OCCASIONAL PAPER NO. 300

Records of the Zoological Survey of India

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A REVIEW OF STUDIES ON THE CHROMOSOMES OF GRASSHOPPERS IN INDIA (1928-2006)

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INTRODUCTION

Three hundred ninety-three publications (including 200 abstract papers) have been recorded on Indian grasshoppers. 117 species of the grasshoppers have been utilized which revealed prolific choice of this insect as study material. Grasshoppers have also been extensively used in screening mutagenic and carcinogenic potential of a variety of chemicals and drugs (for total information please refer publication *viz.*, Singh, A. K. 2002. *A Catalogue of Experimental Studies on Grasshopper Chromosomes in India 1928 to 2000. Rec. zool. Surv. India, Occ. Paper* No. **197** : 1-44.)

The present review is a subject-wise presentation of the findings on the chromosomes.

GENERAL CYTOGENETICS

A Karyology

Karyological characterization of grasshopper species for cytotaxonomical study, and detection of gross aberrations in chromosome number, morphology and sex-chromosome mechanism were the objectives outlined by the earlier cytologists. The group has long been recognized as chromosomally conservative. Majority of species have 19 XO♂; 20 XX♀ (Pyrgomorphidae) and 23 XO♂; 24 XX♀ (Acrididae) with all the members as rods. A variety of deviations from this uniformity have however been recorded in literature (White1973; Hewitt 1979).

A serious problem encountered in karyology is genetically controlled variation in contraction within the chromosome complement of a cell. In addition, an "artificial" variation can also be introduced by the experimenter *viz.*, on excessive concentration and length of pretreatment with colchicine leading to differential contraction of euchromatin and heterochromatin (Smith 1965). On reliability of banding methods now in extensive use in karyology, utility of correct determination of chromosome length and arm length have been reduced. Underlying gross structural heterozygosities are also detected considerably.

Study of the karyological features of a species and its populations are essential for understanding the evolution of the chromosomal changes in them.

i) Chromosome number and morphology

Cytogenetic investigations on animal chromosomes in India started as early as 1928. It was Asana who initiated it with his observations on the spermatogenesis of a grasshopper species *viz., Poecilocera picta.* The species has 2n = 19 with XO sex chromosomes in males.

In 1931 Asana studied the spermatogenesis of 6 species belonging to the 4 subfamilies *viz.,* Tryxalinae, Oedipodinae, Catantopinae and Pyrgomorphinae. He and his associate Mahabale in 1945 reported spermatogenesis of the desert locust *Schistocerca gregaria.* Asana in collaboration with Makino (1934) described the spermatogonial metaphase chromosomes of *Poecilocerus pictus.* With a cytotaxonomical approach Asana *et al.* (1939) analysed the chromosome complements of 6 species belonging to the subfamilies Acridinae, Oedipodinae and Catantopinae. They found that small dot like chromosomes were in no way characteristic of a subfamily. One, two or three such pairs were although recorded, but not uniformly in all the species studied under a subfamily.

Rao made fairly extensive studies during 1932 to 1940 to trace chromosomal relationships in Indian Pyrgomorphinae. His comparative account of the chromosomes of eight genera of Indian Pyrgomorphinae (Rao 1937) is considered as the first comprehensive study of cytotaxonomical nature in India. He discovered that the size of chromosomes varied considerably in different genera. However, on analyzing the measurements of meiotic metaphase II chromosomes for sizes and seriation, there was some degree of uniformity within a subfamily. In this study he has shown that simple elimination of two pairs from the usual twenty three chromosomes of the complement in Acrididae does not explain fully karyologically any phylogenetic relationship of Pyrgomorphinae with Acrididae.

Ray-Chaudhuri along with his collaborators started cytogenetic studies on grasshoppers in the late 1940s and made a substantial contribution particularly in the field of radiation cytogenetics. He had first started research in *Drosophila* genetics at the Zoology Department, Calcutta University. But due to several technical limitations he switched over to work on the chromosomes of grasshoppers and other arthropods. Later he had revived his interest in *Drosophila* genetics with the help of Prof. J.B.S. Haldane who had reestablished *Drosophila* genetics laboratory for him in the Department of Zoology, Calcutta University. By this time research laboratories in universities tackled a variety of problems of fundamental importance. Srivastava in Allahabad, Sharma in Chandigarh, Manna in Kalyani, Rajasekarasetty in Mysore, Sharma, T in Banaras and Yadav in Kurukshetra, have been the major contributors in this field.

Ray-Chaudhury and Dutt (1947) presented a comparative study of the chromosome structure and behaviour in three different genera, *viz.*, *Spathosternum, Oxya* and *Phloeoba.* They showed that an indirect proportionality between length and number of chiasmata existed in all the species studied, and terminalization coefficients failed to show any significant increase from diplotene to metaphase I. They reported presence of a chiasma in the short arms of the ring bivalents of *Oxya* sp. There is however controversy existing regarding occurrence of chiasma in the short arms of acrocentric chromosomes.

Dutt (1948a) on cytological investigation of genus *Phlaeoba,* recognized two different forms and assigned them as forms A and B. He sent the specimens to B.P. Uvarov of Imperial Bureau of Entomology in London for identification, who identified them as two separate species but had not assigned specific names to them. However, B.R. Seshachar of Bangalore identified them as one species *Phlaeoba angustidorsis.* The chromosomes in both the forms were almost identical. Nonetheless, the long and medium bivalents of the form B had definitely shown higher chiasma frequency even when the forms were studied.

Dutt in 1955 carried out a comparative analysis of karyotypes of 9 species of grasshoppers. The chromosomes of all the species were acrocentric. The chromosomes of metaphase II stage were measured in each case. He tried to homologize the chromosomes among the species under each subfamily, and the structural changes undergone by some of the chromosomes were detected. Some meiotic features were also recognized as distinct in these species. Dutt (1966) studied the chromosomes of *Schistocerca gregaria* and contradicted the findings of White (1934) after observing no significant reduction in the number of chiasmata between, diplotene to metaphase I due to terminalization. In 1967, in another locust species *Locusta migratoria,* he described the chromosomes giving their size classes.

Manna (1954) described somatic and meiotic chromosomes of fifteen species belonging to four subfamilies. On the metrical study of metaphase II chromosomes, he analysed and grouped them into different size classes. Based on the graphs plotted for the relative lengths of these chromosomes, he inferred that some of the chromosomes in these species had undergone marked rearrangements in the course of evolution. But his attempt to deduce some phylogenetic relationships met with very limited success. Studies on chiasma frequency and terminalization coefficient had also been made. He reported anaphase bridges, fragments and supernumeraries in the natural populations.

Manna (1969) while discussing his findings in grasshoppers during his presidential address at Section of Zoology and Entomology of 56th Session of Indian Science Congress had remarked "Cytotaxonomical characterizations of the different subfamilies or genera of short-horned grasshoppers have great limitations because of the orthodox chromosome number and meiotic pattern. However, metrical analysis of chromosomes, chiasma frequency studies, etc. (Powers, 1942; Dutt, 1948b; Manna, 1954), have yielded supportive evidences when systematists found difficulty in distinguishing morphologically some closely related species"

Chatterjee (1958) reported presence of only 15 acrocentric chromosomes in *Eyprepocnemis* sp. collected from different places in Patna. She also recorded one or two small supernumeraries in some of the spermatogonial metaphases. In her meiotic study she provided evidence of the presence of a paracentric inversion heterozygous condition in some of the individuals. In the congeneric species *E. alacris*, Manna (1954) however had reported 23 acrocentric chromosomes. The reduction in chromosome number in the species from Patna is unique owing to the acrocentric nature of the chromosomes of the complement. It deserves a thorough investigation of the population to reveal the mechanism involved. Interestingly, in another finding reported in an abstract by Chatterjee (1960), 2n = 13 was recorded in *Spathosternum* sp. If confirmed, it would be the lowest chromosome number found in an Indian species so far. In the complement two were metacentrics and the rest acrocentrics. She also noted presence of an unequal bivalent and occurrence of syndiploidy showing triploid and tetraploid condition during meiotic metaphase I.

Sharma *et al.* (1962) reported breakdown of meiotic stability in two geographically separated populations of *Chrotogonus trachypterus.* The anomalies on the basis of which the breakdown was estimated in different individuals were suppression of pachytene pairing, anaphase laggards, unequal bivalents, and variable number of chromosomes in different cells of their testes. Polyploid cells and anaphase bridges were also found to be of wide occurrence in both the wild populations. They commented on the genetic consequences of such abnormal cells and argued that there would be reduction in fertility. They summarized the anomalies and reviewed the consequences of such anomalies reported in literature. It was pointed out that chromosomal variability due to suppression of pachytene pairing was an extremely rare phenomenon. They assumed that the suppression of pachytene pairing could be due to inbreeding since the individuals collected were from an isolated locality with only a limited possibility of introgression from outside. In regard to the origin of dicentric bridges it was found more or less similar to the conditions described by Manna (1954) and John *et al.* (1960) who classified the bridges with or without a fragment which have different modes of origin. For the chromosomal variability, they assumed the following reasons: (i) meiotic non-disjunction, (ii) mitotic non-disjunction, (iii) random behaviour of univalents during anaphase I, (iv) failure of spindle elongation and (v) failure of cleavage during mitosis. Authors were tempted to conclude that chromosomal variability had resulted from a very close breeding or inbreeding.

Sharma *et al.* (1965) reported chromosomal variability in the male germ cells of *Chrotogonus trachypterus* from Ottu (Punjab). This included supernumerary chromosomes and chromosomal instability. Under the chromosomal instability category they found (i) aneuploidy, (ii) polyploidy, (iii) suppression of pachytene pairing, (iv) anaphase laggards, (v) chromatid separation, (vi) non-disjunction, (vii) anaphase I bridges, (viii) chromosomal breakages, (ix) extra fragment and (x) understained regions. Earlier in a population of this species from Delhi, Sharma *et al.* (1962) had described consequences of breakdown of mitotic and to some extent meiotic stability. The Delhi population had also revealed most of those variations found in the population from Punjab but differed mainly in the presence of a pair of megameric chromosomes and in some individuals occurrence of supernumerary chromosomes. They assumed the origin of the supernumeraries from the megameric chromosomes and suggested a scheme in support of it. They distinguished extra elements (polysomy) from the supernumeraries on certain features. They observed variability in an individual from zero to ten extra chromosomes. They also observed inhibition of spindle elongation and consequently absence of cytokinesis as one encounters in grasshoppers after X-ray irradiation.

Sharma *et al.* (1974) investigated the mode of size variation of chromosomes in *Chrotogonus trachypterus*. Owing to differential coiling of the chromosomes, a considerable variation in length was observed within a cell as well as between cells in an individual. The sex-chromosome was mainly considered which showed a variation in size from its usual position to a place between 1st to 5th and even up to 7th or 9th in the complement. Another feature was the presence of heteromorphic bivalents. In most of the cells only one or two such bivalents were observed. In rare cases however three heteromorphic bivalents were found in a cell. They observed that different cells of an individual did not show a definite pattern. A third aspect observed by them was variation in condensation of autosomes in different cells of an individual and even in cells of different individuals. This indicated that contraction of the chromosomes in a cell did not proceed in a uniform manner. Several reasons were assigned for the differential coiling as (i) variation in the nature of cells at different stages of development, (ii) variation in the cellular environment even at the same stage of development, and (iii) variation in the genotype of individual cells. The authors specifically mentioned that the difference shown by the sex chromosome in response to changes in the environment or genotype was also true for the autosomes. The heterozygous condition of genes on the chromosomes of *Tulipa* (Southern, 1967) and *Hyacinthus* (Bajer, 1959) suggested for their differential coiling was not accepted as the cause in case of orthopteran X chromosome, where differential coiling was observed more commonly. But they provided no other explanation. The heteromorphism observed was also not considered likely due to heterozygosity.

Sharma *et al.* (1974) described the karyotype and meiotic behaviour of bivalents of *Tryxalis indica*, in which loss of chiasmata was more between diplotene and diakinesis than between diakinesis and metaphase I. They also noted that the number of chiasmata was proportional to the lengths of the bivalents. On the basis of the morphological details of the chromosomes of tetraploid epithelial cells they suggested endomitosis as the mechanism of their origin.

In a paper published by Sharma *et al.* (1983), chromosomal behaviour in *Oedaleus abruptus* and *Locusta migratoria* were described, besides characterizing their karyotypes. In both the species they encountered variations in the number of chromosomes both at intra and inter-individual levels. The variation in aneuploid cells ranged from 18 to 25 and the reason suggested for variation was non disjunction during mitotic anaphase. They also studied effects of intrinsic and extrinsic factors on the chiasma frequency, and calculated the coefficients of terminalization and non terminalization. On the slight difference observed in the mean chiasma frequency between the individuals of *O. abruptus,* they assumed that it could be due to variation in the temperature of their natural surroundings, since individuals of *L. migratoria* of a breeding room did not show any significant difference. But it is interesting to note that Nolte (1967) had shown that chiasma difference in locusts reflected the degree of gregarization. It was found that those swarming in fields had an increase in their chiasma frequency as compared to those in cages.

Rahiman and Rajasekarasetty (1967a) also investigated intraspecific karyotypic variation in five populations of *Poecilocera picta.* Since the species being semi gregarious, it was assumed that inbreeding or very close breeding was in operation in the populations. In their study, categorization of various anomalies and their behavioural pattern in meiosis was one aspect, and their resultant effect on the breeding potential was another aspect. In their attempt to investigate the population cytology of this species, they recognized populations which were statistically significant in difference in the incidence of anomalies. These different populations of this species exhibiting a considerable degree of karyotypic plasticity were given the status of chromosomal races by them.

Rahiman and Rajasekarasetty (1967b) examined chromosomal behaviour in *Locusta migratoria* and reported several numerical and structural anomalies like B-chromosomes, tetraploidy, precocious metaphase formation, bivalent segregation, anaphase bridges and translocation heterozygosity etc. They analysed the anomalies carefully. In the bivalent segregation, where two members of a bivalent moved to the same pole, they noticed that this anomaly remained highly localized in the cysts. The frequency of translocation heterozygosity observed by them was very low.

Gururaj and Rajasekarasetty (1967-68) studied the chromosomal variation in a random sample of 50 males of a natural population of *Chrotogonus oxypterus* and compared the findings with those reported in congeneric species *trachypterus* by Sharma *et al.* (1962). They also observed (i) univalent formation, (ii) non staining gaps, (iii) B-chromosomes, (iv) anaphase laggards, (v) bridges, (vi) multivalent associations of chromosomes, (vii) bivalent segregation, (viii) precocious despiralization of metaphase chromosomes and (ix) spontaneous clumping, pycnosis and fragmentation of chromosomes. They discussed genetic implications of the anomalies. Unlike others, in the species the univalents were formed due to desynapsis as no failure of pairing was observed in early stages. The different bivalents were involved but the degree of univalent formation was not so high as in *trachypterus,* though supernumeraries were found mitotically unstable. They speculated supernumeries to provide some selective advantage to those individuals which possessed them. They did not observe true dicentric bridges. Occurrence of pseudobridges involving the long bivalents was assumed to be caused by terminal stickiness or delayed terminalization and uncoiling of homologues. On the origin of unequal bivalents in the species they opined that spontaneous terminal deletion was involved in one of the homologues in the pre-meiotic mitotic divisions during which time the fragment was lost and the homologues exhibited unequal bivalent nature in meiosis. This however raises the question of loss of telomeric end of a chromosome and how it subsequently gains stability. Anomaly of bivalent segregation was also observed in *oxypterus*, while in *trachypterus* it was not noticed. On such malfunctioning detected in only some of the cells, the authors suggested that the forces operating in the switching-over process of monosyntely to disyntely was either non functional or malfunctioning in bivalent segregation.

Hussain and Rajasekarasetty (1968-69) extended their study of chromosomal variation in *Poecilocerus pictus*. In the population of Chitradurga town of Karnataka state, non-staining gaps, unequal bivalents and multivalent associations in polyploid cells were encountered which were not observed in the earlier populations. Whereas supernumeraries and long chromosomes characteristic of Mysore and Bangalore populations were not present in this population.

On such finding where some of the anomalies were commonly shared by all the populations while a few were specific to certain population, the authors remarked that even though there was chromosomal equivalence to greater extent among the different populations, karyotypic divergence seemed to be operative independently in each population.

Rajasekarasetty (1969) reported mitotic and meiotic behaviour of chromosomes of five species *viz.*, *Schistocerca gregaria, Oedaleus senegalensis, Catantops pinguis innotabilis, Gastrimargus africanus orientalis* and *Acrotylus humbertianus*. He characterized the karyotypes of each of the species. In meiotic study several anomalies were reported like non-staining gaps, unequal bivalents, multivalent associations due to chromosome interchanges, univalent formation, bivalent segregation, polyploidy, supernumerary chromosomes, bridges and precocious uncoiling. Besides the qualitative description of the anomalies, survey for their statistical analysis at the population level was also done.

Kumaraswamy and Rajasekarasetty (1975) described the karyotype of *Poecilocerus pictus* and published its idiogram. They designated the first 7 pairs as telocentric and the last two as subtelocentric chromosomes. The sex chromosome was distinct as the longest telocentric in the complement. They pointed out the difference found in its karyotype from the one reported earlier.

In their last paper Kumaraswamy and Rajasekarasetty (1978) proposed two hypothetical line of karyotypic evolution from cryptosacci (23 chromosome species) to chasmosacci (19 chromosome species). In this context, they cited the chromosomal structure of three species studied by them *viz.*, *Acrida turrita* (23, all telocentric), *Poecilocerus pictus* (19, telocentric and subtelocentric) and *Chrotogonus oxypterus* (19, all telocentric). In their fig. 4, the diagrammatic presentation depicts how from 23 all telocentric to 19 telo and subtelocentric and all telocentric forms have evolved through chromosomal repatterning. In this paper they have summarized the views of the earlier workers.

Chatterjee and Pradhan (1970) investigated the chromosomes of *Sikkimiana darjeelingensis* which had as in other acridids 23 acrocentrics in the males. They described the X chromosome belonging to the small size group. Manna (1954) had however reported it belonging to the medium size. Chatterjee (1971 and 1972) in two abstracts reported the number of chromosomes of *Oxya uvarovi, Xenocatantops humilis, Phlaeoba antennata* and *Atractomorpha burri.*

The chromosomes of *Eyprepocnemis roseus, Ceracris deflorata, Aulacobothrus luteiceps* and *Hieroglyphus banian* were investigated by Chatterjee *et al.* (1971). In a composite graphical presentation of the length of each chromosome in these species, they inferred occurrence of chromosomal rearrangements during the course of evolution.

Kacker and Singh (1976 and 1977) reported the chromosome numbers and sex chromosome constitution of 12 species of grasshoppers, some of them were collected from different localities of Andaman Islands. Singh and Kacker (1980) in an overall view on "Cytotaxonomical Research" have included the reports on Indian species of grasshoppers.

Mittal and Khurana (1971) analysed 88 individuals of *Ceracris deflorata* of subfamily Tryxalinae and reported breakdown of the meiotic stability in an abstract.

