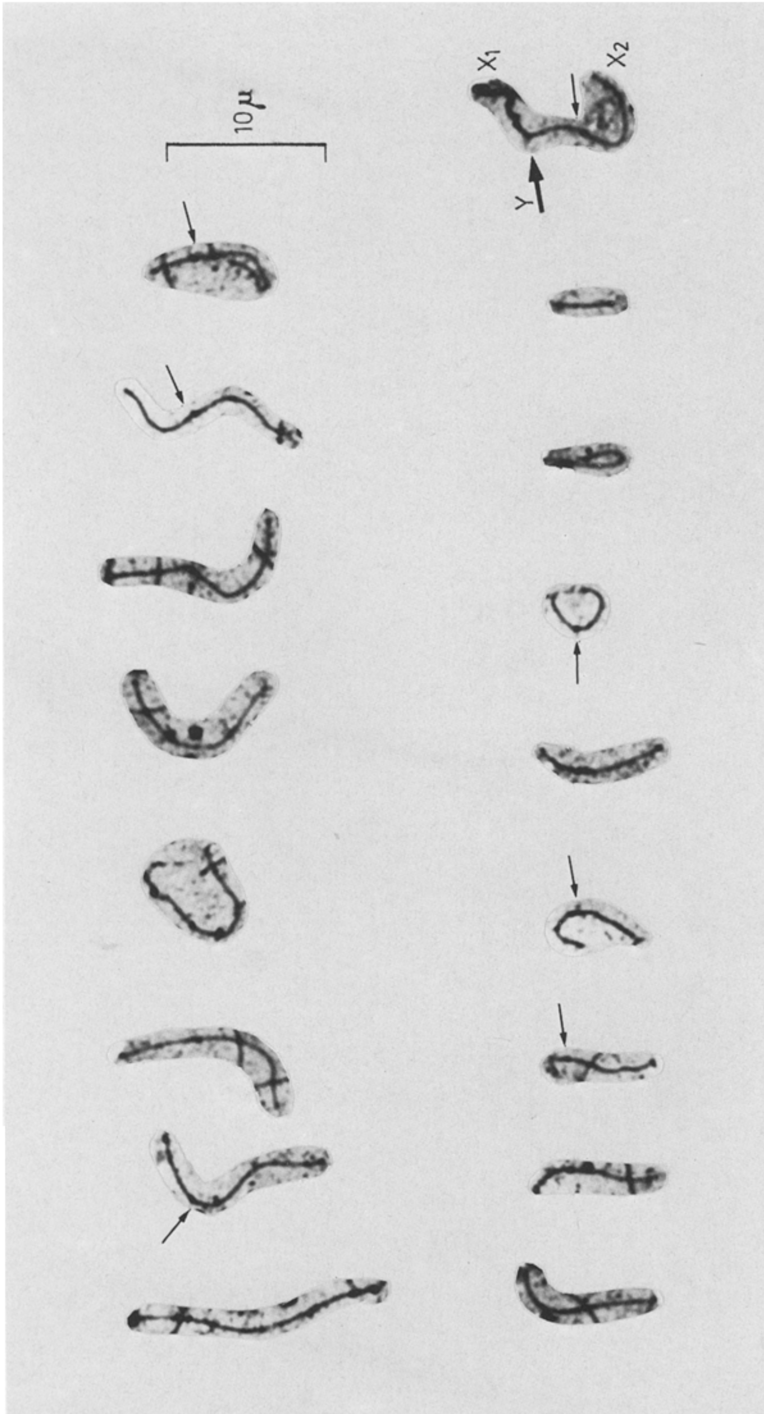

Fig. 6. Pachytene karyotype of the synaptonemal complexes. The *thick arrow* denotes the point of association between the sex chromosomes. The *small arrows* indicate kinetochores

Table 1. Relative lengths ($L^{R\%}$) of individual SCs measured from 22 pachytene cells. The lengthwise position of the $\widehat{AY}$ bivalent in individual plates is indicated in the parenthesis. The measurements do not include the $L^{R\%}$ of the X_1 chromosome

