TABLE 2

Comparison of results obtained by serological test (IHA) with dried blood on filter paper and stool samples under microscope (only from those persons found serologically positive).

IHA positive samples				Stool examination/criteria % positive
Total	Male	Female	Total	
104 (66.35%)	69 →	35 (33.65%)	104	Male 58 (55.77%) + ve cysts* = 34 (49.28%) + ve trophoz.** = 22(31.88%) + ve cyst and the trophoz. = 2 (2.9%) −ve cyst and the −ve trophoz. = 11 (15.94%) Female 27 (25.96%) + ve cyst* = 15 (42.86%) + ve trophoz** = 11 (31.43%) + ve cyst and the +ve trophoz. = 1 (2.86%) −ve cyst and −ve trophoz. = 8 (22.86%)

Total samples: 648 (males 406, females 242) *Cyst = 4 nucleated, chromatoid bars, and double walled, with glycogen mass under Lugol's iodine preparation, measuring above 10 / μm in diam; ** Trophozoite = with RBC in the cytoplasm; +ve = positive; −ve = negative.

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THE USE OF G-BANDING TECHNIQUE IN THE CHROMOSOME STUDIES OF A MILLIPEDE SPECIES—*SPIROSTREPTUS ASTHENES*

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ABSTRACT

The chromosomes of *Spirostreptus asthenes*, a millipede from Southern India, has been studied by air-drying and banding techniques. In this cytologically difficult material it was possible to get well-spread and banded metaphases by pretreatment with hypotonic Na citrate (0.016 to 0.021 M) followed by ASG staining. Both mitotic and meiotic tissues were studied.

INTRODUCTION

INTEREST in the chromosome cytology of Diplopoda has been renewed in recent years and several species of Indian Diplopoda are cytologically known at present[1,2]. An attempt is made here to introduce the combined use of different cytological techniques including intrahaemocoelic injection of colchicine, hypotonic pretreatment[3], air drying[4], and

acetic-saline-Giemsa (ASG) techniques[5] for the study of chromosomes in a millipede species, *Spirostreptus asthenes*.

Material and Methods

Adult males of *S. asthenes* (family Spirostreptidae) constituted the material for the present study. These were collected during the monsoon season from Alagarkovil hilly tracts, near Madurai, South India.

Testes were dissected in normal saline 5 hr after intrahaemocoelic injection of colchicine (0.01%). Testes were pretreated for about an hour, at room temperature, in one of the hypotonic solutions, such as potassium chloride (KCl), sodium chloride (NaCl) and sodium citrate (Na citrate), at varying molarities. The use of 0.075 M KCl[6], which has proved to be a standard solution for the preparation of mammalian metaphase chromosomes was found unsuitable for this species. But, KCl at 0.125 to 0.150 M gave good results in the preparation of diplotene chromosomes. The use of 0.016 to 0.021 M Na citrate, however, gave the best results in the preparation of meiotic as well as mitotic metaphase chromosomes.

The methods of Rothfels and Siminovitch[4] were employed with modifications for the preparation of air-dried slides. Testes material was thoroughly minced in hypotonic solution and centrifuged at 800–1000 rpm for 5 min each time. Two changes were given in the hypotonic solution and the cellular material was fixed in freshly prepared 3:1 methanol acetic acid for 30 min at room temperature. After two changes in the fixative for 10 min each time and centrifugation, a thin cell suspension was prepared from the cell button by adding 2 ml of the fixative. Using a Pasteur pipette, a few drops of the cell suspension were placed on each of the alcohol cleaned slides which were kept immersed in ice-cold water (at 4° C). The slides were dried on a hot plate at 60° C and stained in 2% Giemsa working solution, consisting of 1 ml of Giemsa stain and 1 ml of sodium phosphate buffer at pH 6.8 in 48 ml of deionized water, for about 5 min at room temperature. The slides were then rinsed in deionized water, air/heat dried and finally mounted in Euparal.