Mittal and Soni (1974) reported XO type of sex mechanism in *Thisiocetrus pulchra* Bolivar collected from Chandigarh. In this species Ray-Chaudhuri and Guha (1952) had discovered neo-Xy mechanism from an individual collected from kolkata. Keeping in view their distantly separated sites of collection (Kolkata and Chandigarh), authors presumed that there occur two different races of this species, one having the neo-Xy and the other with primitive XO : XX.

Ved Brat and Sethi (1974) investigated polymorphism for meiotic characters in *Chrotogonus trachypterus* which has a very restricted mobility. These hoppers are isolated in small pockets hence homozygosity is expected to get established due to higher possibility of sibmating. The study was aimed to find out how its genotype interacts to overcome this. They found that distribution and frequency of chiasmata in different cells of an individual varied, consequently chiasma frequency might have influenced recombination potential in such populations. In the study 21 histograms depicted distribution of chiasmata, mean frequency per cell, and its variances in 21 individuals. The frequency, variance and the distribution of chiasmata within and between different members of the breeding group revealed their genotypic differences. It was shown that in case of low chiasma frequency there was a distinct preference for chiasma formation at distal region while in case of two chiasmata, one was proximal and the other distal. Thus, interference distance was found influenced by chiasma frequency. Authors remarked that though the findings indicated something interesting, it could be properly assessed only if the biology of the grasshopper was well understood.

Yadav *et al.* (1978) reported chromosomal dimorphism in a population of *Tryxalis indica.*

Yadav and Yadav (1983a) carried out a comparative analysis of the karyotypes of five species and have found them chromosomally distinct from each other. The aim was to analyse closely related species for an insight into the process of evolution. In the context, they have mentioned the mechanisms involved in the evolution of chromosome number of few cases of Acridid and Pyrgomorphid species. On the inconsistency of the relative lengths of the chromosomes authors have opined that deletions and duplications have also played a role in the evolution of karyotype. Yadav and Yadav (1985) discovered two submetacentric X-chromosomes in two females of *Paraconophyma kashmiricum.* They proposed its origin by two centric fusions. Yadav and Yadav (1986) studied the chromosome numbers and sex-determining mechanisms of 29 species of Acridid and Pyrgomorphid grasshoppers worked out by them and described them in tabular form.

Aswathanarayana *et al.* (1981) reported the presence of 9th pair as a metacentric element in a population of *Gastrimargus africanus orientalis.* The variation indicated the possibility of pericentric inversions.

Sharma and Dogra (1987) summarized karyotypic details of three Acridids *viz., Cyrtacanthacris tatarica, Acrida exaltata* and *Spathosternum prasiniferum.* A diploid set of 23 (XO) acrocentrics have been reported in them. Their complement has been identified into long, medium and short size classes, and the sex~chromosome as the longest unpaired element in each of them. Differences in their genome size have also been shown.

Umadevi and Aswathanarayana (1987) reported interesting structural variations in the chromosomes *of Coptarca punctaria.* Based on the chromosomal composition they have categorised karyotypes of the individuals into 3 types, *viz.*, (i) metacentric 9th pair (ii) 6th pair heteromorphic (meta-/acro-) and 9th pair homomorphic (submeta-/submeta-) and in (iii) 9th pair heteromorphic (meta-/submeta-) 10th pair (meta-/acro-). The structural changes were the same in all the tissues of an individual. The chromosomes have been depicted in their karyotypes. Based on this study it is clear that all Indian acridids are not having acrocentric chromosomes alone.

ii) Constitutive heterochromatin and C-banding

Chromosome banding is of immense practical value in identifying individual chromosome and their different parts. With the advent of C-banding it became possible to differentially stain constitutively heterochromatic regions at condensed stages when such regions were otherwise normally indistinguishable. A good deal of C-band heterogeneity both within and between individuals of some species, have been observed recently in species studied *viz., Atractomorpha crenulata, Spathosternum prasiniferum, Tristria pulvinata, Oxya fuscovittata, Oxya hyla, Gesonula punctifrons, Chondracris rosea, Patanga succincta, Dittopternis venusta, Acrida exaltata, Ceracris nigricornis and Phlaeoba infumata* (Singh unpublished).

Chatterjee (1975) studied constitutive heterochromatin in four species *viz., Gesonula punctifrons, Oxya fuscovittata, Spathosternum prasiniferum* and *Atractomorpha crenulata.* She described its pattern in the regular and supernumerary chromosomes of the complements. She studied the behaviour of facultative and constitutive heterochromatin of the sex-chromosome besides localization of chiasmata in the megameric bivalents. She reported that the megameric chromosome was C-band positive throughout the stages of mitosis and meiosis. The X-chromosomes on the other hand, was C-band positive only at the centromeric region in both somatic as well as spermatogonial metaphases. However, during meiotic prophase the entire X remained deeply stained with C-banding procedure. She noted chiasma formation in the C-band negative region of the 9th megameric bivalent of *O. fuscovittata* and the 4th megameric bivalent of *G. punctifrons* which corroborated with the view of Fox *et al.* (1973) that the chromosomal regions which are C-band positive form few or no chiasma. On the differences in the staining intensity of the intercalary and centromeric heterchromitic bands, she attributed it to the varying inherent differences between them. The supernumeraries in the two species had centromeric heterochromatin. Thus her study had shown the utility of banding in cytotaxonomical investigation in grasshoppers.

Kumaraswamy and Rajasekarasetty (1976a) studied the C-band pattern of *Acrida turrita.* In this study they stated to have confirmed the telocentric nature of the chromosomes of this species. In another publication Kumaraswamy and Rajasekarasetty (1976c) investigated the C-band pattern of male meiotic chromosomes of *Poekilocerus pictus.* Large sizes of C-band were revealed as centromeric, interstitial and telomeric blocks in majority of its chromosomes. The sex-chromosome was shown to have six prominent bands distributed throughout its length. On this particular feature the authors designated it as "Zebra" chromosome. In most of the meiotic stages, centromeric and telomeric heterochromatic blocks became more conspicuous than that of the intercalary regions. Based on the banding patterns they suggested that inversions and translocations were involved in the karyotype evolution of this Acridid.

Majumdar *et al.* (1979) reported uridine incorporation in the constitutive heterochromatin of grasshopper *Gesonula punctifrons.* The 5th pair of autosomal bivalent in the male germ cells, which is constitutive heterochromatic, incorporated 3H-uridine as actively as in any other pair. Thus its involvement in RNA synthesis during first meiotic prophase of the male germ cells was shown. Whereas, Fox. *et al.* (1974) had shown in the male germ cells of *Chorthippus parallelus* and *Myrmeleotettix maculatus* that all the chromosomes actively incorporated 3H-uridine except the whole of the X and the distal half of M5 pair which however not being C-band positive, are presumably not constitutive heterochromatin. They discussed the findings and speculated that repeated sequences of some complex kind might code for some important proteins whose biological significance was not ascertained. The findings of Singh *et al.* (1976) was especially mentioned in this context.

The distribution of constitutive heterochromatin was studied by Yadav and Yadav (1983b and 1993) in 8 species, *viz., Atractomorpha crenulata, Phlaeoba infumata, Gelastorrhinus filatus, Oxya velox, Oxya hyla, Spathosternum prasiniferum, Parahieroglyphus biliniatus, Gastrimargus transversus.* In a table of their publication (1993) distribution pattern of C-bands of 17 species have been incorporated. They also tried trypsin treatment for G-banding but they noted very poor response. In *P. infumata* all the chromosomes had C-band in the centromeric regions and also an additional band in only three chromosome pairs. In *A. crenulata* only the first pair had shown a band besides the centromeric one. They remarked that the presence of second C-band or telomeric bands could be taken as species specific. On treatment with trypsin some of the chromosomes in *A. crenulata* exhibited flares at the centromeric ends only while the C-banded distal and interstitial segements were not affected which reflected heterogeneity of constitutive heterochromatin. Only in a few somatic cells of a female of *A. crenulata*, a sub-metacentric chromosome was observed. Since the chromosome number was reduced, its origin was suggested through centric fusion. C-banding had shown its dicentric nature therefore they proposed its origin with breakages involving the centromeres, meaning there by the imperceptible short arm of the acrocentric chromosomes. Another interesting observation by them was a large difference in the C-band pattern of two congeneric species *O. velox* and *O. hyla hyla.* In their normal karyotype also, they identified two or three large sized chromosomes, respectively. On C-band staining the two species were distinct. The difference was in the additional band on their large chromosomes as well as in the band-size difference in their short chromosomes. The marker X-chromosome of *O. velox* had a faint intercalary band. The authors emphasized that variation in heterochromatin was helpful in distinguishing various lower taxonomic categories like species and populations. Another two species *S. prasiniferum* and *P. biliniatus* of the same subfamily had also shown difference in the C-band pattern. Both the species had centromeric bands but the main difference was shown by the X-chromosome. In *S. prasiniferum* presence of a large intercalary and a telomeric band was there but in *P. biliniatus,* the smaller chromosomes had exceptionally large centromeric blocks besides heteromorphism in their telomeric band. In *S. prasiniferum,* the 2nd longest pair and two medium pairs exhibited heteromorphism for telomeric bands where only one member of each pair showed its presence. *G. filatus* and *G. transversus* representing two different sub-families also revealed great difference in their C-band pattern. *G. filatus* was distinct with large blocks of centromeric heterochromatin on smaller chromosomes besides intercalary and telomeric bands on some of its elements (Yadav and Yadav, 1988).

Hota and Patnaik (1989) have also reported their findings on the C-band pattern of 7 species of Acrididae *viz.*, *Morphacris fasciata* Thunberg, *Catantops innotabilis* (Walker), *Cyrtacanthacris tatarica* (Linnaeus), *Eyprepocnemis alacris* (Serville), *Aiolopus tamulus* (Fabricius), *Oxya fuscovittata* (Marschall) and *Oxya nitidula* Walker.

iii) HOECHST '33258' Fluorescence banding

Das *et al.* (1979) carried out the analysis of relationship of chromosomal condensation and differential Hoechst 33258 fluorescence staining in the chromosomes of *Spathosternum prasiniferum.* It was observed that at least in the grasshopper the C-band reaction was indicative of compositional specificity of the heterochromatin, but the H 33258 specific staining was more related to the state of condensation of chromosomes. They observed that at metaphase of the mitotic and meiotic divisions when the chromosomes were maximally compact, all the chromosomes fluoresced brightly without revealing any differentially fluorescing regions.

Whereas at pachytene and diplotene only the centromeres of the autosomal bivalents and the entire X-chromosome had shown bright fluorescence. On the basis of the observation it was stated that the bright fluorescence of X as a whole and of the autosomal centromeres at certain restricted stages of the cell cycle in *S. prasiniferum* was certainly not explicable by AT-specificity alone. They also studied the effect of H 33258 on the growing cells of testis and hepatic caeca (in vitro) which failed to show any induction of decondensation of the autosomal centromere regions as well as of the X-chromosome, though it was reported earlier by other workers that H 33258 selectively decondensed AT rich regions in the mouse and *Drosophila* (Hilwig and Gropp 1973; Gatti *et al.* 1976). The grasshopper chromosomes were completely unaffected by this drug. In an experiment to understand the underlying mechanism, staining with H 33258 after removal of histones was done. The over all fluorescence was rather dull, but at pachytene and diplotene the entire X-chromosome and a few of the autosomal centromeres were still discernible because of their relatively brighter fluorescence. They inferred that the Hoechst drug fluorescence was greatly affected by protein removal. They therefore proposed that modulations of chromosomal proteins in some way affect binding of the dye with the chromatin to bring about the observed differential fluorescing patterns.

In a study of the H 33258 fluorescence pattern of the mitotic and meiotic chromosomes of six species of grasshoppers, *viz.*, *Atractomorpha crenulata, Spathosternum prasiniferum, Tristria pulvinata, Oxya fuscovittata, Oxya hyla,* and *Gesonula punctifrons* by Singh (unpublished) no longitudinal differentiation of the condensed metaphase chromosome was noticed. The chromosomes of *Oxya fuscovittata* are shown in Plate fig. 5. It could be noted that the secondary constrictions showed no fluorescence (arrow). It is known that only specific chromosomes in a complement are involved in the formation of nucleoli. Thus NORs bearing chromosomes of *O. fuscovittata* can also be recognized in H 33258 staining and serve as a marker element.

iv) Nucleolus organizing regions (NORs)

Yadav and Yadav (1987) also did silver staining following the technique of Bloom and Godpasture (1976) to locate NORs in the chromosomes of six species. The feature of localization of NORs in the complements was also utilized in the cytotaxonomic evaluation of a species. While studying NORs they observed that the NORs were seen in only a few of the several spermatogonial metaphases examined. The active NORs were mostly located either on one of the homologues or on one chromatid of a homologue, and the NORs showed proximal, sub-proximal or interstitial locations. They corroborated the earlier findings by others that inactive NORs are not stained by silver staining methods. They found no correlation between the localization of NORs and C-bands. In *Oedaleus abruptus* and *Parahieroglyphus biliniatus* the NORs were located at two positions. They felt convinced that NORs on chromosomes could be used as marker for distinguishing closely related species. Another aspect which the authors discussed was the constraints in the evolution of karyotype imposed by the presence of only one pair of NORs. They assumed that in such a karyotype the chromosome pair with the NORs undergoes no abrupt structural changes like translocations, deletions etc.

v) Lampbrush type chromosomes

Srivastava studied fairly extensively in the 50's and 60's the structure of meiotic chromosomes of grasshoppers, and published 6 papers. He also made a significant contribution by reporting the "Lampbrush" fibres of the chromosomes at pachytene to diakinesis stages in *Chrotogonus incertus*. He tried to provide explanation for fibres giving "Lampbrush" appearance (Srivastava 1951). Srivastava (1954) also attempted to trace relationship of the chromomeres with "lampbrush" development in *Chrotogonus incertus*. He also studied chromosomes in spermatogonial division and in all meiotic stages and noted occurrence of supernumeraries and syndiploidy. He discussed various aspects of the structural organisation of chromosome like chromomeres, lampbrush fibres, chromosome matrix, and heterochromatin besides reporting the presence of supernumeraries and syndiploidy. He distinguished the matrix from the chromonema at the second meiotic anaphase and telophase. Srivastava (1957) continued his studies on a more suitable acridid grasshopper species *Dissosteira carolina* L. He observed endopolyploidy in the cells of testicular sheath and suggested their origin by endomitosis. He further focused on the chromomeres and lampbrush fibres and opined that the chromomeres were definite granules on chromosome threads at the leptotene stage and was not resolvable into gyres of chromatic helix, and lampbrush fibres appeared on bivalents at pachytene and persisted up to diakinesis. They were not loop-like and were not shed into the nucleoplasm, but they appeared to be withdrawn into the body of the bivalent.

Srivastava (1968) in his presidential address of Zoology and Entomology Section at the Fifty-fifth session of Indian Science Congress discussed in detail the structure of chromosomes from various angles on different materials.

In the paper by Sharma and Parshad (1957), etiology of lampbrush appearance of meiotic chromosomes in grasshoppers besides in gryllids and bugs were worked out. They correlated the onset of lampbrush appearance in these insects with two physiological events *viz*., the growth of spermatocytes, and depolymerization of nucleic acid.

After analysis of overall findings they inferred a common nature of growth represented by the diffused lampbrush elements in heteroptera where it is accompanied by more or less complete depolymerization of the nucleic acids. Whereas in orthoptera due to incomplete depolymerization of the nucleic acids the lampbrush appearance of the chromosomes results They described that the approach to a state of complete depolymerization of the nucleic acids and very slight growth of the auxocyte at the early diplotene in the grasshopper, *Poecilocerus pictus*, result in a comparatively rare occurrence of the lampbrush fibres. The depolymerization of nucleic acid concept however is untenable in view of the progress made in the knowledge of organization of eukaryotic chromosomes.

While discussing about the cytochemical nature of the lampbrush chromosomes of *Chrotogonus trachypterus* Sharma *et al.* (1964) have postulated existence of RNA-protein envelop surrounding the chromosomes during pachytene to diakinesis which gradually becomes thinned out and disappear finally by metaphase. According to them fibres arise from this matrix and have regarded them as structures representing the metabolic activity of the chromosomes.

vi) Different techniques used in the study of grasshopper chromosomes

To study grasshopper chromosomes the old technique of sectioning testis continued even up to 1965-66 (Rajasekarasetty, 1965; Desai *et al.* 1966). Ray- Chaudhuri and Pyne (1954) were the first in India to use the squash technique and since then the aceto-carmine squash technique has been most extensively used by different workers. The squash preparations were made permanent either by removing the coverslips by immersing the slides in n-butyl alcohol for 6 to 8 hours or by quick-freeze method. Use of colchicine, hypotonic treatment, etc. was also made to obtain better dispersion of chromosomes. In general, the workers found the combination of colchicine, hypotonic, and air-dried preparation very good and have used it regularly.

Some workers tried other procedures too. Ray Chaudhuri *et al.* (1952) attempted Electron microscopic study of early spermatocyte chromosomes of grasshopper. They followed the squash preparation technique of light microscopy, but the testis was squashed on colloidion coated slides. The suitable portions of the film were subsequently stripped off on water surface and mounted on grids for electron microscopic observation. They found this method yielding consistently fairly good micrographs.

Mangalangi and Subramaniam (1963) reported suitability of haematoxylin squash technique by studying preparation from *Poecilocerus pictus.* Kumaraswamy and Rajasekarasetty (1976b) tried preparation of chromosomes from hepatic caeca of Acridids and Tettigoniids. According to the procedure after colchicine and hypotonic treatments the material was fixed, and it was minced well on slide. The minced caeca were then scrapped off slide and collected in a centrifuge tube and spun at 750 RPM for 5 minutes. After decanting the supernatant, freshly prepared fixative was added to the cell pellet, and the cell suspension was dropped on slides and flame dried. The slides were stained in Giemsa and mounted in DPX. They claimed that the chromosome preparations were better than those prepared from testes. They pointed out the advantage of getting hepatic caeca chromosomes from females.

Saha (1978) in a technical note provided a method for dissecting out testes lobes from the abdomen of grasshoppers. He made a slight superficial incision on the mid dorsal line of the anterior part of the abdomen with very fine sharply pointed scissors, then fully bathed the grasshopper in normal saline and injected a fine jet of freshly prepared Clark's fluid with terrific speed in to the abdomen by a very fine pipette. He gently squeezed the abdomen which resulted in the eruption of the lobes of the testes from the dorsolateral sides. They were taken out by a pair of fine forceps or needles and cleaned in normal saline. Through this method of dissection and fixation Saha got better pictures of the grasshopper chromosomes.

Kacker *et al.* (1986) emphasized the importance of C-banding, staining of NORs and Hoechst 33258 fluorescence banding in cytotaxonomic studies. Chromosomal features obtained by those methods by one of the authors (Ashok K. Singh) have been presented in Plate (Figs. 1-6; pl. refer legend also). Fine quality pictures speak of the usefulness of the techniques.