Plate No.	SC No.																
	$\widehat{AY}$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	5.7 (9th)	13.3	7.3	7.2	6.9	6.7	6.5	6.5	6.3	5.5	4.8	4.5	4.4	4.3	4.1	3.3	2.3
2	8.1 (2nd)	10.6	7.2	6.7	6.5	6.5	6.5	6.5	6.3	5.8	5.6	5.6	4.8	4.7	3.6	3.3	3.3
3	6.2 (9th)	11.0	8.2	7.4	7.1	7.0	6.5	6.5	6.4	5.8	5.0	4.4	4.4	3.7	3.6	3.4	3.4
4	7.1 (5th)	8.4	8.3	7.9	7.1	7.0	6.5	6.1	6.0	5.8	5.6	5.5	4.9	4.7	4.0	2.9	2.1
5	5.1 (9th)	9.7	8.8	7.9	7.5	7.1	7.0	6.8	6.2	5.0	4.9	4.6	4.3	4.0	4.0	3.6	3.2
6	4.9 (9th)	18.7	8.4	7.8	7.0	6.0	5.5	5.4	5.4	4.9	4.5	4.5	4.2	4.1	3.9	2.5	2.1
7	5.2 (9th)	8.7	7.8	7.7	7.4	7.2	7.0	6.9	6.0	5.2	5.1	5.0	4.7	4.7	4.3	4.2	2.6
8	6.0 (9th)	11.0	9.0	7.8	7.0	7.0	6.9	6.2	6.2	5.9	5.0	4.8	4.6	4.5	3.2	2.8	2.1
9	6.2 (8th)	8.2	8.1	7.7	7.5	6.4	6.3	6.3	6.0	5.6	5.4	5.3	4.9	4.7	4.2	3.8	3.5
10	6.5 (6th)	9.8	8.5	7.2	6.9	6.7	6.5	6.4	6.4	5.7	5.5	5.3	4.6	4.0	3.6	3.3	2.7
11	5.3 (9th)	9.6	9.0	7.7	7.3	7.0	6.8	6.7	5.9	5.2	5.1	4.5	4.5	4.3	3.8	3.6	3.4
12	5.5 (9th)	8.6	8.4	8.3	8.1	8.1	8.0	5.9	5.8	5.4	5.4	5.2	4.7	3.9	3.4	2.7	2.6
13	5.7 (9th)	8.2	8.1	7.9	7.5	7.4	7.3	6.5	6.4	5.7	5.7	5.3	4.7	3.7	3.5	3.4	3.0
14	5.7 (10th)	8.2	7.6	7.4	7.3	7.2	6.5	6.5	6.0	5.8	5.7	5.4	5.1	4.3	4.2	3.8	3.2
15	5.6 (9th)	10.1	8.6	7.5	7.2	6.5	6.4	6.4	5.7	5.5	5.3	5.0	4.6	4.1	3.8	3.8	3.7
16	6.3 (9th)	7.7	7.6	7.6	7.2	6.7	6.7	6.6	6.3	6.1	5.8	5.0	4.7	4.6	4.0	3.6	3.1
17	5.8 (10th)	8.3	7.5	7.4	7.3	6.9	6.5	6.2	6.1	6.0	5.5	5.4	5.4	4.2	4.2	4.0	3.3
18	6.4 (9th)	7.8	7.7	7.4	7.4	7.2	6.8	6.7	6.6	5.3	5.2	5.2	4.8	4.6	4.4	3.5	2.9
19	6.2 (9th)	7.9	7.8	7.7	7.4	6.8	6.8	6.5	6.2	6.0	5.7	5.5	5.4	4.2	3.4	3.2	3.2
20	5.5 (9th)	8.7	8.2	7.9	7.8	6.8	6.5	6.4	6.1	5.3	5.0	4.8	4.7	4.2	4.1	4.0	3.7
21	5.8 (9th)	9.5	8.0	7.6	7.6	6.6	6.3	6.2	6.0	5.6	5.6	5.4	4.8	4.7	4.0	3.2	3.1
22	6.3 (9th)	8.1	7.9	7.5	7.3	7.3	7.1	7.0	6.8	6.2	4.5	4.4	4.4	4.0	3.9	3.6	3.2
Average	5.9 (9th)	9.6	8.1	7.6	7.3	6.9	6.7	6.4	6.1	5.6	5.3	5.0	4.7	4.3	3.9	3.4	3.0