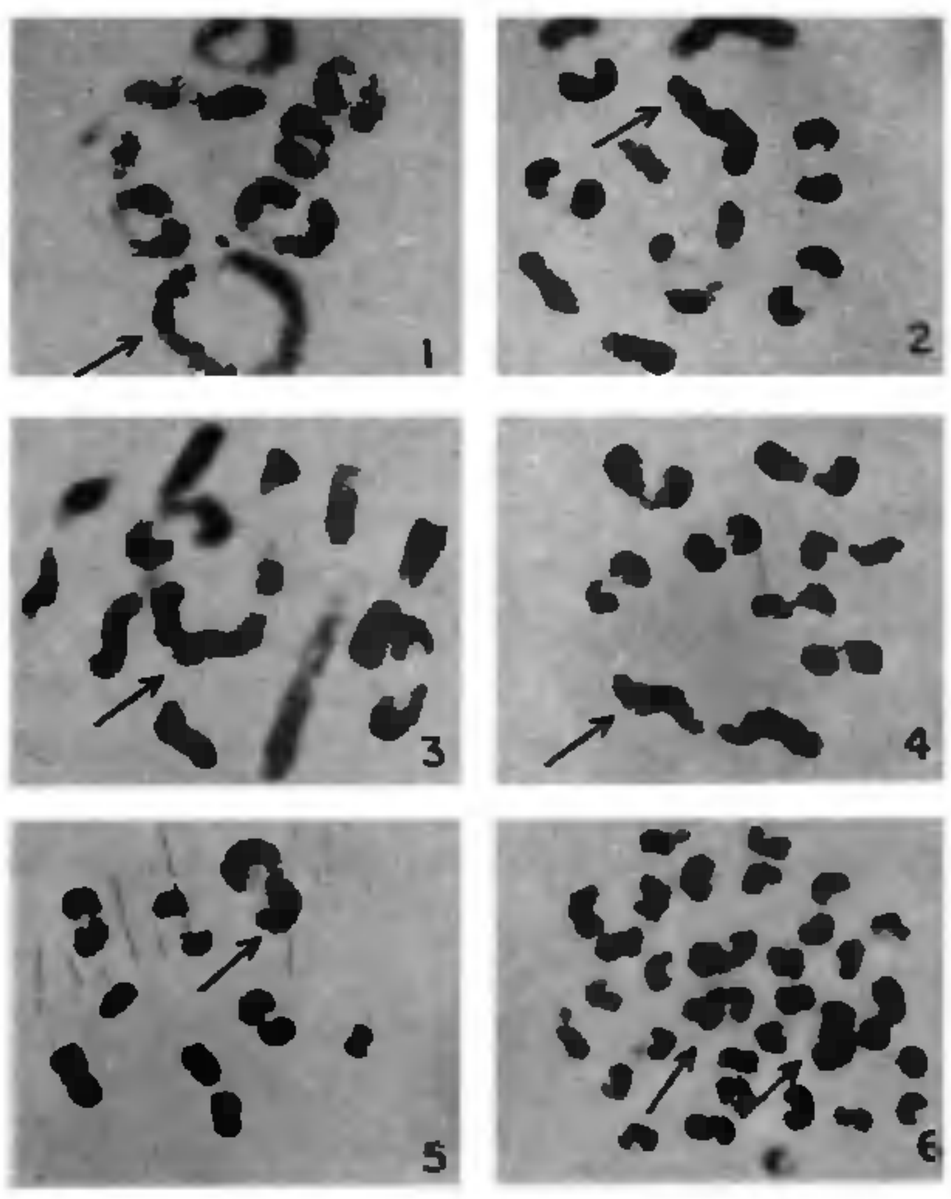
The acetic-saline-Giemsa (ASG) banding technique of Sumner *et al*[5] was employed with minor modifications. One week-old slides were selected and incubated in 2 × SSC (saline sodium citrate) (0.3 M NaCl and 0.03 M trisodium citrate) for 1 hr in water bath at 60° C. The slides were rinsed in deionized water before staining. The slides were then stained, air/heat dried and mounted as before.