De and Ghosh (1990) tried to demonstrate core structure of meiotic chromosomes in grasshoppers by silver staining. They observed the core structure best revealed in diakinesis and metaphase I.

B Sex chromosomes

i) Normal sex chromosome constitution

Dutt (1950) examined the behaviour and nucleic acid cycle of the sex chromosome of *Chrotogonus* sp., a Pyrgomorphid grasshopper. The heteropycnotic cycle of the sex chromosome of this species was found to differ from the existing accounts.

Ghosh and Rao (1983) tried to make out functional status of the allocyclic X in the male germ cells of *Schistocerca gregaria* by autoradiographic technique after 3H uridine incorporation. They found positively heteropycnotic X not incorporating uridine thus reflecting transciptionally inactive condition. Isopycnotic and negatively heteropycnotic state of the X however showed transcriptional activity. They reported that negatively heteropycnotic X was found to be hyperactive.

Ghosh and Rao (1986) studied DNA replication in the somatic cells (hepatic caeca) of *S. gregaria* by tritiated thymidine autoradiography. But the Xs in the female revealed synchronous DNA replication and thus assumed to be equally active as other autosomes, while in the male the single X replicated at a faster rate than in the female complement. They also worked out the duration of cell cycle (in vivo) by analysis of proportion of labelled mitosis at different intervals after treatment. The entire cycle was of 12-13 hours duration, of which the S phase was about 7 hours, the G_2 of 6 hours and G_1 being hardly perceptible.

ii) Neo-X and Neo-Y

Ray-Chaudhuri and Guha (1952) discovered the neo-X and neo-Y sex chromosome complex in the grasshopper *Thisiocetrus pulcher*. The report was based on only two individuals. They examined the pairing behavior and worked out the difference in the nucleation cycle of the neo-Y and its homologue that is the long arm which is translocated on the original X which formed the shorter arm. On the basis of this difference observed they suggested that the XY mechanism was fully established in the species for which a long period must have elapsed.

Manna and Chatterjee (1963) made detailed study of meiosis in the XO type males and in the neo-X and neo-Y males of *Eyprepocnemis* sp. They however wrongly described the generic name as *Euprepocnemis*. They considered two alternative possibilities fission or fusion for the origin of polymorphic sex-chromosomes in this species. When fusion of the X with an autosome gives rise to the metacentric neo-X and the autosomal homologue transformed into the neo-Y, the XO type male is taken as the primitive condition. While if the neo-X and neo-Y type male condition is the primitive one then fission results in the formation of the XO males. Later in 1967 Manna argued in favour of the neo-X and neo-Y condition as the primitive one in grasshoppers but White (1973, page 615) rejected Manna's interpretation as entirely illogical.

Yadav *et al.* (1981) in one individual out of 600 specimens of *Tryxalis indica* analyzed observed neo-XY system. A large-sized autosome was fused with the X resulting in a submetacentric neo-X and its homologue the neo-Y In meiotic prophase the original X exhibited positive heteropycnosis and the neo-Y was relatively more intensely stained and appeared more condensed than its homologue. Since the neo-Y was associated at terminal region with its homologue, they assumed progressive heterochromatinization of the neo-Y as compared to the autosomal pair of the XO males.

Yadav and Yadav (1985) discovered two submetacentric X-chromosomes in two females of *Paraconophyma kashmiricum.* They proposed its origin by two centric fusions.

Yadav and Yadav (1986, 1990a) reported two cytotypes with XO or neo-XY in morphologically similar individuals of *Eyprepocnemis alacris* and *Choroedocus capensis* existing in the same population. In another species *Thisiocetrus nobilis* all the 6 individuals studied also had neo-XY They recognized the fusion products and the changes that have developed during the course of evolution specially the heteropycnotic behaviour and heterochromatinization of neo-Y in these species. In contrast, the neo-X has exhibited virtually no outward sign of any such modifications. They have observed that in XO individuals the length of X was higher than in the neo-XY individuals of *E. alacris* and *C. capensis.* Authors precisely discussed more or less entire aspect of neo-XY system in grasshoppers.

Yadav and Yadav (1999) have presented a comprehensive account of evolution of sex chromosome systems in Acridoidea based on the literature. In Acridoidea 48 species are recorded with neo-XY system and 7 with $X_1 X_2 Y$ (refer their table 2). In table 1, they have presented variation in the size of X chromosomes (i. e., size relationship with autosomes) in XO male species investigated by them.

C Supernumerary chromosomes

Ray-Chaudhuri and Manna (1951) on the occurrence of a supernumerary chromosome in *Aiolopus* sp., stressed the need of recording data from a large number of specimens collected from different geographical localities. They emphasized it as a prerequisite to understand the mechanisms of chromosomal evolution. B. P. Uvarov of British Museum Natural History, London had identified the specimens studied by them as *Aiolopus* sp. 'A' and *Aiolopus* sp.

'B' A supernumerary chromosome present in 2 out of 26 individuals of *Aiolopus* sp. 'B' also attracted them to study its cytology which was identifiable in meiotic cells by its univalent nature. It was never observed pairing with autosomes, while in 45 out of 80 diplotene nuclei it was associated with the X. The supernumerary showed a random segregation at anaphase I and very regular equational division at anaphase II. Because of the limited data the authors did not comment on the origin and on the role played in the population dynamics of the species.

Ray-Chaudhuri and Guha (1955) made an extensive study for a number of years on the meiotic behaviour and population dynamics of the supernumerary chromosomes in the two population of *Aiolopus* sp. They opined that spread or disappearance of the supernumerary in course of successive generations depended not only on its survival value during the operation of natural selection but also during gametogenesis and thus suggested that study of meiosis carrying the supernumeraries was essential. On the ancestry of the supernumeraries they concluded that the same supernumerary was present in both the populations and it was presumed that the chromosome mutation might have occurred in the common ancestors of the two populations studied. On the basis of difference in behaviour observed as compared with the sex chromosome, they inferred that the supernumerary was of autosomal origin. However, evidence of its pairing with any of the autosomes could not be provided, although they had looked for such cases. The authors analysed the segregation of the supernumeraries and their relationship with the movement of the sex chromosome and also worked out its accumulation mechanism through male line and found, that it was not impossible for the supernumeraries to remain restricted to the male line only.

Sharma *et al.* (1962) in *Chrotogonus trachypterus* from Delhi population assumed the origin of the supernumeraries from the megameric chromosomes and suggested a scheme in support of it. They distinguished extra elements (polysomics) from the supernumeraries on certain features.

Puttanna published 3 short papers (1964, 1968 and 1971) on supernumerary chromosomes. In 1964 he reported the behaviour of one or two supernumeraries in the individuals of a pyrgomorphid *viz.*, *Orthacris* sp. He observed association of supernumerary with the X-chromosome in early stages of meiosis. He was also of the opinion that supernumeraries might confer adaptive value to the individuals carrying them. After studying the wild populations of seven species, Puttanna (1971) speculated that chromosomal aberrations might result due to some intrinsic causes rather than by influence of environmental factors or other variables.

Gururaj and Rajasekarasetty (1971) investigated the meiotic behaviour and meiotic stability of B-chromosomes in two populations of *Acrotylus humbertianus*, and analysed their influence on the chiasma frequency. They first characterized their behavior in mitotic and meiotic divisions, and it was interesting that in one population meiotic stability of the B- chromosome was not completely established, while in the other it had attained stability. On their influence on chiasma frequency, statistical analysis revealed that presence of the B-chromosomes had increased the chiasma frequency irrespective of the number of Bs present. The increase had been contributed by the long and medium sized bivalents.

Yadav and Yadav (1990b) recorded B chromosomes in eight acridoid species. 6 belonging to Acrididae (*Aiolopus thalassinus*, *Ceracris deflorata*, *Gelastorrhinus filatus*, *Paraheiroglyphus biliniatus*, *Spathosternum prasiniferum*, *Tristria pulvinata*) and 2 to Pyrgomorphidae (*Chrotogonus trachypterus* and *Orthacris orthacris* sp.). Their number varied from 1 to 4 in different species and in each case depicted random segregation. In 'C' banding, Bs were found heterochromatic in nature on which they pleaded that neutralization of accessory genes by heterochromatinization is perhaps necessary to the evolution and accumulation of Bs.

We have also observed persistence of supernumerary chromosomes in different numbers in the individuals of *Patanga succincta*. In Plate fig. 7 this shows three minute supernumerary elements of which two are in a bivalent configuration and the third one as univalent associated to it (Bs). In this species supernumeraries were rarely found to be associated with the sex element. Retention in the population even after their established non-Mendelian inheritance indicates their certain biological significance in the genetic system of this species which needs to be investigated.

D Chromosomal anomalies in natural populations

i) Polysomy

Sharma *et al.* (1967) continued their studies on *Chrotogonus trachypterus* and reported numerical variability in another three widely separated populations from Chandigarh, Gurdaspur and Amristsar. They encountered a large number of polysomic individuals with mostly one, less frequently with two and only in one case with four extra autosomes. The polysomics were common in four of the five populations investigated, and these populations lacked individuals with supernumeraries. Interestingly, the polysomics were less common in the Ottu population which had individuals with supernumerary chromosomes. Thus, the finding is different from that of *Myrmeleotettix maculatus* (Hewitt and John, 1965) in which three polysomic individuals were found which also possessed B-chromosomes. The authors had commented that polysomy in B-chromosome containing individuals might be because of other reason rather than the influence of the presence of the B-chromosomes. They discussed about 'polysomy' and ruled out the possibility of the role of polysomy in the origin of higher chromosome number of Acrididae and other families of Acridoidea from low chromosome number of the Pyrgomorphidae and Pamphagidae.

ii) Polyploid cells

Ray-Chaudhuri and Bose (1948) examined meiosis in diploid and tetraploid spermatocytes of *Atractomorpha* sp. In the polyploid cells various types of multiple chromosome complexes were observed. They classified the tetraploid cells into (A) multinucleate and (B) uninucleate types. The later was further categorized into two (i) with the two heterochromatic sex chromosomes lying close to each other, and (ii) with the two sex chromosomes lying far apart. The uninucleate tetraploid cells were occurring rarely.

Dutt (1951) noticed polyploidy (4n) in one of the testicular follicles of *Oedaleus abruptus*. Dutt (1952) also reported polyploidy in the spermatocytes of some more Acridids. He believed that polyploid cells were formed by fusion of nuclei in a syndiploid tissue prior to metaphase I.

Srivastava (1957) in *Dissosteira carolina* L. observed endopolyploidy in the cells of testicular sheath and suggested their origin by endomitosis.

Sharma *et al.* (1974) on the basis of the morphological details of the chromosomes of tetraploid epithelial cells of *Tryxalis indica*, suggested endomitosis as the mechanism of their origin.

Rajasekarasetty (1965) while studying sperm-dimegaly in *Gastrimargus* species reported the importance of studying the size and structure of spermatids. On a closer look of diploid spermatids, he noted two centrioles and two tails which were retained even during later stages of spermateleosis. In case of sperm-dimegaly, bigger sperm contained diploid set instead of haploid. On their functional significance two possibilities were suggested, (i) upon fertilization might produce triploid individuals and (ii) might contribute nucleoprotein material to the egg. The first possibility was not tenable since such individuals in *Gastrimargus* were not found; the second one though appeared plausible was not confirmed by direct observation.

Bhunya and Behera (1975) observed tetraploid cells in spermatogonial prophase I, metaphase I and anaphase I besides normal diploid meiocytes in the same individual of *Poecilocerus pictus*. Different types of quadrivalents were present while the two Xs always remained as univalents in the tetraploid cells.

iii) Unequal bivalents

Gururaj and Rajasekarasetty (1967-68) studied the chromosomal variation in a random sample of 50 males of a natural population of *Chrotogonus oxypterus* and compared the findings with those reported in congeneric species *trachypterus* by Sharma *et al.* (1962). On the origin of unequal bivalents in the species they opined that spontaneous terminal deletion was involved in one of the homologues in the pre-meiotic mitotic divisions during which time the fragment was lost and the homologues exhibited unequal bivalent nature in meiosis. This however raises the question of loss of telomeric end of a chromosome and how it subsequently gains stability.

Elayidom (1971) reported an unequal pair of long chromosomes in *Spathosternum prasiniferum* which he recognized as a characteristic feature of the species. The difference in the length of the two chromosomes of the pair was found statistically significant. The existence of such a pair in high percentage of the individuals is in contrast to a small percentage of such individuals reported by White (1954) and Nur (1961). It was the first record from India of an unequal pair of long chromosomes in acrididae. The significance of its presence in majority of individuals is not clear.

Bhunya (1971) also reported an instance of unequal pair in an individual of *Spathosternum prasiniferum.* About the origin of the unequal pair he opined that it could be through the loss or deletion of some interstitial part of the short element since pairing was always restricted to terminal end of the chromosomes, and if there was a chiasma it was localized near the terminal end.

Yadav and Yadav (1989) on his investigations in the natural populations of six species of grasshoppers recorded several anomalies *viz.*, polyploidy, interlocking of bivalents, multivalent associations and translocations, chromatid gaps and breaks, unequal bivalents, precocious uncoiling, anaphase bridges, and stickiness and fragmentation. They opined that anomalies unique to hoppers can be forced in to their genetic system by the environmental pollutants frequently used in the agricultural fields and near by areas inhabited by these hoppers. They suggested that the chromosome complement as a unit may resist intoxication more effectively than an individual chromosome.

E Structural heterozygosity in natural populations

Sarkar (1955) reported a translocation heterozygote in a wild population of *Gesonula punctifrons,* and studied meiotic behavior in it. On comparing with the normal chromosome complement, she found the largest chromosome was unpaired and there was a corresponding increase in the number of medium- sized elements by one. It was explained that a reciprocal translocation had occurred between one of the longest chromosomes and medium-sized one and thus giving rise to two medium-sized chromosomes. She observed various types of meiotic configurations at metaphase I and also estimated the frequency of expected viable gametes in such a structural heterozyote. She also examined the location of chiasmata in the multivalents at the middle and late diplotene stages.

Sharma *et al.* (1963) studied meiotic behavior in a translocation heterozygote individual of *Chrotogonus trachypterus.* Since no spermatogonial metaphase was available for analysis, the chromosomes involved in the reciprocal translocation could not be identified. Multivalent configurations were analysed at metaphase I and the frequency of distribution of various types of multivalents like rings and chains vertical and transverse U-and V-shaped in 88 cells were studied. They observed a progressive decrease in the frequency of cells with multivalents from diplotene /diakinesis to metaphase I and assigned terminalisation of the distal chiasmata as probable reason.

F Karyotype evolution and cytotaxonomy

Rajasekarasetty (1963) dealt the chromosomal dynamics of *Gastrimargus* spp. and stressed the essentiality of anomalies for repatterning of karyotypes and their subsequent survival. He advocated that absolute karyotype stability may lead to an evolutionary blind alley, while relative stability alone can afford scope for further genotypic change.

Sharma (1966) in his Presidential address of section of Zoology and Entomology at Fifty third session of Indian Science Congress presented an account of animal cytotaxonomy. He stated that the chromosome cytology had not so far become a routine in taxonomic studies.

In the mean time workers in the field have however noted several modes of karyotype evolution in Acridoidea. Manna (1972) found heterochromatinization a common cause for the origin of diversified chromosomal constitutions in some orthopteroid species. This concept was mostly based on his observations on *T. pulvinata* where, he stated that certain chromosomes were degenerating and getting changed into supernumeraries. Yadav and Yadav (1996) found this species an excellent example for demonstrating karyotypic diversity in action depicting cytotype 2n (male) = 23 to 19 with XO sex mechanism. Apart from 'degeneration hypothesis' they found meiotic instability and polyploidy in the species suggestive of simultaneous chromosomal rearrangements.

Yadav and Yadav (1994) have recorded the following cytoevolutionary pathways in Acridoidea based on the literature, 1. Inversions, 2. Interchanges and translocations, 3. Centric fusion and fission, 4. Unequal bivalents and supernumerary segments, 5. Degeneration of heterochromatic chromosomes, 6. Heterochromatin distribution and 7. Patterns of silver staining.

EXPERIMENTAL CYTOGENETICS

A Parthenogones

Sharma, T. (1962) made an interesting observation on the development of parthenogonic haploid embryos grown from the unfertilized eggs laid by virgin females of the grasshopper *Aiolopus* sp. reared in the laboratory. He dissected a random sample of 41 eggs for the study of chromosomes of neuroblast cells. Some eggs were kept undisturbed for hatching of parthenogonic individuals. Only two nymphs hatched out from the eggs and both of them were females. One of them died at the embryonic moult while the other died after four days of hatching. In a total of 41 eggs dissected, only 3 eggs were found with normally developing embryos which had a large number of mitotically active neuroblast cells. He found that the neuroblast cells of one of the embryos were predominantly haploid whereas the other two had a higher frequency of diploid cells. He discussed two possibilities for the high mortality of the embryos developing from unfertilized eggs : (i) due to formation of mosaics of haploid and diploid tissues as assumed by King and Slifer (1934), and (ii) due to action of lethal genes present in hemizygous and homozygous parthenogones as suggested by Darlington in 1932. King and Slifer had observed poor viability of the embryos in the parthenogenetically produced second generation, and on this they remarked that the action of lethal genes as thought by Darlington (1932) appeared less probable because the second generation from viable, apparently lethal free parthenogones should not have had that poor viability. Sharma also found Darlington's suggestion not convincing and argued that if the very few viable, apparently lethal-free individuals were developed from diploid eggs derived from tetraploid oogonial cells, then parthenogenetically produced second generation from such parthenogones would be of equally poor viability either because of mosaics of haploid and diploid tissues or for the action of lethal genes. In support of his argument he mentioned the presence of an appreciable number of mainly tetraploid cells in certain individuals observed in his breeding experiment and also the findings of Lewis and John (1959) where 20% of polyploidy cells in two male individuals of inbred locusts were noted. On the occurrence of predominantly haploid neuroblast cells in the parthenogenic embryos, Sharma suggested that in *Aiolopus* two maturation divisions occur in oogenesis without any sperm activation. In one of the haploid metaphase cells, owing to the precocious division of the centromeres, the chromatids had completely separated except in one chromosome. According to him this could be a probable mechanism for the origin of rare parthenogenic males in grasshoppers, where by the precocious division of all the chromosomes at the very first division except the X gives rise to XO condition with diploid autosomal set. He further suggested that due to non-disjunction of the sex-chromosome during oogenesis, a tetraploid cell might give rise to diploid eggs with one sex-chromosome which would develop into rarely occurring parthenogonic males. He however could not ascertain the extent of truly haploid adults and whether the diploid condition is always essential for attaining the adult stage in grasshopper parthenogones.

B Induced structural heterozygotes

Sharma, T. (1966) studied the behaviour of structural heterozygotes during meiosis obtained by irradiating matured males of *Aeolopus* sp. and mating them with virgin females of the same age group. Two individuals from this mated lot could alone be raised upto the adult stage and found to be the structural heterozygotes, where dicentric bridge accompanied with acentric fragment were observed at anaphase I. On critical analysis he found that the bridges were not similar in both the individuals.