somatic complement, the 9th chromosome turns out to be a subtelocentric middle-sized chromosome whose short arm does not display any conspicuous heteromorphism.

Discussion

Soon after the discovery of the XO/XX sex chromosome system in the small Indian mongoose, *Herpestes auropunctatus*, it was made clear that the male determining segment in this species was probably present on an autosome which associated with the X chromosome during meiosis (Fredga, 1964). Subsequently several other species of this genus were also found to have the same sex chromosome system, conventionally called X_1X_2Y ♂/ $X_1X_1X_2X_2$ ♀ (Fredga, 1972). Whereas in *H. auropunctatus* and *H. edwardsi* the Y chromosome component was indistinguishable, a distinctly heteromorphic pair, with one homologue subtelocentric and the other acrocentric, was recorded in *H. sanguineus*. In the latter the Y chromosome was suspected to be on the short arm of the acrocentric homologue of this pair (Fredga, 1970). Influenced by these findings Fredga (1970) assigned the Y chromosome component of *H. auropunctatus* to one of the acrocentrics, the 6th largest chromosome of the complement. However, by applying the surface spreading technique to meiocytes and preparing pachytene karyotypes, we have now obtained a more authentic identification of the AY chromosome. From our observations we conclude that the Y bearing autosome is in fact chromosome 9.

Failure to detect the Y chromosome element in the somatic complement, even after subjecting it to various cytochemical tests for identifying heterochromatin, confirms the elimination of most, if not all, of the heterochromatin known to characterise mammalian Y chromosome in general. However, pairing between the X and a part of the autosome illustrates that besides the sex determining component of Y, a region homologous (or homoeologous) to the X_1 has also been retained in the *H. auropunctatus* male.

The occurrence of such a homologous region between the X_1 and the Y would ensure accurate alignment during zygotene followed by proper disjunction during anaphase. Solari (1973) argues that, in addition to providing a synaptic facility, X_1Y pairing also provides an opportunity for genetic crossing over between these two otherwise nonhomologous chromosomes. Although this claim has yet to be substantiated by adequate genetic and cytological data, the recent demonstration of crossing over, though presumably nonreciprocal, between the X and aberrant Y in Sxr carrier male mice (Singh and Jones, 1982) and drastic reduction in the fecundity due to insufficiency of X-Y pairing in hybrids of *Mus musculus* and *M. molossinus* (de Boer and Nijhoff, 1981) provide some ground for considering X_1Y crossing over as a viable possibility. It would, of course, be imperative that the sex determining element on the Y be so situated as to escape the consequences of crossing over. It may be significant, therefore, that the initial pairing orientation between the X_1 and Y chromosomes in *H. auropunctatus* is side-by-side and not "face-to-face" (Fig. 7). It would be of particular