Photomicrographs were made on 35 mm NP 22 ORWO film using a Zeiss microscope fitted with a 100× achromatic objective and 10× ocular. A dark green filter was used.

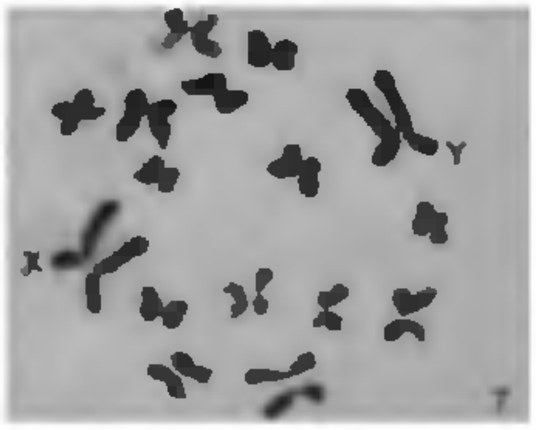
Observations

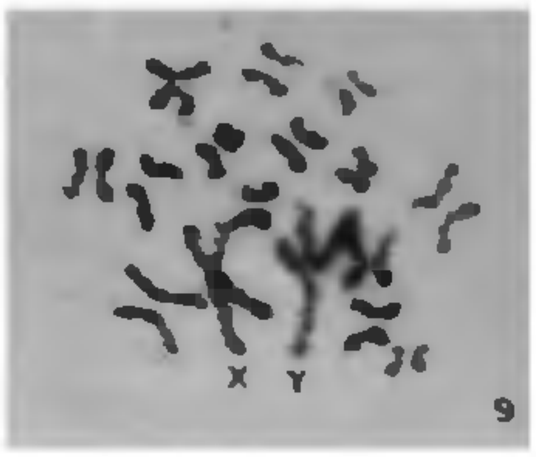
Meiotic chromosomes

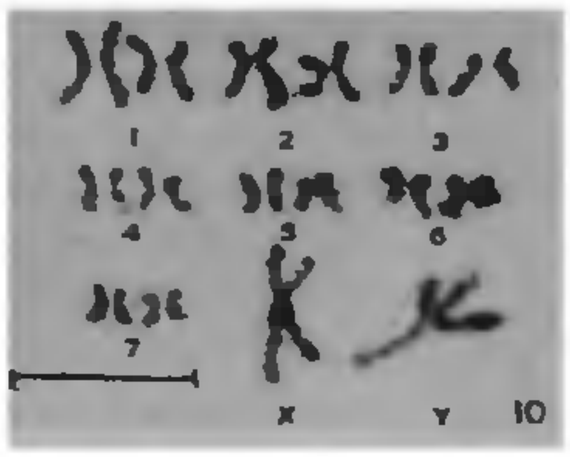
The diploid chromosome number is 16 as revealed by spermatogonial metaphases[7]. At diplotene, the intimately paired homologues undergo repulsion along their entire length and as a consequence, the bivalents open out as circular loops. The sex-bivalent (XY), at this stage, forms a large characteristic circular loop (figure 1). All the bivalents appear dumb-bell shaped during late diakinesis except the sex-bivalent which shows delayed terminalization of chiasmata (figure 2). A characteristic 'premetaphase stretch'



Figures 1-6 Air-dried chromosome preparations of a male *Spirostreptus asthenes* (Giemsa staining). Arrows indicate the sex-bivalent. The bar represents 10 microns in all figures. **1.** A late diplotene configuration showing the sex-bivalent as a large circular loop. **2.** A late diakinetic configuration showing the delayed terminalization of chiasma in the sex-bivalent. **3.** Premetaphase 'stretch'. **4.** Prometaphase stage. **5.** Metaphase I. **6.** Metaphase II (sister groups) showing chromatid separation.