C DNA replication

Sharma *et al.* (1974) estimated DNA in the testis of *Locusta migratoria* and *Acrida exaltata.* The DNA value which was slightly more in *Locusta* than *Acrida* which they attributed to the phase of the cell with a mention about Ris's (1969) finding, who stated that "the amount of DNA in the testis depends upon the phase of the cell i.e. more are the division stages more is the DNA"

Ghosh *et al.* (1987) investigated DNA replication in the spermatogonial cells in *Schistocerca gregaria* and *Gryllotalpa fossor.* Their study demonstrated the presence of at least two discrete populations of spermatogonial cells in these two insects with different inter-mitotic timings. The presence of three types of spermatogonial cells, as in mammals, was suggested earlier by Hannah Alva (1965) in Orthoptera, which he classified as primary, intermediate and secondary types. Ghosh *et al.* (1987) also investigated the replication kinetics of the X-chromosome and bearing of its allocyclic behaviour on replication. They observed a shift in condensation pattern of the X during the course of development of *S. gregaria.* It is positively heteropycnotic in the early fourth instar stage, becomes isopycnotic in the fifth, and finally it becomes negatively heteropycnotic before entering into meiotic prophase. This however needs to be confirmed by critically examining in the developing nymphs in view of the clear observation made by White (1935) in another locust, *Locusta migratoria* where he noted negative heteropycnosis of the X-chromosome in the early spermatogonial division which becomes positively heteropycnotic in the prophase of meiotic first division. According to White such behaviour is probably persisting throughout Acrididae. In *G. fossor* the metacentric X has euchromatic and constitutively heterochromatic arms which never show any differential pycnosity in the spermatogonial cells and remain isopycnotic throughout. In analysis of percent of labelled metaphases as a function of time, they observed an unusual pattern of elevation and depression which made the conventional FLM (fraction labeled mitoses) curve plotting difficult. When they tabulated their results according to time sequence it was observed that both chromosome (first cycle) as well as chromatid (second cycle) labelling appeared in a given sampling hour, thus indicating two different populations of cells with different inter-mitotic timings. On analysing over all findings on the sequence of replication pattern of the allocyclic X of *S. gregaria* including effect of 5 BrdU on X, they suggested that its replication pattern seemed to be dependent on the nature of pycnosity of the X-chromosome. But in *G. fossor* it was independent of the condensation pattern. They therefore commented that the pycnosity pattern of the X-chromosome is unique for each system and the late DNA replication need not always be the property of positive pycnosity.

D Meiotic progression and interference

Meiosis is central to the life of all sexually reproducing organisms. This is the most complex sequence of behaviour in which mechanically active chromosomes are engaged. This is a long process and is supposed to be under continuous control.

Short-horned grasshoppers have been used for the study of meiosis, where it is remarkable for its timing and its condensation and decondensation cycles which begin with long critical introduction, the prophase. Its progression ends with a burst of activity from diplotene through second anaphase during which chromosomes are almost moving and changing forms.

Familiarity with the histology of testis and chronology of development of germ cells is a prerequisite in understanding meiotic progression. In general, testis of grasshopper consists of paired finger-shaped follicles each of which opens at its lower end into one of the two seminal ducts. The whole organ bears a strong resemblance to a bunch of banana. Near the tip of each follicle is an apical cell. A group of primordial spermatogonia and interstitial cells are arranged around the apical cell. Primordial spermatogonial cells divide by mitosis to form secondary spermatogonia which become surrounded by interstitial cells. Each group of secondary spermatogonia with its sheath of interstitial cells is known as a cyst and is protected by a thin membrane. Interstitial cells serve in nursing spermatogonial cells like that of sertoli cells of mammalian testes. As the primordial spermatogonia divide, they first give rise to a pair of cells (secondary spermatogonia) then to quartets, octets etc. The successive divisions of the secondary spermatogonia are always synchronous within the cysts. Thus synchronous divisions of single spermatogonial mother cell results in entire cells of a cyst, which is always a power of two. The cells of each cyst progress uniformly through pre-meiotic, meiotic and post-meiotic stages. The cysts gradually pass down the follicles and eventually become the sperm bundles which are passed out into the vas deferens. The number of generations (divisions) of secondary spermatogonia ranges from 4 to 9 (hence the number of primary spermatocytes per cyst from 16 to 512) in 66 species of grasshoppers as studied by White (1955), and the number of sperms per bundle from 64 to 2048. After completing these due successive divisions of spermatogonia specific for each species, cells in cysts begin meiosis to become primary spermatocytes. Which in turn continue through meiotic division I to become secondary spermatocytes. After completing meiotic division II, these secondary spermatocytes produce haploid spermatids each differentiate into a mature sperm. Thus two successful cell divisions of a primary spermatocyte (diploid cell) give rise to four haploid cells. The nucleus is thus changed from 4 C (DNA value) to 1 C state, by sequential reductional and equational division. The whole process of differentiation of the secondary spermatocytes to mature spermatozoa is termed as spermiogenesis.

The function of apical cell is unknown. Carson (1945) suggested that its prime function may lie in fulfilling some kind of secretory or nutritive role for the primary spermatogonia which surround it.

Our understanding on mitotic-meiotic switch is not clear. Riley and Flavell (1977) and Moens (1987) analysed it to some extent in different animal and plant systems. Moens (1987) opined that in complex organisms it is a genetically programmed step in the life of the organism; while in free living single-celled organisms, as well as in relatively simple multicellular organisms it can occur as a response to environmental conditions. In grasshoppers, build up to meiosis appears gradual where after certain number of mitotic divisions switch over takes place (White 1955).

Prolonged pre-meiotic DNA synthesis (s) is an important step underlying onset of meiosis. Primary spermatocytes undergo a long complex process of meiotic prophase which starts with this pre-meiotic DNA synthetic (s) phase (terminal round of DNA replication) which lasts for many hours in most species. The duplication of most of DNA takes place during this phase. About 0.2% remains to be replicated in zygotene known as "zygotene DNA synthesis" Alberts *et al.* (1983, pl. refer page 613) on the effects of some external factors in the progression of mitotic cell cycle *viz.*, by depriving the cells of essential growth factors, by adding low levels of protein synthesis inhibitors concluded "In every instance, the cell cycle is arrested in the G_1 phase. This finding implies that once a cell has passed out of G_1 it is committed to completing the S, G_2 and M phases. In fact, experiments have shown that the point of no return known as the restriction point (R point or R) occurs late in G_1. After cells have passed this point they will complete the rest of the cycle at their normal rate regardless of external conditions"

Riley and Flavell (1977) found pre-meiotic and pre-mitotic S subject to different controls. Changed pattern of chromosome replication in meiotic S is evident, leading to its prolonged phase. It is likely to be related with the preparation of meiotic chromatin for participation in synaptonemal complexes at zygotene.

The other crucial event of meiotic prophase is homologous chromosome pairing and recombination, extensive RNA synthesis and DNA repair synthesis.

Many components of this genetically controlled process accomplishing evolution and implementing its result in heredity can be interfered by environmental effects, manipulative procedures or by genetic anomalies. These may lead to contraction and congression of chromosomes, pairing and disjunction, formation of spindle, chromosome movement etc. These anomalies generally do not suppress the progression of meiosis. Whereas, protein synthesis inhibited by cycloheximide or DNA synthesis by deoxyadenosine does halt the course of meiosis (Stern and Hotta 1967; Parchman and Stern 1969). Their effects disorganize the synthetic process of the cell which are an integral part of the overall control of the developmental process, while others are not. Thus it is reasonable to presume that progression depends critically on the production of specific proteins and synthesis of RNA and DNA at specific times.

Jain and Singh (1967) in two publications reported the action of Actinomycin D in blocking the functioning of those gene loci in *Schistocerca gregaria* which affect the switch that transform somatic cells to start meiotic division. In their simultaneous observation on RNA synthesis in the interphase cells of fully grown males which they presumed to have completed mitotic divisions, the RNA synthesis was greatly reduced. With increase of the duration of treatment (from 18 hrs. onwards), in addition to spermatocytes showing normal meiosis, a large number of cells were noted in mitotic division resembling those of the spermatogonial cells of immature males. It was opined that on treatment, potential spermatocytes failed to proceed for meiotic divisions and had gone for mitotic ones. On the progression of meiosis blocked by Actinomycin-D on one hand, and synthesis of RNA on the other, they pleaded that RNA synthesis by certain gene loci was involved in the meiotic type of cell development. The moot point that antibiotic in such a case should have checked entry of cells into division rather than to divert them to a particular type of division sequence; they stated "the cells in the follicles with their cycle of spermatogonial mitosis are already rich at the time of treatment in molecules required for a mitotic type of division and can therefore continue along this path for a limited period, even in the absence of fresh synthesis"

On their entries in Tables 1 and 3 we should accept that at the time of Actinomycin-D treatment spermatogonial mitotic divisions were already over, and the cells were in the pre-meiotic 'G'-'S'- phase onwards in the follicles. This cycle i.e. the last round of pre-meiotic synthetic phase is known for its prolonged duration (Mohapatra and Raman 1995 personal communication). Mohapatra and Raman have worked out the chronology of development of spermatogenesis in *Spathosternum prasiniferum* (an acridid species) by incorporation of 3H-thymidine and cellular autoradiography of spermatocyte preparations. Pre-meiotic 'S' was not less than 24 hours and it took 14 days to complete meiosis (temporal sequence of spermatogenesis of this species has been reviewed here under meiotic calendar). White (1935) in *Locusta migratoria* had estimated 48 hrs. to complete cycle from one spermatogonial metaphase to the next, and in *Melanoplus differentialis* Mukenthaler (1964) found that length of a complete cycle of the spermatogonial cells is approximately 28 hrs. So the cells diverted to mitotic division after G_1- S (pre-meiotic synthetic phase) as scored in their table 2 or even those identified in the prophase of meiosis depicting mitotic type of behaviour can not be accepted to have progressed within 30 hours of treatment. White (1955) have already shown that primordial germ cells in cysts of a follicle can go for meiotic divisions only after the completion of a sequence of certain number of spermatogonial mitotic divisions. Thus build up to meiosis in grasshoppers appears gradual. Gradual build up to meiosis has also been reported for mouse (Monesi 1962; Kofman-Alfaro and Chandley 1970) and for *Triturus* (Callan 1972). This negates Jain and Singh's (1967) plea that potential spermatocytes were diverted to mitotic division.

On RNA synthesis in *M. differentialis,* Mukenthaler (1964) summarised that "a high rate of RNA synthesis takes place in the apical cell. RNA synthesis in spermatogonial cells occurs during the entire cell cycle except during the time of maximum contraction of the chromosomes. Considerable amount of RNA synthesis occurs in prophase of meiosis and ceases during diakinesis. Synthesis of RNA resumes after the second meiotic division and continues in the early spermatid until condensation of chromatin occurs" It appears that in RNA synthesis large number of gene loci are selectively activated in advance for future cellular needs of proteins instead of kept awaited. Jain and Singh's (1967) claim of blocking the functioning of particular gene loci by Actinomycin-D remains a blind guess in view of the findings of White (1935 and 1955) Mukenthaler (1964) and Mohapatra and Raman (1995, personal communication).

E Meiotic calendar of a grasshopper species

With the recognition of meiosis as a significant process in gamete differentiation there has been a continuing interest in its temporal aspects.

A detailed understanding of this process is a prerequisite for the critical evaluation of the sensitivity of the germ cells to ionizing radiations and chemicals at varying stages of differentiation. Experiments have been conducted on 70 organisms to estimate duration of total meiosis as well as that of its stages (Bennett, 1977). Duration is one of the most variable aspects of the meiotic process, ranging from less than 6 hrs. in yeast to more than 40 years in human female. Studies on widely unrelated organisms showed that meiosis invariably consisted of the same stages, occurring in the same order. Moreover, it was soon recognized that the impressive regularity of the temporal sequence of meiotic events had an important functional significance. In spite of large variations in the duration of meiosis, two generalizations can be made in animal system (1) Prophase-I is always very long compared with the remaining meiotic stages and (2) duration of meiosis is characteristic of the genotype and the species.

With the obvious exception of variation caused by developmental holds, changes in the duration of meiosis usually involve proportional changes in the duration of all its stages. And the relative proportion of meiotic stages taken by individual stages is clearly much more variable among animal species than in plants (Bennett, 1977). It is also known that male meiosis may have a slightly increased duration in older individuals. It has been shown that the rate of meiotic development is much slower in hibernating than in non-hibernating golden hamster.

The occurrence of a developmental hold reflects the action of an optional control of meiocytic behaviour superimposed upon the mandatory sequence of events which are essential for the completion of the meiotic process. Thus, developmental holds occurring during meiosis have no significance for the meiotic process, instead they indicate adaptation related to the peculiar needs of reproductive development or of the life cycle. Developmental holds are released in various organisms in response to light, temperature and hormonal stimulation.

Factors which affect or determine the duration of meiosis are environmental, nuclear DNA content, ploidy level and the genotype. Clearly the observed duration of meiosis is the product of a complex genotype- nucleotype environment interaction. The nucleotype can be viewed as part of the environment with which the genotype has to function. Thus variation in nuclear DNA content represents an intracellular environmental change.

Experiments to estimate the duration of meiosis and its stages :

Two main procedures have been worked out for timing meiosis namely (1) non-autoradiographic methods and (2) autoradiographic methods.

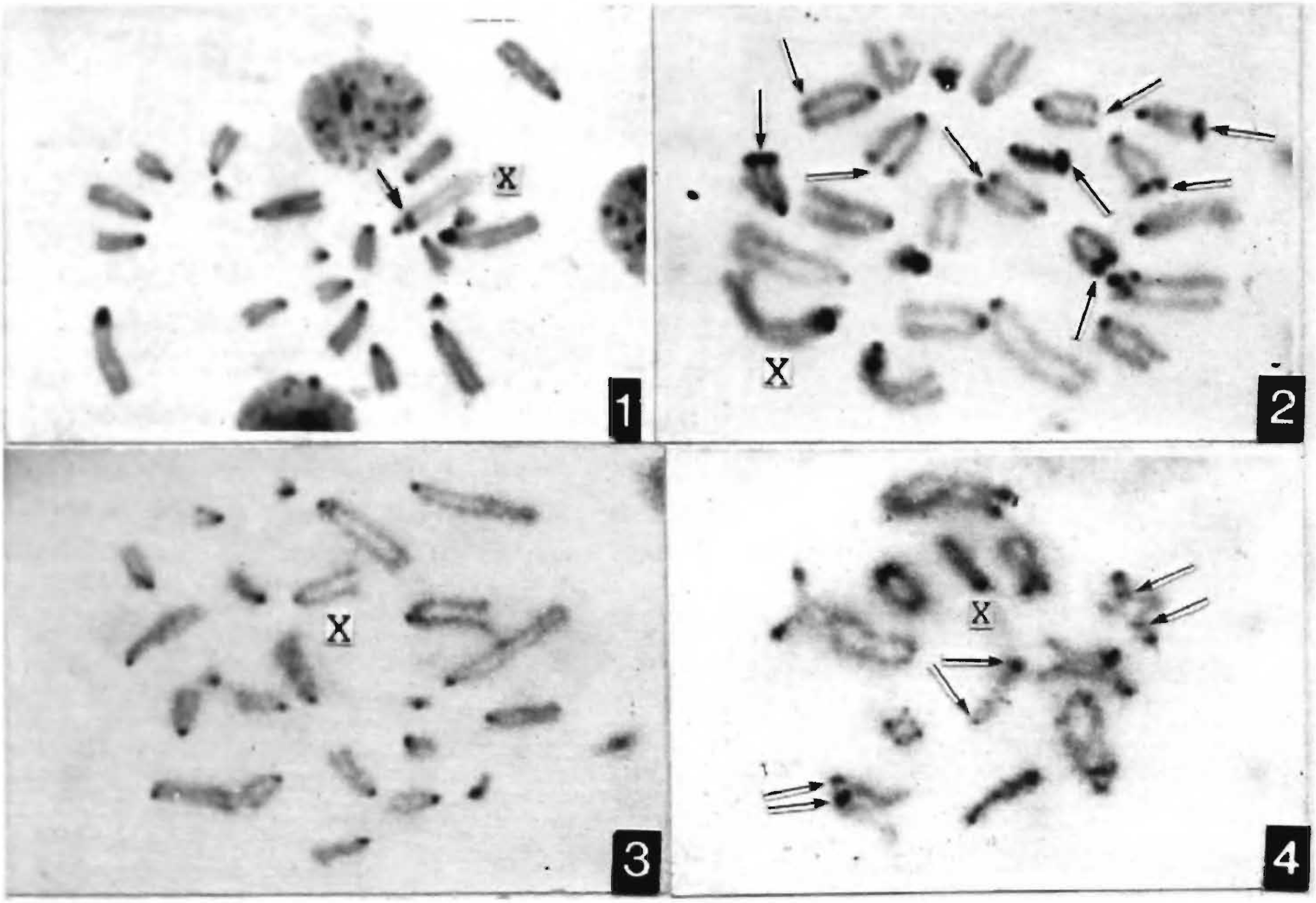
1. **Non-autoradiographic methods** : This involve the existence of two developmental features in the material (i) precise relationship between the stage of meiotic development and the size of reproductive organ or (ii) high degree of synchrony during meiotic development in a population of meiocytes which can be sampled at intervals without affecting the rate of development in the remaining meiocytes. This non-autoradiographic approach was suitable to few plant species alone.

2. **Autoradiographic methods** : Timing of meiosis by this method involves labelling of the pre-meiotic spermatogonials in the pre-meiotic DNA synthetic stage and estimating progression of labelled meiotic chromosomes in to successively advanced stages of meiosis at known intervals by cellular autoradiography. This method has revealed several facts. It has shown that chromosome replication has already occurred before the start of meiosis. Second, it provided a new stage (pre meiotic DNA synthesis stage) which could be precisely identified at a period of meiocytic development when stage identification based on the features visible in the light microscope is very difficult. Thus in many species it allowed the start of meiosis to be more accurately determined.