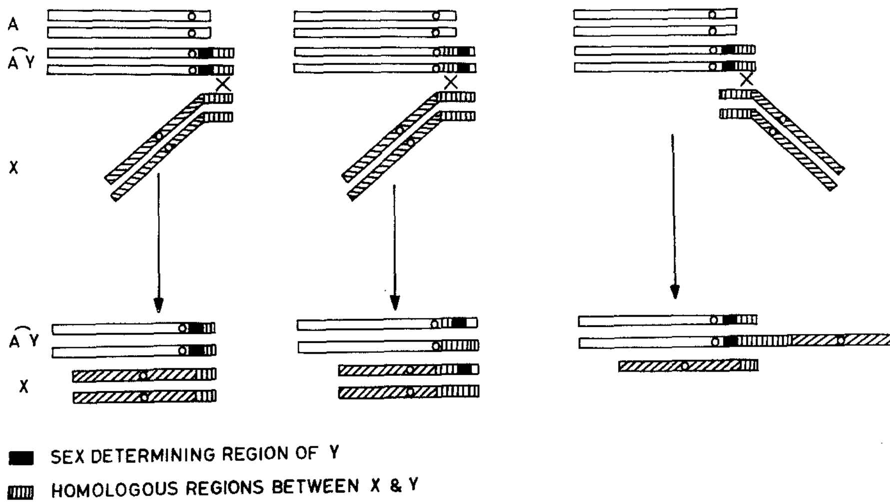

Fig. 7. A diagrammatic representation of the possible locations of the male-determining region on the mongoose Y and their probable meiotic consequences provided the homologous regions between the X and Y undergo crossing over

interest to determine the exact location of the sex-determining Y chromatin in *H. auropunctatus,* possibly by in situ hybridization with an appropriate DNA probe, for example the W-chromosome specific Bkm-like DNA of snakes (Jones and Singh, 1982). On the strength of the present observations it may be suggested that this region in the short arm would be proximal to the centromere while the paired regions would be distal to the centromere and beyond the critical region (Fig. 7). We also suggest that both the sex determining and the X_1-homologous components of the Y are essential for the functional viability of male individual.

The loss of heterochromatin from the Y chromosome of the mongoose apparently causes no unfavourable effects at the individual level. While this fact appears consistent with the general doctrine of the genetic inactivity of heterochromatin, and thus its dispensability from the genome, it is pertinent to ask why Y heterochromatin should be retained in an overwhelming majority of mammals and other animal groups if indeed its presence or absence is of no consequence at either cell or organism level. The present study provides at least one clue to this issue. The formation of a sex vesicle, which is a characteristic feature of early meiotic prophase in most species of mammals, does not occur in *H. auropunctatus.* We are aware of just one other mammal species, the marsupial *Lagorchestes consipicillatus,* which lacks a sex vesicle (Hayman and Sharp, 1981). There is an interesting parallel between this marsupial and *H. auropunctatus* in that the former also carries a translocated Y-autosome sex chromosome system where the Y chromosome component is sandwiched between two autosomes and, as in the mongoose, is too small to be resolved in ordinary cytological preparations. In all probability in *L. conspicillatus* too a part of the Y heterochromatin

has been lost. The coincidence of lack of sex vesicle formation and the partial deletion of heterochromatin from the translocated Y chromosomes suggests that there could be some sort of cause-and-effect relationship between the two phenomena. Here, again the significance of sex vesicle formation vis a vis the meiotic process is far from clear. Nevertheless, its appearance often signals the onset of condensation in both the Y and X chromosome, followed by the genetic inactivity of the X chromosome during spermatogenesis. Also, at this stage the X and Y are brought into intimate association. If the primary role of sex vesicle is to ensure both of these functions, viz., sex chromosome condensation (for inactivity) and spatial approximation (for synapsis and proper disjunction), the observation on mongoose meiosis may be of particular relevance.

Although the X_1, X_2 and Y chromosomes all associate in a majority of meiocytes in *H. autopunctatus,* the X_1 chromosome remains univalent in about 10% of them. Added to this at late metaphase-I 10 of the 24 cells scored (=40%) proved to be tetraploid. The frequency of such cells is obviously far too high to be relegated either to the class of technical artefacts or occasional aberrations of meiosis. We suggest, therefore, that heterochromatin of Y chromosome, though not indispensable, is possibly instrumental in the formation of the sex vesicle and that this, in turn, may be important both in bringing about the pairing and orientation of the sex chromosomes and perhaps also influences the functional state of the X chromosome.

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