Figures 7–10. Air-dried mitotic metaphase chromosomes and karyotypes of a male *Spirostreptus asthenes* (Giemsa staining). The bar represents 10 microns in all figures. **7.** Metaphase plate. **8.** Karyotype prepared from figure 7 showing the size grouping of chromosomes. **9.** Metaphase plate (ASG-stained) showing G-banding pattern. **10.** Karyotype prepared from figure 9.

often succeeds the diakinetic stage (figure 3), which is then followed by a 'prometaphase stage' wherein the sex-bivalent is still seen with incomplete terminalization of chiasma (figure 4). During the first metaphase all the bivalents appear highly condensed and dumbbell shaped (figure 5). In the second metaphase, each half sex-bivalent is recognized mainly by its large size (figure 6).

Mitotic chromosomes

A normal male karyotype comprises 16 chromosomes including the sex-pair (XY). The autosomal pairs have been numbered in a descending order of length form 1 to 7. The autosomal pairs are metacentric and form a continuous series. The sex-pair (XY) is submetacentric and being the largest pair in the complement forms a separate group. The sex-chromosomes are little differentiated from each other, the X being slightly larger than the Y (figure 7,8).

ASG-banding technique

The mitotic metaphase configurations from the investing epithelium of testes as processed by the ASG-technique are presented (figures 9, 10). The diagrammatic representation (figure 11) is based on the patterns observed in a dozen different cells with ASG-technique. Bands are numbered consecutively from one end of the telomere to the other end. Each chromosome in the complement reveals a specific linear pattern of alternating dark and light bands. About 36 major G-bands have been identified in the mitotic metaphase under a light microscope (figure 11 and table 1).