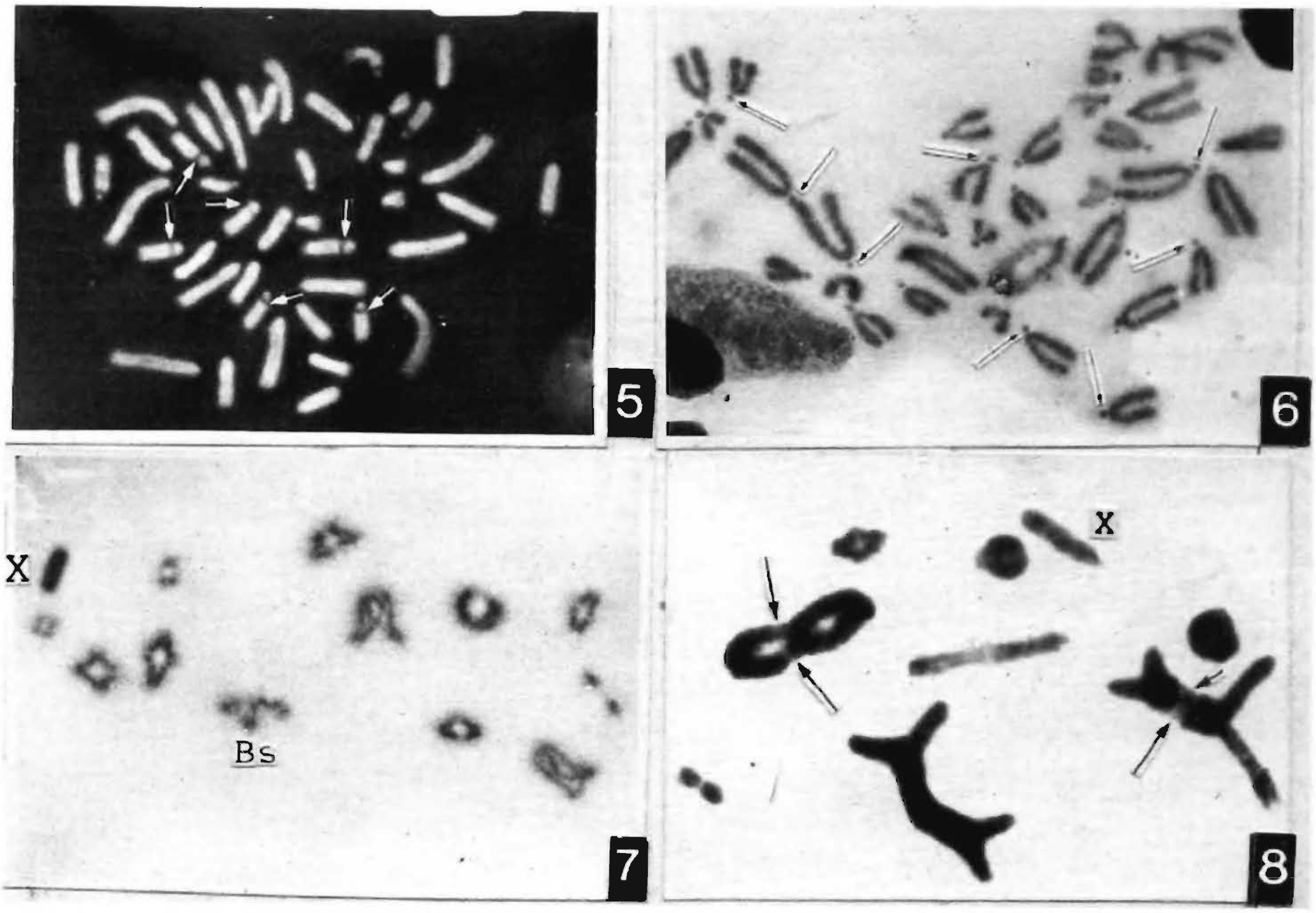
Mohapatra and Raman (1995, personal communication) studied temporal sequence of spermatogenesis of a grasshopper species, *Spathosternum prasiniferum* as a prerequisite for critical evaluation of the radio sensitivity of its different meiotic stages. None of the existing studies on induced chromosomal damage in grasshoppers (White 1937, Creighton and Evans 1941, Bishop 1942, Ray-Chaudhuri and Sarkar 1952, Ray-Chaudhuri 1961 and Gururaj and Rajasekarasetty 1969, 1970) had aimed at stage dependent analysis of the chromosomal aberration formation. Also the meiotic calendar was not established for any grasshopper species available in India. Therefore, these workers have established the meiotic calendar in a commonly available grasshopper by labelling of spermatogonials at the pre-meiotic S-phase with 3H-thymidine and followed their progression by cellular autoradiography. The cells which got labelled at their gonial meiotic S phase, and had undergone mitotic division before developing into the second wave of meiocytes were distinguishable by their poor labelling pattern, and were discarded.

Synchrony of cell division and the fact that the number of cells in a cyst of the grasshopper testis is always a power of 2 were important factors in the scoring of autoradiograms. Uniform labelling of all the bivalents within a cyst was observed except sex chromosome in some cases. They found single pulse labelling sufficient in estimating timing of different stages of spermatogenesis. Progression of spermatocytes was not based only on the first appearance of the more advanced labelled cell type, but also by calculating the percentage of the labelled meiotic stages from the total population of labelled cells. From pre-meiotic S phase to the appearance of labelled mature spermatozoa it took 23-24 days. Difference in the times from the pre-meiotic S to any two meiotic stages gave an estimate of the interval between the stages.

Their sequence of events has been analysed as such : Till ld (one day) after injection more than 50% cells were at pre-meiotic S phase. This frequency reduced to 23% at 2d. Since they had not done sampling between ld and 2d the duration of pre-meiotic S phase has been estimated to be more than 24 hours. With the completion of pre-meiotic S phase, labelled leptotene cells could be recognized as the 1^{st} stage of prophase. This stage was calculated to be of at least 3 days long. On 6d zygotene was found as most frequent among labelled cells. A considerable frequency of early pachytene was also labelled by this day. There after pachytene remained as the most advanced labelled stage till 13d of injecton. Therefore zygotene is quite short, only of 2 days duration, whereas the pachytene is prolonged up to 7-8 days. The appearance of a few labelled diplotenes on 14d was followed shortly. By 14.25d more diplotenes, along with few cells at diakinesis then a good frequency of metaphase I, low frequencies of metaphase II, anaphase I and II appeared simultaneously. This signified that each of these stages were very brief. Diplotene being relatively more prolonged was estimated to be 6-12 hrs. and was followed by metaphase I. From diplotene to anaphase II it took 12 hrs. In addition to these stages, on 14.25d diffused interphase like cells were also observed in a very low frequency and were recognized as diffused spermatids. These were observed without any intermediate or lag period i.e. transformed immediately after second



Figs. 1-4. Chromosome banding is of immense practical value in identifying chromosomes and its parts. With the advent of C-banding it has become possible to differentially stain constitutively heterochromatic regions at condensed stages when such regions are normally indistinguishable from euchromatin. A good deal of C-band heterogeneities both within and between individuals of a species has been investigated. Genome of some closely related species have been recognized on their characteristic C-band pattern, *viz.*, *Tristria pulvinata* (subfamily Tropidopolinae) and congeneric species *Oxya fuscovittata* and *Oxya hyla* (subfamily Oxyinae). In *T. pulvinata* (fig. 1) the sex-chromosome alone has revealed an interstitial band (arrow) and thus functions as the marker element. *O. fuscovittata* (fig. 2) has revealed terminal band of different sizes on four pairs. (arrow). *O. hyla* (fig. 3) contrary to it has not revealed any such band. All the three species belong to similar ecological niche. Fig. 4 is an early diplotene stage of *Phlaeoba infumata*. Heteromorphic C-bands on the natural homologs (arrow) reveal more correct picture of the genotype and help in correcting mitotic pairing. On C-banding, an interstitial band homozygous in nature has also been revealed on a bivalent (arrow). Identification of centromeric ends by the technique has considerably helped in chiasma analysis.



Figs. 5-8. **5.** Hoechst 33258 fluorescence banding has not depicted lengthwise variation of the condensed metaphase chromosomes of *Oxya fuscovittata* (fig. 5). However the secondary chromosomal constrictions have reflected no staining (arrow), which may be viewed as the arrest of the condensation of the region bearing the nucleolus. Only specific chromosomes in any given cell or species are involved in the formation of nucleoli. These NORs bearing chromosomes of *fuscovittata* thus recognized as the marker elements. Etiology of Hoechst fluorescence has been worked out by Das *et al.* (1979) in grasshopper system where increase in bright fluorescence has been correlated with condensation instead of any AT or GC rich specificity of DNA.

6. Chromosomes of *Peripolus pedarius*. Minute short arm of most of the chromosomes are flexing out of primary constriction; a characteristic feature of acrocentrics depicted here. Both acro and telocentrics can be seen in the same preparation.

7. Persistence of supernumerary chromosomes with considerable extent of inter as well as intra individual variation has been recognized in the individuals of *Patanga succincta*. Three minute elements detected are supernumeraries in the cell, where two are in a bivalent configuration and third univalent associated to it (Bs). The elements are moreover not allocyclic at this stage; might have some structural homology in them. In the species they have rarely been found associated with the sex element. Their retention in populations even after their established non-Mendelian inheritance indicates certain significance in the genetic system.

8. A meiotic configuration of 17 chromosomes of *Chorthippus indus* Uvarov (subfamily Gomphocerinae). On analyzing the chromosome complement, the 1st, 2nd and 3rd pairs are recognized as the fusion products of (2+4), (1+8) and (3+7) of the parental complement i.e. 23 chromosome type. Arrow indicates centromeric position of the metacentric chromosomes, a relatively uncoiled region. Here moot point lies in the non correspondence of the fusion products of *indus* with those reported in other species of genus *Chorthippus*. After analizing the observations, reasonable differences appear and thus monophyletic origin of these fusions is suspected.

meiotic division and attained a high frequency on 15d. They have reported it to have progressed to 100% on 16d which decreased to 89% on 18d. Their appearance continued till 23d (51%). On 21d elongated spermatids were found labelled and finally mature sperms appeared on 23d.

Therefore, the total duration of spermiogenesis comes to about 9-10 days and of meiosis 14 days of which 7 days are occupied by pachytene alone.

Authors have summarised the entire sequence of meiosis in a tabular form starting from pre-meiotic 'S' time O (zero) to mature sperm 24d. For analysis of radiosensitivity of different meiotic stages, the time interval required by cells receiving irradiation at different stages of meiosis to progress up to metaphase I / anaphase I has been back calculated. This timing of back calculation is presented in the lower most row of their summarized table. For example a grasshopper sacrificed with 12 hours of irradiation would have metaphases which were treated at stages diplotene and diakinesis. And 14d sample would consist of cells irradiated at pre-meiotic S-phase. It should be noted here that the time indicated here pertains to cells whose development is not arrested.

This study has been carried out on fully developed grasshoppers although their exact age could be variable being randomly collected individuals. However no nymphs were used. This is important because Nelson (1931) had opined that spermatogonia which begin meiosis during the fourth instar take longer to go through meiosis than those which go through the spermatogonial divisions during the sixth instar and adult stages since two instar periods may pass before accumulated pachytene cells resume meiosis in the sixth instar.

The meiotic calendar established for *Spathosternum prasiniferum* by Mohapatra and Raman could be of great use for all those who want to exploit this species for their experimental purposes especially for genotoxic studies on germ cells.

SUMMARY

The review is a subject-wise presentation of findings on the chromosomes of grasshoppers (from 1928 to 2006), by Indian workers. This is separated into two parts, *viz.*, I. General Cytogenetics and II. Experimental Cytogenetics. In General Cytogenetics: Karyology, Sex chromosomes, Supernumerary chromosomes, Chromosomal anomalies in natural populations, Structural heterozygosity in natural populations, and Karyotype evolution and Cytotaxonomy have been dealt. In Experimental Cytogenetics : Parthenogones, Induced structural heterozygotes, DNA replication, Meiotic progression and interference, and Meiotic calendar of a grasshopper species have been incorporated.

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REFERENCES

Alberts, B. *et al.* (ed.) 1983. Molecular biology of the cell. Garland Publishing, Inc. New York : 1-1146.

Asana, J. J. 1928. A preliminary note on the spermatogenesis of *Poecilocera picta* Fabr., Sub-family Pyrgomorphinae, Orthoptera. *Proc. 15th Indian Sci. Congr.* Abstract Part III : 191-192.

Asana, J. J. 1931. Studies on the chromosomes of Indian Orthoptera. *Proc. 18th Indian Sci. Congr.* Abstract Part III : 224.

Asana, J. J. and Makino, S. 1934. The chromosomes of *Poecilocera picta* Fabr. (A short-horned grasshopper). *J. Univ. Bombay*, **2** : 50-52.

Asana, J. J., Makino, S. and Niiyama, H. 1939. A chromosomal survey of some Indian insects II. A comparative study of the chromosomes of six species of Acrididae. *Jap. J. Genet.* **15** : 251-260.

Aswathanarayana, N. V., Ashwath, S. K. and Manjunatha, K. R. 1981. On the occurrence of metacentrics in a south Indian grasshopper. *Curr. Sci.* **50** : 293-294.

Bajer, A. 1959. Change of length and volume of mitotic chromosomes in living cells. *Hereditas* **45** : 579-596.

Bennett, M. D. 1977. The time and duration of meiosis. *Phil. Trans. R. Soc. Lond.* B. **277** : 201-226.

Bhunya, S. P. 1971. Natural heterozygotes in a species of grasshopper, *Spathosternum prasiniferum. J. Cytol. and Genet. Congr. Suppl.* : 241-245. (*Proc. First All India Congr. Cytol. and Genet.* 1971).

Bhunya, S. P. and Behera, H. N. 1975. Occurrence of tetraploidy in the male germ cells of a grasshopper, *Poecilocerus pictus* (Acrididae-Orthoptera). *J. Cytol. and Genet. Congr. Suppl.* : 29-32 (*Proc. Second All India Congr. Cytol. and Genet.* 1975).

Bishop, D. W. 1942. Spermatocyte chromosome aberrations in grasshoppers subjected to X-irradiation during embryonic stages. *J. Morphol.*, **71** : 391-429.

Bloom, S. E. and Goodpasture, C. 1976. An improved technique for selective silver staining of nucleolar organizer regions in human chromosomes. *Hum. Genet.*, **34** : 199-206.

Callan, H. G. 1972. Replication of DNA in the chromosomes of eukaryotes. *Proc. R. Soc. Lond.*, B **181** : 19-41.

Carson, H. L. 1945. A comparative study of the apical cell of the insect testis. *J. Morph.*, **77** : 141-161.

Chatterjee, A. K. 1971. Some cytological informations about two species of grasshoppers (Orthoptera : Acrididae). *Proc. 58th Indian Sci. Congr.* Abstract Part III : 625.

Chatterjee, A. K. 1972. On the chromosomes of two species of grasshoppers. *Proc. 59th Indian Sci. Congr.* Abstract Part III : 415.

Chatterjee, A. K. and Pradhan, S. 1970. Chromosomes of male *Sikkimiana darjeelingensis* Bolivar (Orthoptera, Acrididae) *Science & Culture*, **36** : 560-561.

Chatterjee, C. 1975. Constitutive heterochromatin in grasshoppers. *J. Cytol. Genet. Congr. Suppl.*: 48-53.

Chatterjee, K., Majhi, A. and Barik, S. K. 1971. A study of chromosomes during meiosis in four species of Indian grasshoppers. *Caryologia*, **24** : 447-455.

Chatterjee, N. 1958. Cytological studies on spermatogenesis of *Eyprepocnemis* sp. *Curr. Sci.*, **27** : 98-99.

Chatterjee, N. 1960. Studies on structure and behaviour of chromosomes during spermatogenesis of *Spathosternum* sp. *Proc. 47th Indian Sci. Congr.* Abstract Part III : 491.

Creighton, M. and Evans, B. H. 1941. Some effects of X- rays on the germ cells of *Chorthippus longicornis* (Orthoptera). *J. Morph.* **69** : 187-205.

Darlington, C. D. 1932. Recent Advances in Cytology, (First edition) London : Churchill ; Philadelphia : Blakiston.

Das, B. C., Raman, R. and Sharma, T. 1979. Chromosome condensation and Hoechst 33258 fluorescence in meiotic chromosomes of the grasshopper *Spathosternum prasiniferum* (Walker). *Chromosoma*, **70** : 251-258.

De Deepesh, N. and Ghosh, R. 1990. Revealation of chromosome core structure by silver-staining and fluorescence microscopy. *Proc. International Symposium on Chromosome Research : From Microscope to Molecule,* University of Calcutta, Calcutta : 22.

Desai, V. B., Sirsi, M., Shankarappa, M. and Kasturibai, A. R. 1966. Studies on the toxicity of *Abrus precatorius* L. Part I – Effect of aqueous extracts of seeds on mitosis & meiosis in grasshopper, *Poecilocera picta. Indian J. Exp. Biol.*, **4** : 164-166.

Dutt, M. K. 1948a. A cytological investigation on the genus *Phloeoba* (Acrididae). *Proc. Nat. Inst. Sci. India*, **14** : 13-20.

Dutt, M. K.1948b. Meiosis and chiasma formation in *Tristria pulvinata* Uvarov (Catantopinae, Acrididae). *Proc. zool. Soc. Beng.*, **1** : 49-57.

Dutt, M. K. 1950. Studies on chromosome behaviour and sex chromosome in *Chrotogonus* sp. (Pyrgomorphinae, Acrididae). *Proc. zool. Soc. Bcng.*, **3** : 109-117.

Dutt, M. K. 1951. Polyploidy in a short-horned grasshopper. *Curr. Sci.*, **20** : 327-328.

Dutt, M. K. 1952. Polyploidy in the spermatocytes of some Acridids. *Proc. 39th Indian Sci. Congr.* Abstract Part III : 312.

Dutt, M. K. 1955. On the chromosome complement and meiosis in nine species of Indian grasshoppers. *Proc. zool. Soc. Calcutta*, **8** : 111-129.

Dutt, M. K. 1966. Chromosomes of *Schistocerca gregaria* (Forskal). *Curr. Sci.*, **35** : 497.

Dutt, M. K. 1967. Cytological studies on *Locusta migratoria. Sci. Cult.*, **33** : 384-385.

Elayidom, N. B. 1971. An unequal pair of long chromosomes as the characteristic of *Spathosternum prasiniferum* Walk. (Acrididae, Orthoptera). *Cytologia,* **36** : 26-33.

Elayidom, N. B. and Subramaniam, M. K. 1966. Some interesting cytological features of male *Spathosternum prasiniferum* Walker. *Curr. Sci.*, **35** : 357-358.

Fox, D. P., Carter, K. C. and Hewitt, G. M. 1973. Giemsa banding and chiasma distribution in the desert locust. *Heredity*, **31** : 272-276.

Fox, D. P., Hewitt, G. M. and Hall, D. J. 1974. DNA replication and RNA transcription of euchromatic and heterochromatic chromosome regions during grasshopper meiosis. *Chromosoma*, **45** : 43-62.

Gatti, M., Pimpinelli, S. and Santini, G. 1976. Characterization of *Drosophila* heterochromatin. I. Staining and decondensation with Hoechst 33258 and Quinacrine. *Chromosoma*, **57** : 351-375.

Ghosh, S. N. 1985. Studies on insect sex chromosomes: Functional aspects of the X-chromosome in *Schistocerca gregaria* (Forskl). Ph. D. Thesis, University of Delhi.

Ghosh, S. N., Bhattacharya, M. and Rao, S. R. V. 1987. DNA replication in the spermatogonial cells of *Gryllotalpa fossor* and *Schistocerca gregaria* (Orthoptera). *Insect Sci., Applic.*, **8** : 359-364.

Ghosh, S. N. and Rao, S. R. V 1983. Functional relationship of the allocyclic X- chromosome in *Schistocerca gregaria. Proc. XV International Congress of Genetics,* New Delhi : 151.

Ghosh, S. N. and Rao, S. R. V. 1986. Cell proliferation kinetics and replication of the X-chromosome in somatic cell of *Schistocerca gregaria* (Desert locust). *Proc. Tenth All India Cell Biology Conference,* Gujarat University, Ahmedabad : 78.

Gururaj, M. E. and Rajasekarasetty, M. R. 1967-68. Chromosomal variation in a natural population of *Chrotogonus oxypterus* (Acrididae-Orthoptera). *Half-yrly J. Mysore Univ.* (B), **21** : 31-40.

Gururaj, M. E. and Rajasekarasetty, M. R. 1969. Effects of X-rays on the meiotic cells of *Poecilocera picta* (Acrididae Orthoptera) II. Inter-relationships of aberrations. *Ind. J. Hered.*, **1** : 35-44.

Gururaj, M. E. and Rajasekarasetty, M. R. 1970. Effect of X-rays on the meiotic cells of *Poecilocera picta. Ind. J. Hered.*, **2** : 103-112.

Gururaj, M. E. and Rajasekarasetty, M. R. 1971. Impact of B-chromosome polymorphism on the endophenotype of *Acrotylus humbertianus* (Acridoidea : Orthoptera). *J. Cytol. and Genet. Congr. Suppl.*: 151-158. (*Proc. First All India Congr. Cytol. and Genet.* 1971).

Hannah-Alva, A. 1965. The pre-meiotic stages of spermatogenesis. *Advances in Genetics*, **13** : 157-226.

Hewitt, G. M. 1979. Animal Cytogenetics, Vol. **3**, Insecta 1. Orthoptera. Gebrüder Borntraeger, Berlin Stuttgart : 1-170.

Hewitt, G. M. and John, B. 1965. The influence of numerical and structural chromosome mutations on chiasma conditions. *Heredity*, **20** : 123-135.

Hilwig, I. and Gropp, A. 1973. Decondensation of constitutive heterochromatin in L-cell chromosomes by a bibenzimidazole compound ("33258 Hoechst"). *Exptl. Cell Res.* **81** : 474-477.

Hota, J. and Patnaik, S. C. 1989. A study of chromosomes and their C-band patterns in seven species of Acrididae (Orthoptera). *Caryologia*, **42** : 81-89.

Hussain, B. H. and Rajasekarasetty, M. R. 1968-69. Chromosomal variation in the spermatogenesis of grasshopper, *Poecilocera picta* (Acrididae- Orthoptera). *Half-yrly J. Mysore Univ.*, (B) **22** : 47-55.

Jain, H. K. and Singh, U. 1967. Actinomycin D induced chromosome breakage and suppression of meiosis in the locust, *Schistocerca gregaria. Chromosoma*, **21** : 463-471.

Jain, H. K. and Singh, U. 1967. Effect of Actinomycin D on chromosomes at meiosis in a locust. *Proc. Oxford Chromosome Conference* : 12.

Jain, H. K. and Singh, U. 1969. RNA synthesis and chromosome behaviour in *Schistocerca. Chromosome Today* **2** : 70-74.

John, B., Lewis, K. R. and Henderson, S. A. 1960. Chromosome abnormalities in a wild population of *Chorthippus brunneus. Chromosoma*, **11** : 1-20.

Kacker, R. K., Kulkarni, P. P. and Singh, A. K. 1986. Techniques for the chromosome preparation for the cytotaxonomic studies. IN : Manual. *Collection Preservation, Identification of Insects and Mites of Economic Importance*, Zoological Survey of India, Calcutta. (Director, Zoological Survey of India, Ed.) : 241-250.

Kacker, R. K. and Singh, A. K. 1976. Cytotaxonomic studies in Orthoptera I. Chromosome number in six species of grasshoppers from Himachal Pradesh. *Newsl. zool. Surv., India*, **2** : 117-118.

Kacker, R. K. and Singh, A. K. 1977. Cytotaxonomic studies in Orthoptera II. A study of chromosomes in eight species of grasshoppers. *Newsl. zool. Surv. India*, **3** : 53-54.

King, R. L. and Slifer, E. H. 1934. Insect development. VIII. Maturation and early development of unfertilized grasshopper eggs. *J. Morph.*, **56** : 603-620.

Kofman-Alfaro, S. and Chandley, A. C. 1970. Meiosis in the male mouse. An autoradiograhpic investigation. *Chromosoma*, **31** : 404-420.

Kumaraswamy, K. R. 1977. Contribution to the cell biology of Orthoptera. Ph. D. Thesis, University of Mysore, Mysore.

Kumaraswamy, K. R. and Rajasekarasetty, M. R. 1975. The chromosomal architecture of *Poekilocerus pictus* (Acrididae : Orthoptera). *Chromosome Inf. Serv.*, **19** : 26-28.

Kumaraswamy, K. R. and Rajasekarasetty, M. R. 1976a. Pattern of C-banding in *Acrida turrita* (Acrididae : Orthoptera). *Curr. Sci.*, **45** : 762-763.

Kumaraswamy, K. R. and Rajasekarasetty, M. R. 1976b. A modified technique for grasshopper chromosome preparations. *Entomon*, **1** : 83-84.

Kumaraswamy, K. R. and Rajasekarasetty, M. R. 1976c. C-banding in male meiotic chromosomes of *Poekilocerus pictus* (Acrididae : Orthoptera). *Proc. Indian Acad. Sci.* **83**(B) : 139-142.

Kumaraswamy, K. R. and Rajasekarasetty, M. R. 1978. Chromosomal repattering in Acrididae. *Cytobios*, **19** : 21-26.

Lewis, K. R. and John, B. 1959. Break down and restoration of chromosome stability following inbreeding in a Locust. *Chromosoma*, **10** : 589-618.

Majumdar, K., Chatterjee, C. and Ray-Chaudhuri, S. P. 1979. Uridine incorporation in constitutive heterochromatin in grasshopper. *Nucleus*, Calcutta, **22** : 149-152.

Mangalangi, N. K. and Subramaniam, M. K.1963. A. haematoxylin squash technique for the study of grasshopper chromosomes. *Curr. Sci.*, **32** : 364-366.

Manna, G. K. 1954. A study of chromosomes during meiosis in fifteen species of Indian grasshoppers. *Proc. zool. Soc. Calcutta*, **7** : 39-58.

Manna, G. K.1967. Cytological analysis of the sex chromosome from testis cells of grasshoppers-a review. *Nucleus, Calcutta*, **10** : 64-80.

Manna, G. K. 1969. Some aspects of chromosome cytology (Presidential address). *Proc. 56th Indian Sci. Congr.* : 185-214.

Manna, G. K. 1972. Heterochromatization, a common cause for the origin of diversified chromosomal constitutions in some Orthopteroid species. *Proc. natn. Acad. Sci. India,* **42**(B) : 55-56.

Manna, G. K. and Chatterjee, K. 1963. Polymorphic sex chromosomes in *Euprepocnemis* sp. I. The meiosis in the XO type male and in the neo-X and neo-Y type male. *Nucleus, Calcutta*, **6** : 121-134.

Manna, G. K. and Mazumder, S. C. 1965. Supernumerary or degenerated chromosomes (?) in a chromosomally aberrant cryptosacci grasshopper, *Tristria pulvinata* Uvarov. *Experientia*, **21** : 139-142.

Mittal, O. P. and Khurana, R. K. 1971. Breakdown of the meiotic stability in short- horned grasshopper. *Proc. First All India Congr. Cytol. and Genet.* Punjab University, Chandigarh : 60.

Mittal, O. P. and Soni, R. M. 1977. Chromosomes in the male germ-cells of two species of grasshoppers. *Research Bulletin (Science) of the Panjab University*, **28** : 205-206.

Moens, P. B. 1987. Introduction to meiosis. IN: Meiosis. Moens, P. B. (edited). *Academic Press* : 1-17.

Monesi, V. 1962. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. *J. Cell Biol.*, **14** : 1-18.

Muckenthaler, F. A. 1964. Autoradiographic study of nucleic acid synthesis during spermatogenesis in the grasshopper, *Melanoplus differentialis. Experimental Cell Research*, **35** : 531-547.

Nelsen, O. E. 1931. Life cycle, sex differentiation, and testis development in *Melanoplus differentialis. J. Morph. Physiol.*, **51** : 467-525.

Nolte, D. J. 1967. Phase transformation and chiasma formation in Locusts. *Chromosoma,* **21** : 123-139.

Nur, U. 1961. Meiotic bebaviour of an unequal bivalent in the grasshopper *Calliptamus palaestinensis* Bdhr. *Chromosoma,* **12** : 272-279.

Parchman, L. G. and Stern, H. 1969. The inhibition of protein synthesis in meiotic cells and its effect on chromosome behaviour. *Chromosoma,* **26** : 298-311.

Powers, P. B. A. 1942. Metrical studies on the spermatogonial chromosomes of Acrididae (Orthoptera). *J. Morph. Philadelphia,* **71** : 523-576.

Puttanna, C. R. 1964. Role of supernumeraries in the population dynamics of *Orthracris ruficornis* (Family : Acrididae). *Proc. Combd. 51st and 52nd Indian Sci. Congr.* Abstract Part III : 445-446.

Puttanna, C. R. 1968. Meiotic accidents in the HG population of *Poecilocera picta* (Insecta: Acrididae). *Proc. 55th Indian Sci. Congr.* Abstract Part IV : 27.

Puttanna, C. R. 1971. Principles of casuality and chromosomal aberration in the natural populations of grasshoppers. *Proc. 58th Indian Sci. Congr.* Abstract Part IV : 15-16.

Rahiman, M. A. and Rajasekarasetty, M. R. 1967a. Intraspecific karyotypic variation in different populations of *Poecilocera picta* (Simon). *J. Cytol. and Genet.,* **1** : 32-53.

Rahiman, M. A. and Rajasekarasetty, M. R. 1967b. Nuclear phenotype of *Locusta migratoria. La Cellule,* **66** : 301-310.

Rajasekarasetty, M. R. 1963. Chromosomal dynamics of *Gastrimargus. Nature Lond.,* **197** : 622-623.

Rajasekarasetty, M. R. 1965. Sperm- dimegaly in *Gastrimargus* sp. (Family: Acrididae, Order: Orthoptera). *Half-yearly J. Mysore Univ.,* **18** : 1-4.

Rajasekarasetty, M. R. 1969. Chromosomal abnormalities in wild populations of some Acridids. *Indian J. Hered.,* **1** : 125-141.

Rao, T. R 1932a. Chromosome number in Pyrgomorphinae. *Curr. Sci.,* **1** : 41-42.

Rao, T. R. 1932b. Further notes on the chromosomes of Pyrgomorphinae. *Curr. Sci.,* **1** : 101.

Rao, T. R. 1933. Spermatogenesis in *Orthacris* (Acrididae). *Proc. 20th Indian Sci. Congr.* Abstract Part III :

Rao, T. R. 1934a. On the spermatogonial divisions in *Aularches miliaris* L. *Proc. Indian Acad. Sci.,* (B) **1** : 19-30.

Rao, T. R. 1934b. Polymegalous spermatids in a grasshopper. *Curr. Sci.,* **2** : 239-241.

Rao, T. R. 1934c. Chromosomal aberrations occuring in unirradiated grasshoppers. *Half-yearly J. Mysore Univ.,* **7** : 5-14.

Rao, T. R. ·1937. A comparative study of the chromosomes of eight genera of Indian Pyrgomorphinae (Acrididae). *J. Morph.,* **61** : 223-255.

Rao, T. R. 1940. Chiasma frequency and chromosome length in closely related members of Pyrgomorphinae (Acrididae). *Proc. 27th Indian Sci. Congr.* Abstract Part III : 159.

Ray-Chaudhuri, S. P. 1961. Ionizing radiations and the induction of chromosome mutations in the germ cells. (Presidential address). *Proc. 48th Indian Sci. Congr.* : 1-23.

Ray-Chaudhury, S. P. and Bose, I. 1948. Meiosis in diploid and tetraploid spermatocytes of *Atractomorpha* sp. (Acrididae). *Proc. zool. Soc. Beng.* **1** : 1-12.

Ray-Chaudhuri, S. P., Dasgupta, N. N. and Guha, A. 1952. A new method for the study of the chromosomes of grasshoppers by the Electron microscope. *Proc. 39th Indian Sci. Congr.* Abstract Part III : 310

Ray-Chaudhuri, S. P. and Dutt, M. K. 1947. A comparative study of the chromosome structure and behaviour in three different genera of Indian grasshoppers. *Proc. R. Soc. Edinb.*, **62** : 292-298.

Ray-Chaudhury, S. P. and Guha, A. 1952. X and neo-Y mechanism of sex-determination in the grasshopper, *Thisiocetrus pulcher. Nature, Lond.*, **169** : 78.

Ray-Chaudhuri, S. P. and Guha, A. 1955. Supernumerary chromosomes in two populations of the grasshopper, *Aiolopus* sp. B and their behaviour during spermatogenesis. *J. Genet.* **53** : 363-378.

Ray-Chaudhury, S. P. and Manna, G. K. 1951. Chromosome evolution in wild populations of Acrididae Part I. On the occurrence of a supernumerary chromosome in *Aiolopus* sp. *Proc. Indian Acad. Sci.* (B), **34** : 55-61.

Ray-Chaudhuri, S. P. and Pyne, C. K. 1954. Time-intensity factor in the production of dicentric bridges with Gamma rays of radium during meiosis in the grasshopper, *Gesonula punctifrons. Science, N. Y.*, **119** : 685-686.

Ray-Chaudhuri, S. P. and Sarkar, I. 1952. Frequency of dicentric bridges in meiosis in the grasshopper *Gesonia punctifrons*, produced by different dosages of X-rays. *Science, N. Y.*, **116** : 479-482.

Riley, R. and Flavell, R. B. 1977. A first view of the meiotic process. *Phil. Trans. R. Soc. Lond.*, B **277** : 191-199.

Ris, H. 1969. In : "Handbook of Molecular Cytology" (A. Lima-de-Faria, ed.) Am. Elsevier, New York : 221-250

Saha, A. K. 1978. A new technical advance in dissection, fixation and preservation of grasshopper testes for cytological permanent squash preparation of chromosomes. *Chromosome Inf. Serv.*, **25** : 3.

Sarkar, I. 1955. A translocation heterozygote in the grasshopper *Gesonula punctifrons. J. Hered.*, **46** : 157-160.

Sharma, G. P. 1966. Chromosomes in relation to taxonomy and phylogenetic relationship of the animals. (Presidential address). *Proc. 53rd Indian Sci. Congr.* : 1-12.

Sharma, G. P., Gupta, M. L. and Randhawa, G. S. 1967. Polysomy in *Chrotogonus trachypterus* (Blanchard). (Orthoptera : Acridoidea : Pyrgomorphidae) from three more populations and its possible role in animal speciation. *Research Bulletin (N. S.) of the Panjab University*, **18** : 157-163.

Sharma, G. P., Handa, S. M. and Mandhar, N. 1974. DNA in the testis of *Locusta migratoria* and *Acrida exaltata* (Acrididae : Orthoptera). *Research Bulletin (N. S.) of the Panjab University*, **25** : 121.

Sharma, G. P., Handa, S. M. and Mandhar, N. 1983. Chromosomal studies on two species of grasshoppers (Acrididae : Orthoptera). *Research Bulletin (Science) of the Panjab University*, **34** : 191-199.

Sharma, G. P., Mittal, O. P. and Kaur, S. 1974. Size variation and differential coiling of chromosomes in *Chrotogonus trachypterus* (Blanchard). *Research Bulletin (N. S.) of the Panjab University*, **25** : 21-25.

Sharma, G. P., Mittal, O. P. and Yadav, J. S. 1974. Chromosome behaviour in an Indian grasshopper *Tryxalis indica* Thunb. *Research Bulletin (N. S.) the of Panjab University*, **25** : 1-4.

Sharma, G. P. and Parshad, R. 1957. Lampbrush chromosomes in insects. *Nature, Lond.*, **180** : 147.

Sharma, G. P., Parshad, R. and Aggarwal, B. M. 1964. Cytochemical analysis of the lampbrush fibres in the chromosomes of *Chrotogonus trachypterus* (Blanchard) (Orthoptera : Acridoidea : Pyrgomorphidae). *Research Bulletin (N.S.)of the Panjab University*, **15** : 47-49.

Sharma, G. P., Parshad, R. and Bedi, T. S. 1962. Breakdown of the meiotic stability in *Chrotogonus trachypterus* (Blanchard) (Orthoptera : Acridoidea : Pyrgomorphidae). *Research Bulletin (N. S.) of the Panjab University*, **13** : 281-308.

Sharma, G. P., Parshad, R. and Bedi, T. S. 1963. A translocation heterozygote in *Chrotogonus trachypterus*. *J. Hered.*, **54** : 62-65.

Sharma, G. P., Parshad, R. and Gupta, M. L. 1963. On the seasonal variation in the mean chiasma frequency per nucleus in the wild population of *Chrotogonus trachypterus* (Blanchard) (Orthoptera : Acridoidea : Pyrgomorphidae). *Research Bulletin (N. S.) of the Panjab University*, **14** : 313-315.

Sharma, G. P., Parshad, R. and Gupta, M. L. 1965. Chromosomal variations in the male germ cells of *Chrotogonus trachypterus* (Blanchard) (Orthoptera : Acridoidea : Pyrgomorphidae) from Ottu (Punjab). *La Cellule* **65** : 295-314.

Sharma, O. P. and Dogra, A. K. 1987. On the chromosomes of three Acridid species (Acridoidea). *Proc. Sixth All India Congr. Cytol. and Genet.*, University of Jammu, Jammu : 110-111.

Sharma, T. 1962. Parthenogonic haploid embryos in the grasshopper, *Aiolopus* sp. *Sci. Cult.* **28** : 345-346.

Sharma, T. 1966. Study on dicentric bridges during meiosis in X-ray induced structural heterozygotes of grasshopper. *Proc. of the Seminar of International Cell Biology*, Bombay : 282-285.

Singh, A. K. 2002. A catalogue of experimental studies on grasshopper chromosomes in India 1928-2000. *Rec. zool. Surv. India, Occ. Paper* No. **197** : 1-44.

Singh, A. K. and Kacker, R. K. 1980. Cytotaxonomical research. IN: *State of Art Report, Zoology, Zoological Survey of India.* (Director, Zoological Survey of India, Ed.) : 278-293.

Singh, L., Purdom, I. F. and Jones, K. W. 1976. Satellite DNA and evolution of sex chromosome. *Chromosoma*, **59** : 43-62.

Smith, S. G. 1965. Heterochromatin, Colchicine and Karyotype. *Chromosoma*, **16** : 162-165.

Southern, D. I. 1967. Species relationships in the genus *Tulipa. Chromosoma*, **23** : 80.