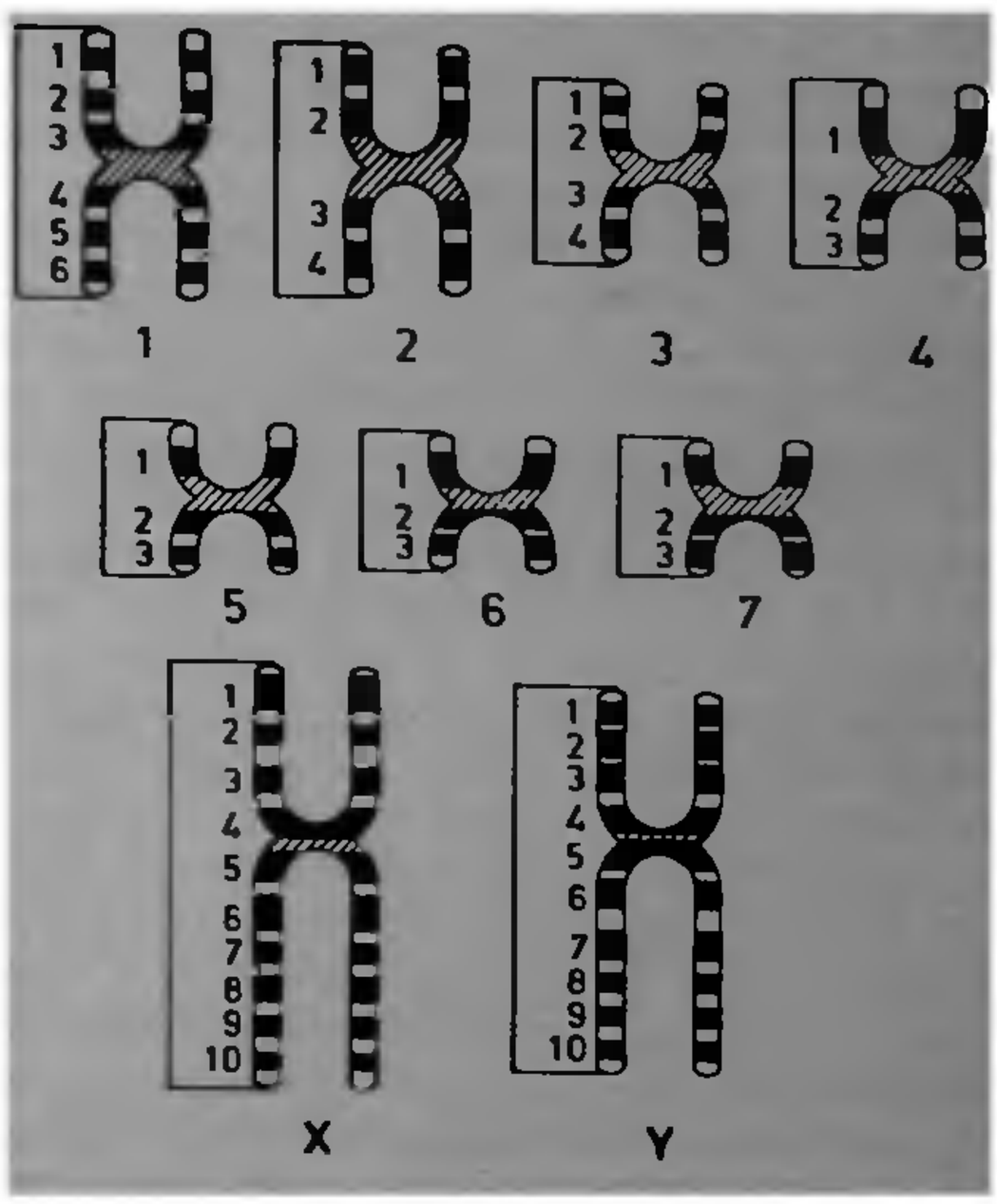


Figure 11. Diagrammatic representation of chromosome bands of *Spirostreptus asthenes* as observed with ASG-staining technique.

Discussion

The application of air drying and G-banding techniques has provided the useful biologic probes into the

TABLE 1

Approximate number of major bands in the mitotic metaphase complement of Spirostreptus asthenes.

Mitotic metaphase chromosomes	No. of major bands
Chromosome 1	6
2	4
3	4
4	3
5	3
6	3
7	3
Sex-chromosome X/Y	10
Total	36

structure of diplopod chromosomes. The general picture that emerges from the present cytological studies is that the sex-determining mechanism in the males of *S. asthenes* is still in a primitive state, sex-chromosomes being little differentiated from the autosomes. This observation agrees with the studies[8] on sex-chromosomes of a variety of placental mammals, that X and Y chromosomes have evolved from a homologous pair of autosomes. Further, in all the species of Diplopoda thus far investigated, including this species, the heteromorphic nature of the sex-pair (XY) could not be unequivocally proved since the chromosome cytology of meiosis as well as mitosis in the female sex has not yet been studied in any diplopod species owing to difficulties in chromosome preparation.

The introduction of G-banding technique which has been intitiated for the first time to study diplopod chromosomes has proved itself useful in the identification of chromosomal pairs in *S. asthenes*.

The banding pattern observed in the two homologous sets are similar and their centromeric regions stain densely by Giemsa banding techniques as in the case of humans and rodents [9,10] but unlike in cattle and sheep[11], where the centromeric regions of several chromosomes including the sex-pair remain nearly unstained. The Giemsa banding method has also helped to distinguish X and Y chromosomes as the banding patterns differ in them to some extent. The Y chromosome shows more of constitutive heterochromatin when compared to the X chromosome which is revealed in the ASG-banded sex-pair. This condition is possibly associated with the genetic inertness commonly associted with Y chromosome of diverse groups of organisms.

Since chromosome banding techniques provide the means to study a number of behavioural situations of chromosomal heterochromatin, the need for such intensive studies on diplopod chromosomes can hardly be over-emphasized.

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