Srivastava, M. D. L. 1951. 'Lampbrush' fibres in the chromosomes of *Chrotogonus incertus* Bolivar. *Nature, Lond.*, **167** : 775-776.

Srivastava, M. D. L.1954. Studies on the structure of the chromosomes of *Chrotogonus incertus* Bolivar (Acrididae). *J. Genet.*, **52** : 480-493.

Srivastava, M. D. L. 1957. Studies on the structure and behaviour of the chromosomes of *Dissosteira carolina* L. (Acrididae : Orthoptera). *La Cellule*, **57** : 271-279.

Srivastava, M. D. L. 1960. Meiotic aberrations in the spermatogenesis of *Poecilocerus pictus* (Acrididae- Orthoptera). *La Cellule*, **61** : 67-77.

Srivastava, M. D. L. 1968. The structure of chromosomes (Presidential address). *Proc. 55th Indian Sci. Congr.* : 1-14.

Stern, H. and Hotta, Y 1967. Chromosome behaviour during development of meiotic tissue. IN: The control of nuclear activity (L. Goldstein, ed.), Ingle Cliffs, New Jersey: Prentice Hall : 47-76.

Umadevi, K. and Aswathanarayana, N. V. 1987. *Coptarca punctaria* (Walker), an unique Indian Acridid with chromosomal polymorphism (Order : Orthoptera, Class : Insecta). *Curr. Sci.*, **56** : 317-318.

Ved Brat, S. and Sethi, S. K. 1974. Polymorphism for meiotic characters in grasshopper. *Nucleus, Calcutta*, **17** : 28-32.

White, M. J. D. 1934. The influence of temperature on chiasma formation. *J. Genet.*, **29** : 203-215.

White, M. J. D. 1935. The effects of X-rays on mitotis in the spermatogonial divisions of *Locusta migratoria* L. *Proc. Roy. Soc. London*, B **119** : 61-84.

White, M. J. D. 1937. The effect of X-rays on the first meiotic division in three species of orthoptera. *Proc. roy. Soc.*, B **124** : 183-196.

White, M. J. D. 1954. Animal cytology and evolution. (Second edition) Cambridge University Press.

White, M .J. D. 1955. Patterns of spermatogenesis in grasshoppers. *Aust. J. Zool.*, **3** : 222-226.

White, M. J. D. 1973. Animal Cytology and Evolution. Cambridge University Press, London : 1-961.

Yadav, A. S. 1983. Chromosome studies of some hoppers. Ph. D. Thesis, Kurukshetra University, Kurukshetra.

Yadav, A. S. and Yadav, J. S. 1999. Evolution of sex chromosome systems in Acridoidea (Orthoptera). IN : *Some Aspects on the Insight of Insect Biology* (R. C. Sobti and J. S. Yadav Eds.) : 195-204.

Yadav, J. S., Karamjeet and Yadav, A. S. 1981. Occurrence of neo-XY system in *Tryxalis indica* Thunb. *Caryologia*, **34** : 311-316.

Yadav, J. S., Rishi, K. K. and Virdy, K. K. 1978. Chromosomal dimorphism in the natural population of a grasshopper *Tryxalis indica* Thunb. (Tryxalinae: Acrididae : Orthoptera). *Proc. Third All India Congr. Cytol. and Genet.* Haryana Agricultural University, Hissar : 18.

Yadav, J. S. and Yadav, A. S. 1982. Breakdown of meiotic stability in *Tristria pulvinata* Uvarov (Acrididae : Orthoptera). *J. Anim. Morphol. Physiol.*, **29** : 202-210.

Yadav, J. S. and Yadav, A. S. 1983a. Karyotypic analysis of five species of grasshoppers (Acridoidea : Orthoptera). *J. Anim. Morph. Physiol.*, **30** : 42-48.

Yadav, J. S. and Yadav, A. S. 1983b. Analysis of hopper chromosomes with banding techniques. I. *Phlaeoba infumata* Brunner (Tryxalinae: Acrididae) and *Atractomorpha crenulata* F. (Pyrgomorphinae : Acrididae). *Biologia*, **29** : 47-53.

Yadav, J. S. and Yadav, A. S. 1985. Centric fusions in an Indian grasshopper (Acrididae: Orthoptera). *Cell and Chromosome Research*, **8** : 43-44.

Yadav, J. S. and Yadav, A. S. 1986. Chromosome number and sex-determining mechanisms in thirty species of Indian Orthoptera. *Folia biol.* (Krakow), **34** : 277-284.

Yadav, J. S. and Yadav, A. S. 1987. Localisation of NORs in spermatogonial metaphase chromosomes of six species of grasshoppers. *Genetica,* **74** : 155-160.

Yadav, J. S. and Yadav, A. S. 1988. Distribution of constitutive heterochromatin in six species of grasshoppers. *Proc. International Symposium on Recent Advances in Cytogenetic Research,* University of Kurukshetra, Kurukshetra : 106.

Yadav, J. S. and Yadav, A. S. 1989. On chromosomal aberrations in the natural populations of some grasshoppers. *Genet. Iber.,* **41** : 37-46.

Yadav, J. S. and Yadav, A. S. 1990a. X-autosome fusion in Catantopine grasshoppers (Acridoidea: Orthoptera). *Cytobios,* **61** : 21-30.

Yadav, J. S. and Yadav, A. S. 1990b. B chromosomes in some Indian grasshoppers (Acridoidea: Orthoptera). *Nucleus, Calcutta,* **33** : 73-77.

Yadav, J. S. and Yadav, A. S. 1993. Distribution of C-heterochromatin in seventeen species of grasshoppers. *Nucleus, Calcutta,* **36** : 51-56.

Yadav, J. S. and Yadav, A. S. 1994. Cytoevolutionary pathways in Acridoidea. IN : *Perspectives in Entomological Research* (O. P. Agrawal, Ed.). Scientific Publishers Jodhpur : 191-209.

Yadav, J. S. and Yadav, A. S. 1996. Karyotypic diversity in grasshopper *Tristria pulvinata* Uv. IN : *Some Facets of Biodiversity* (R. K. Kohli, N. Jernath and D. Batish Eds.). M/s Research Periodicals and Book Services Inc. : 229-247.

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THE NUCLEAR PHENOTYPE OF *XENOCATANTOPS HUMILIS* (SERVILLE) (ORTHOPTERA : ACRIDIDAE : CATANTOPINAE), *W.S.R.* TO SUPERNUMERARY SEGMENTS

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Supernumerary segments

One of the principal forms of heterochromatin variation in natural populations of Orthopterans is the presence of supernumerary segments in polymorphic states. These small blocks may be sited proximally, distally or in an interstitial position on the chromosomes. In prophase of meiosis they reveal their characteristic heterochromatic features and alter the chiasma pattern of their bivalents. Thus through meiosis provides genetic variation for a population. The variation is then greatly increased by fertilization, when new pairings of homologues occur. The species is therefore by their own mechanism of genotype evolution avoid extinction by removing their maladapted genotypes from population and getting selected the new ones in the ever changing environmental factors.

INTRODUCTION

Important aspects of the nuclear phenotype of an organism are the chromosome numbers, their sizes, extent of contraction during cell divisions, and chiasma pattern. Apart from karyotypes and idiograms, features visible on the chromosomes during mitotic and meiotic divisions are also taken into consideration. These include nucleolar organizer regions (NORs), gaps, constrictions, heteropycnotic and allocyclic segments, megameric chromosomes, ditactic bivalents, subchromatid structure, behaviour of unequal bivalents and supernumerary chromosomes. Some of these features are briefly introduced here.

Supernumerary chromosomes : These chromosomes are in addition to the normal complement. They may be present in some individuals of natural populations but not in others. Supernumeraries are not homologous or may be partly homologous to certain members of the regular set. They often display non-disjunction at anaphase of mitosis, therefore their frequency varies from one organ to the other. Supernumeraries rarely depict nucleolus organizers and, therefore lack secondary constrictions.

NORs : Cytologists have shown that the nucleolus is organized at a specific site on the chromosomes. These sites are located in the so called "achromatic secondary constriction regions" One chromosome pair or many pairs may get engaged in nucleolus formation. This is a constant feature of a karyotype. The nucleolus organizer regions are usually very strikingly marked since these are negatively heteropycnotic to such a degree that the remaining portion of the chromosome, the so-called satellite, seems to have been removed from the rest of the chromosome like a chromosome fragment (Schulz-Schaeffer, *et al.* 1980). In Acridids, this feature can easily be located on their chromosomes.

Gaps : A narrow unstained region in chromosome which may occur in one chromatid or more rarely isolocally in both. They do not represent discontinuities in the chromosome. There is always a difficulty in identifying chromatid gap from break. At Chatham conference in 1971, it has been recommended that "any discontinuity of the chromatids that is equal to or greater than the width of a chromatid be considered "unequivocal" and recorded as break. Lesser lesions may be tabulated as chromatid gaps, for accessory information only" (refer Moorhead *et al.*, 1974 and Satya Prakash *et al.*, 1981). Due to non-availability of better criteria, this is being adopted by most cytologists. At anaphase, a chromosome with a gap does not loose its fragment.

Constriction : This is an unspiralized region of fixed position on the metaphase chromosome. Diameter of the segment is less than elsewhere, and their DNA content is low per unit of length. Kinetochores and NORs are located in constrictions.

Allocyclic segment : The process of coiling of chromosomes is not always uniform in all the members of a complement at a given stage of the nuclear division. The regions in chromosomes which are out of coiling cycle are termed allocyclic. The regions most frequently subject to allocyclic behaviour are the centromeres, telomeres and NORs, while the chromosomes as a whole allocyclic are the supernumeraries and sex-chromosomes. The commonest type of allocycly is that the concerned segments appear over condensed in comparison to regions which follow the standard cycle. This is called positive heteropycnosis. The opposite allocyclic state, negative heteropycnosis, is less frequent and is detectable at all times during cell division. It is also found that the same region can behave differently in different cycles of nuclear division (refer Rieger *et al.* 1976). Heterochromatin is mostly found out of step or allocyclic in its condensation behaviour relative to euchromatin.

Megameric chromosome : Autosomes which possess a conspicuous heterochromatic segment or with several such segments are termed megameric. These segments are characteristically heterochromatic in male meiotic prophase and for this reason is found frequently associated with the similarly condensed X-chromosome. Almost all the species of grasshoppers possess in their karyotypes one pair of medium size or small autosome with megameric feature (White, 1973).

Ditactic bivalent : Bivalents with chiasma in the short arm of the so called acrocentric chromosomes of grasshoppers (refer White, 1973 and Hewitt, 1979).

Subchromatid structure : A chromatid at metaphase when found divided longitudinally following routine pretreatments, suggests that half-chromatids exist in chromosomes. This favours bineme model, where chromatids are made up of two DNA strands or subchromatids. According to the unineme model, each chromatid at prophase or metaphase consists of a single spirally coiled DNA duplex together with associated RNA and protein (White, 1973).

Unequal bivalents : Occurrence of unequal homologous pairs is not a rare feature in grasshoppers. They become distinct in their meiotic configurations. The larger homologue incorporate extra region which is called supernumerary segment. In other cases an unequal pair may be resulted from the loss of a chromosome segment from one of its homologues. The supernumerary segments are clearly akin to supernumerary chromosomes. Such segments are not indispensable because other individuals in the same population without these extra segments develop normally. Supernumerary segments being part of regular members of the karyotypes are inherited in conventional manner. Supernumerary segments are usually heteropycnotic in nature.

MATERIALS AND METHODS

Fifteen males and three females comprised the material for the present investigation. The species is one of the commonest grasshoppers of India, found in shrubs as well as in agricultural fields. Females are very similar to males except larger in size. The grasshoppers of the subfamily Catantopinae are distributed in tropical and sub-tropical zones of the whole world (Dirsh, 1975).

Sex	Code Number of Individuals Studied	Site of Collection
♀	319	Renuka Lake (Himachal Pradesh)
♀	586, 587	Singla, District Darjeeling (W.B.)
♂	256, 330, 335, 341, 346, 370, 375, 379, 383, 384, 471	Sukna Forest, District Darjeeling (W.B.)
	491, 492	Sivoke, District Darjeeling (W.B.)
	565, 591	Singla, District Darjeeling (W.B.)

Each individual of the species studied are tagged with a code number and details of the locality and maintained in Cytotaxonomy Research Laboratory, Zoological Survey of India, Kolkata.

Technique used in Chromosome Preparation

For arresting cells at metaphases, selected male and female individuals were injected with colchicine at a concentration of 0.05% and with an amount of 0.03 to 0.04 ml in each case and thereafter they were kept alive for at least 4 hrs. in insect cages. Chromosomal preparations were made from the cells of testes and hepatic caeca. The tissues were dissected out and cleaned in 0.67% sodium chloride solution. The testes were pretreated (hypotonic treatment) in 0.9% sodium citrate solution for 45 minutes and hepatic caeca for 1 hr. in the same solution, diced and fixed in freshly prepared methanol-acetic acid (3 : 1) with three changes of 15 minutes. A slightly more hypotonic treatment and fixation was preferred for the somatic cells. In the field, the tissues were fixed in small tubes, brought to laboratory and stored at 4°C. For preparation of the slides, the fixed tissues were transferred to 50% acetic acid till it became soft. The materials were then teased and squashed in one or two drops of 50% acetic acid. The slides were stored in vapours of 50% acetic acid overnight in cold. Next morning the slides were brought at room temperature and immersed in a solution of 3:1 methanol-acetic acid for an hour. The cover slips were removed with the sharp edge of a blade, while immersed in the medium and dried at room temperature in a dust free chamber. Staining was done in 2% Giemsa [E. Merck (India) Private Ltd.] prepared in phosphate buffer (pH 6.8). After differentiating in phosphate buffer (pH 6.8), slides were rapidly air dried under a lamp, cleaned in xylene and mounted in DPX.

Chromosome morphology and nomenclature were made according to the system of Levan *et al.* (1964). In order to compare the chromosome pairs, histogram was constructed from the relative length.

OBSERVATIONS

Karyological details

Diploid Number Chromosome Morphology and Sex Chromosome Constitution

Diploid number (2n) was 23 in males and 24 in females. All the chromosomes of the complement were acrocentric (Figs. 1-4). In the males, identification of the sex chromosome (X) was done in metaphase I and metaphase II cells. In anaphase I cell the univalent nature of the X made it easy to identify (Figs. 27 and 28), and it was relatively more darkly stained in metaphase II (Fig. 3). The X was the 6th largest with 8.5% relative length in the complement. In some cells, subchromatid-like visible structure was seen in this element (Fig. 9, arrow). In an anaphase II, this feature was also revealed in an autosome (Fig. 10, arrow). Some times one or two autosomes or their chromatids showed difference in condensation (Figs. 11 and 12, arrow).

Karyotype

In 6 males and 1 female, 25 karyotypes were studied. 8 individuals were investigated for meiotic progression for which their 48 meiotic karyotypes were analysed.

Relative length (RL) in percentage

1	2	3	4	5	6	7	8	9	10	11	X
14.26	12.22	10.90	10.27	9.25	8.23	7.34	6.56	5.03	4.42	3.16	8.50

Idiogram

In the idiogram, the X chromosome was the 6th largest member in the complement. The chromosomes could not be grouped into any size class because of gradual gradation in their size (Fig. 8).

Gaps / Constrictions

Gaps and constrictions were noted in anaphase I and metaphase II chromosomes of 3 male individuals. In a metaphase II cell of one individual (No. 491), the 1st and 5th largest elements had a gap in the distal region of one of their chromatids (Fig. 5, arrow). In this cell, the 3rd element had also revealed a gap in the proximal region of one of its chromatids (arrow). In another cell of this individual, centromeric constriction of the largest element and a satellite region at the terminal end of one chromatid of the X chromosome was distinct (Fig. 6, arrow). In another individual (No. 492), a gap was noted in the distal half region of one of the chromatids of 5th and 7th largest chromosomes (Fig. 7 arrow). In a diplotene cell of an individual (No. 492), the X chromosome also had a gap situated at the terminal end (Fig. 15 arrow).

Supernumerary Chromosomes

Two types of supernumerary chromosomes were recognized in 3 individuals (No.384, 471 and 565) collected from two different localities. The first type was observed in 1 out of 8 diakinetic cells of an individual (No. 384) collected from Sukna forest, in the foot hills of Darjeeling, Himalaya. The bivalent formed by the supernumerary chromosomes was slightly bigger in size than the smallest bivalent, and it had apparently a chiasma at the distal end (Fig.20). Its staining behaviour was comparable to that of the regular bivalents. In another individual of the same locality (No. 471), the supernumerary bivalent was present in 2 cells at metaphase I out of 6 diplotene, diakinetic and metaphase I cells studied (Figs. 21 and 22). These cells at different stages were belonging to different cysts. This signified that inter and intra-cyst variations of supernumerary bivalent had occurred. In side view of a metaphase I plate, this bivalent was not placed outside the equatorial plate (Fig. 22). This feature had reflected its stability during meiotic division. In both these metaphase cells, the 5th and 6th bivalents were found unequal.

The second type of supernumerary chromosome was observed in an individual (No. 565) collected from another locality, namely Singla, in the district Darjeeling which is near Sikkim border. This supernumerary element was scored in 2 out of 5 diakinetic cells, and in 1 out of 2 spermatogonial metaphase cells. This was distinctly smaller than the smallest bivalent of the normal complement, and was thus designated as mini Bs of this species (Figs. 1 and 32, arrow). In both the diakinetic cells, and in the spermatogonial metaphase cell, this supernumerary element was found less stained as compared to the normal smaller bivalents. This staining feature had also reflected their usual euchromatic nature. The supernumerary homologues were associated with a terminal chiasma. Unequal bivalents were not found in any of the cells of this individual (No. 565). In another individual (No. 591) also from the same locality Singla, both the features that is the supernumerary chromosomes and the unequal bivalents were not found.

Sex chromosome Behaviour in Diploid and Tetraploid Cells during Meiosis

Behaviour of the two sex chromosomes was studied in the occasionally present tetraploid cells. In a cell at the zygotene stage, both the sex chromosomes were similarly condensed, and were lying apart, one at the periphery of the cell, and the other in the center (Fig. 13). In the adjoining diploid cell of the same stage, the sex chromosome was at the periphery. In another tetraploid cell of this individual (No. 471), at the pachytene stage, both the sex chromosomes had depicted staining of equal intensity with sharp out line (Fig. 18). Presence of multivalents in the cell had indicated that the polyploid cell was formed prior to the zygotene stage (Fig. 18, arrow). This was probably derived through the failure of segregation of the homologues at spermatogonial division. In a tetraploid cell of another individual (No. 341), both the sex chromosomes were of comparable staining intensity with the bivalents, and there were no multivalents formed in the cell (Fig. 29). This cell might have formed through fusion of two primary spermatocytes. The behaviour of the two sex chromosomes in the tetraploid cells was in contrast to what happens in normal diploid oocytes, where both their sex chromosomes are not heteropycnotic and they form a true bivalent held together by chiasmata (White, 1973).

The sex chromosome in the diploid cells of the male individuals had shown gradual variation in its morphology. In a zygotene cell, it was enlarged in size. In a visibly diffuse stage of zygotene, one can also follow its course of chromatids from the centromere to the telomeric end (Fig.14). With the progression of meiosis, it showed gradual condensation, and by the diplotene stage, rod-like configuration was formed (Fig. 15). A gap at the terminal end was also distinct at this stage (Fig. 15, arrow). It was worth recording the differential staining property of the sex chromosome beginning with the metaphase I stage. First, the centromeric region of this chromosome was found to be isopycnotic (Fig. 23, arrow). After that, in another 3 cells at the late metaphase I stage, the entire length of the sex chromosome was found totally allocyclic as compared to the bivalents. In all the 3 cells, the sex chromosome had attained negatively heteropycnotic state to a considerable extent (Figs. 24, 25 and. 26), but in one cell the centromeric end of the sex chromosome was visible as a minute darkly stained dot (Fig. 24, arrow). With the onset of anaphase I stage, this element had attained staining intensity comparable to that of the bivalents (Figs. 27 and 28).

Unequal bivalents

In several individuals of this species, one of the homologues of the two bivalents were consistently larger in their different meiotic configurations. These elements were designated as the unequal bivalents. 43 meiotic karyotypes from 6 individuals collected from Sukna forest, in the district Darjeeling and 1 collected from Sivoke, situated 15 kms. away from Sukna forest, had consistently two unequal bivalents which were the 5th and 6th largest elements in their meiotic karyotypes (Fig. 33). Whereas in 4 meiotic karyotypes of an individual (No. 565) from Singla in the district Darjeeling, no such unequal bivalents were present (Fig. 30). The additional segment present in the larger homologue of both the bivalents were located in the distal region and showed heteropycnotic behaviour (Fig. 33 arrow).

All the 86 unequal bivalents investigated were associated with a single chiasma (monochiasmate). In 83 instances the chiasma was located in the interstitial region. Though interstitial in position, the chiasma in the bivalents showed variation from near proximal to near distal locations. Such localization of chiasma resulted into equational segregation of the extra region at the first meiotic division. In three cases, chiasma was found terminal in position (Fig. 33, bivalent no. 29, 36 and 55). This provided evidence of a minute euchromatic region beyond the additional region, and its involvement in the chiasma formation with the corresponding end of the smaller homologue. Such localization of chiasma resulted into reductional segregation of the extra region.

The variation in the chiasma distribution of the unequal bivalents was compared with that of the corresponding normal bivalents of the individual (No. 565), collected from Singla. The homomorphic bivalents of this individual had shown uniformity in chiasma localisation. All had interstitial chiasma which resulted into uniform cross configurations at the diakinesis stage (Fig. 30). On the basis of this consistent feature observed in the homomorphic bivalents, it was presumed that, heterozygosity due to the increase of chromatin material in one of the homologues could affect chiasma distribution in the unequal bivalents. The 11th bivalent being unequal was noted in a diplotene cell of the individual no. 383, collected from Sukna forest (Fig. 19).

Behaviour of Megameric Bivalent

The 9th bivalent was found to be a megameric element of the species. It was noted with a conspicuous heteropycnotic segment at the distal region. Characteristic feature of this megameric bivalent was distinct at early stages of prophase. At a diffuse stage of the zygotene, this bivalent was found precociously condensed, and had attained a more or less diplotene configuration (Fig. 14, M). The bivalent had shown interstitial and proximal chiasmata. Its heterochromatic segments were distributed throughout, but the distal one was prominent. With the progression of meiosis, the distal heterochromatic segments formed a prominent block at diplotene and diakinesis (Figs. 15, 16 and 17). In anaphase I cells, this bivalent had shown early separation (Figs. 27 and 28).

Chiasma Distribution

The table below gives an account of the distribution of chiasmata in each bivalent. Each bivalent has been arbitrarily divided into three equal parts as proximal (P) interstitial (I) and distal (D).

Bivalents	1	2	3	4	5	6	7	8	9	10	11
Chiasma, Location & Number	P 05	P 05	P 09	P 17	P 18	P 20	P 15	P 01			
	P,I,01	P,I, 04									
	P,I,D 03	P,I,D 05		P,I, 02							
	P,D 27	P,D, 11	P,D 07	P,D 04							
	I 02	I 11	I 17	I 17	I 24	I 24	I 21	I 15	I 19	I 08	I 15
		2I 02	2I 01								
	I,D 08	I,D 07	I,D 08	I,D 02							
	D 02	D 03	D 06	D 06	D 06	D 04	D 12	D 32	D 29	D 40	D33
Total	P=36	P=25	P=16	P=23	P=18	P=20	P=15	P=01			
	I=14	I=31	I=27	I=21	I=24	I=24	I=21	I=15	I=19	I= 08	I=15
	D=40	D=26	D=21	D=12	D=06	D=04	D=12	D=32	D=29	D=40	D=33
	90	82	64	56	48	48	48	48	48	48	48

Thus, 628 chiasmata scored in 528 bivalents, showing 154 proximal(P), 219 interstitial (I), and 255 distal (D) in position.

DISCUSSION

Sub-Chromatid Structure

Chromatids of a sex-chromosome in a metaphase II cell, and, of an autosome, in anaphase II cell had depicted two filaments as their sub-chromatids with evidently split telomeres (Figs. 9 and 10, arrow). Both the filaments were separated through-out but were joining at the centromere. This feature suggested that half-chromatids exist in Acridid chromosomes. In the present investigation, this feature has been revealed with conventional air-dry preparation of the chromosomes and then their staining by the Giemsa stain. Long back Mickey (1946) had reported the presence of multiple strands in the sex-chromosomes of an Acridid namely *Romalea* sp. During anaphase I, this chromosome exhibited the typical repulsion of chromatids, thus presenting the familiar 'V' configuration. In his camera lucida drawing, the sub-chromatids of the sex chromosome were loosely coiled about each other and which individually had shown a spiral structure (Pl. refer his fig. 2). Egozcue (1973) offered evidence suggesting that half- chromatids exist in human chromosomes also. In his paper, it has been shown that banding patterns on sub-chromatids were identical to those described for whole chromosomes

(Pl. refer his figs. 2 and 3). Apart from these findings, Vosa (1968) revealed sub-chromatids in early mitotic anaphase chromosomes of the root tip cells of the *Crocus chrysanthus*, by hydrolyzing the chromosome in .1 N HCL at 60° C for 20-30 seconds. And Becak (1974) revealed half chromatids in chloroform treated metaphase chromosomes of tetraploid (4n = 44) and diploid (2n = 22) *Odontophrynus americanus* and diploid *Fleutherodactylus binotatus* (2n = 22). Both are Anuran species. Gimenez-Martin (1968) investigated the structural levels of the chromatids of root tip cells of a plant species namely *Scilla non-scripta* with or without 8-oxyquinolene pretreatment. The sub-chromatids were distinct in his preparations (Pl. refer his figs. 1-4).

Living cells were also studied in this context and provided conclusive evidence that half-chromatids do exist in the metaphase chromosomes. Bajer (1965) had taken motion pictures of living chromosomes in the endosperm of *Haemanthus*. His pictures of living cells showed metaphase chromosomes spliting into half-chromatids. The split becomes more obvious as the cells progress to anaphase where half-chromatids can be clearly seen. He stated that by telophase, four units could be found in a chromatid. Since these studies were carried-out on living cells, the phenomenon could hardly be attributed to an artifact of fixation. Wolf (1969) had summarised the findings regarding strandedness of chromosomes, and found that evidences obtained by light microscopy were mostly favouring multi- stranded nature of chromosomes. But some experiments with the lamp brush chromosomes of a newt indicated that its chromosome might very well be single stranded. Thus, with our findings and on the preponderance of the cytological evidence it is concluded that most of the chromosomes are not single stranded.

NORs, Gaps and Constrictions

Only specific chromosomes in any species are involved in the formation of nucleolus (Nucleolus is an RNA-rich, spherical body associated with a specific chromosomal segment, the nucleolus organizer (refer King and Stansfield 1990). These special regions (i.e., NORs) on the chromosomes are recognized as "secondary chromosomal constrictions" and the chromosomes bearing them are referred to as nucleolar chromosomes (Busch and Stemetana 1970). The bladder like nucleoli is prominent structure in interphase and prophase nuclei. In the present investigation the NOR has been located at 4th bivalent in a diplotene cell of an individual No. 341 (Fig. 17). The arrow on the bivalent indicates nucleolus which is persisting as a spherical structure, when there is a progressive condensation of the bivalent. At this prophase stage of meiosis, the NOR is visible as chromosomal knob, instead of "secondary chromosomal constriction" This situation indicates that the formation of ribosomes has ceased and NOR has become condensed to such a degree that it is visible as a "knob" to which the inactive nucleolus is attached. So this 4th pair is found to bear the NORs. This feature is distinct at this stage alone where bivalents are also visible with their lampbrush fibers. It has been reported that unique sequences are transcribed during the lampbrush stage of oogenesis, alongwith repetitive sequences (Davidson and Hough 1971). Therefore this lampbrush stage of the spermatogenesis is supposed to be the chromosomal sites of transcription and has a bearing with the nucleoli formation.

The process of formation of the secondary constriction in prophase or metaphase chromosomes is viewed as arrest of the condensation of the region. At the critical time when the nucleolus disintegrates, there is insufficient time for completing condensation of the chromatin in the region of the nucleolus organizer. As a result, there is a region of extended or dispersed chromatin which mark the position of the nucleolus organizer. If the nucleolus disintegrates early, i.e., at the rate that is equal to or greater than the rate of chromosomal condensation, the nucleolus organizer region will not appear as a secondary constriction.

Visibility of any non centric constrictions either nucleolar or non nucleolar in mitotic and meiotic stages of this species and that too heterozygous in nature depended upon the dynamics of the chromosomal condensation. Thus there may be large, small or absence of secondary constrictions even in the regions where they are usually seen. In the present observation, such variability has been revealed, for example in fig. 5, arrow indicate an unstained gap in one of the chromatids of the 1st, 3rd and 5th longest chromosomes. In fig. 7, the 5th and 7th longest elements have revealed a gap in the distal half region of one of their chromatids. The sex chromosome has also depicted this feature. In fig. 6, a metaphase II stage, a minute satellite is visible at the tip of one of the chromatids of the sex-chromosome, which is separated by a minute sharp gap, and in fig.15, at diplotene arrow indicate gap in the distal region of this chromosome leaving the satellites of both the chromatids which is prominently displayed. The sex-chromosome revealing the satellite is not found associated with nucleolus in the cells. Centromeric constriction is also noted in the largest element in fig. 6 (arrow). The elongation of this so called centromeric constriction at this metaphase II stage is considerably large. On lack of evidence i.e., they are also NORs, the gaps on the metaphase II chromosomes and centromeric constrictions may be taken as additional information of the karyotype. The number of nucleolar organizers is constant per diploid genome. Thus it seems that the NOR of the 4th largest element is not active at the metaphase II stage. Or, it has been demonstrated that with several NORs in cells, one can functionally dominate (out-compete) another.

The scoring of the gap was considered to be "highly subjective" by some workers and therefore unsuitable for proper analytical work (Schinzel and Schmid, 1976). In the present investigation the methanol / acetic acid fixed and air dried preparations stained in Giemsa have provided quite clear view of NOR, gaps, constrictions and satellites and all have been distinguished unambiguously.

Differential Spiralization

It is found that process of condensation is always not uniform or synchronous in all the elements at a given stage of the mitotic and meiotic cycle. This has been located in two cells at metaphase II stage (Figs. 11 and 12) where two chromatids are under condensed and are lightly stained (arrow). Similar phenomenon is visible in a bivalent of one cell at diakinesis (Fig. 31. bivalent no. 1) where it is out of coiling cycle, but one end of the bivalent is having comparable staining intensity with the other normal region in the cell. In the cell, all the elements are visible as euchromatic even though this heterogeneity in the condensation has been depicted. Dyre (1963) has recorded the basic difference between two components of a chromosome namely euchromatin and heterochromatin. Euchromatin is recognised as the whole or part of every chromosome complement which follows the almost universal cycle of contraction in which spiralisation is at a minimum during interphase and at a maximum at metaphase. Heterochromatin may be defined as any chromosome or part of a chromosome which, during the meiotic or mitotic cycle at any stage in the life history of an organism, shows a cycle of condensation which is different from that of euchromatin. It follows that at any given stage a segment of heterochromatin may or may not be heteropycnotic. Those segments recognised by the different timing and amplitude of their cyclical staining reaction have been termed "allocyclic" by Darlington and La Cour (1940).

Here one chromatid and a major part of the bivalent is lagging behind in the coiling cycle but can not be termed 'allocyclic' because this has been found in one or two cells alone. We know that each germ cell is part of an isogenetic clone of synchronously developing germ cells and a consortium of germinal and somatic (sertoli) elements. This of course depicts that chromatids of a chromosome and bivalents are independently coiled

Dyer (1963) found that on low temperature treatment, metaphase chromosomes of many species of plants revealed allocyclic segments of under spiralised heterochromatin. This undergoing a different cycle of condensation or coiling than the normal regions and revealed structural heterozygosity in pair of chromosomes. Allocycly is therefore sensitive to cold treatment. In *Xenocatantops humilis*, supernumerary segments have shown allocyclic feature of heterochromatin (without any treatment) in certain cells (pl. refer fig. 33).

Unequal Bivalents and Supernumerary Chromosomes

A pair of homologues may be heteromorphic for a number of reasons, but we may distinguish two primary categories. The first is that of pericentric inversion heterozygosity, where two chromosomes are equal and homologous but have different morphology. The second is where one member has more material than the other and addition or deletion making the homologues unequal (Hewitt 1979). In *X. humilis* the second type of heteromorphic homologues has been readily identified in their meiotic configurations where particular chromosome carries a segment of sufficient length which is easily visible under a microscope.

The 5th and 6th have been identified as unequal bivalents. The extra region in the larger homologue has been displayed in the early meiotic stages as heterochromatic segment (for example Fig. 33 nos. 9, 19, 20, 29, 36, 45, 49, 51, 54 and 55). Variation of supernumerary segments in natural populations have resulted in polymorphic states where a particular homologue occurs in three forms, depending on whether extra heterochromatic segments are found on neither (basic homozygote), both (structural homozygote) or as in the present case on only one of them (structural heterozygote).

In order to compare meiotic bivalents, all have been precisely arranged and numbered serially (Fig. 33). The additional chromatin of each one is facing downwards for uniformity in presentation apart from those where chiasma is formed within the additional segment or just beyond that. All the 58 bivalents are monochiasmate (having single chiasma). The bivalents can be grouped in to two categories according to their configuration.

1. Cross configurations with equal and unequal arms.

·2. Rod configurations with chiasma proximal, distal and even terminal in nature.

A tentative grouping of bivalents into seven types on the basis of similarity of their configurations is presented below :

1. In the first type, in all cross configurations chiasma is located between centromere and distally sited extra segment leading to their equational segregation at first meiotic division. In some stages the extra segment appears heterochromatic. Bivalents numbered 14, 16, 18, 20, 27, 28, 32, 33, 34, 37, 38, and 56 fall under this group.

2. In the second type, all have unequal cross configurations. The extra segment is distally sited and chiasma is interstitial closer to the centromeric end leading to their equational segregation. The bivalents numbered 1, 3, 5, 7, 9, 12, 13, 15, 19, 21, 25, 26, 30, 39, 40, 42, 49, and 51 are placed under this type.

3. In the third type, equational segregation of the segment is evident where chiasma is located close to the segment. Bivalents numbered 4, 6, 10, 11, 17, 22, 43, 44, 45, 47 and 53 are placed under this type.

4. The fourth type consisting of bivalents 2, 8, 24, 31, 41 and 48 is the type where all bivalents have more or less rod configurations. The extra element in all appears to be distal on the larger element. A chiasma is close to the centromeric end leading to their equational segregation.

5. In the fifth type each configuration leads to reductional segregation of their supernumerary segment. In these elements, extra segment is located quite distal and chiasma appears to be quite terminal in them. Bivalents numbered 23, 35, 36 and 55 are of this type.

6. The sixth type include bivalents 50 and 54. In these, segments are distinct as heteropycnotic knobs on the chromatids. The chiasma is quite close to the centromere leading to their equational segregation.

7. This type has been recognized at an early diplotene of individual number 370 (Bivalent No. 29). In this configuration it is interesting to observe that heterochromatic segment, appears to have extended beyond the paired euchromatic region and folded backwards, with its tip pairing with the distal end of the short homologue. This does indicate that a very short euchromatic segment extends beyond heterochromatic block which is in chiasmate association with the euchromatic end of the short homologue. Since the segment is positively

heterochromatic from the onset of meiosis and remains so until diakinesis, it can not make a chiasmate association with the equivalent region of its euchromatic homologue. An alternative explanation for this configuration is nose to nose synapses, is not that convincing. In another bivalent (No. 36), heterochromatic end of the larger element is found associated with euchromatic end of its homologue. However in either case the segregation is reductional at first anaphase.

Nur (1961) reported an equivalent case involving short bivalents heterozygous for a supernumerary segment in *Calliptamus palaestinensis.* He has suggested that the association was chiasmate with their presumed euchromatic ends. John (1973) agreed to Nur's interpretation of interstitial location of heterochromatic segment. However, he has pointed out that no direct evidence was offered that these segments were interstitial. The conclusion was an inference based on the occurrence of both reductional and equational segregation of the inequality. In his material *Chorthippus jucundus* (an equivalent case) he ruled out this explanation and quoted example of several types of supernumerary segments existing in *Cryptobothrus chrysophorus.* One of these present in the smallest S_{11} pair is clearly interstitial, exhibiting a substantial terminal segment of euchromatin. No chiasma has ever been found in this segment and on which he concludes that "if chiasmata do not form in a case like this where a very substantial euchromatic segment is present, it is difficult to believe that a chiasma maintains the association in *Calliptamus"* An alternative explanation put forward by him is that, the fold back pairing observed by Nur (1961) is nothing but a massive tandem duplication of the terminal portion of the short arm, so that the end of the segment retains a homology with the end of the normal homologue. The same kind of fold back pairing is also noticed in the pachytene of 370 in the present study. The segment is positively heterochromatic, hence it can not make a chiasmate association with the equivalent region of its euchromatic homologue. On the associated normal homologue and that of its enlarged partner in meiotic prophase of *Calliptamus palaestinensis* and *Chothippus jucundus,* Hewitt (1979) commented that "This would be expected if the extra segment is a duplication of the terminal region of the basic chromosome but this could also be due to a 'non-homologous' association as is often observed with heterochromatic regions" Hewitt further stated that "many of the figures of unequal bivalents also show an internal segmentation of the extra material, which clearly suggests duplication as the originating process" Hewitt quoted some species namely, *Phrynotettix magnus* (Wenrich 1916), *Stethophyma gracile* (Carothers 1931; Shaw 1970), *S. lineatum* (Shaw 1970), *Schistocerca gregaria* (Shaw 1971) and *Chortoicetes terminifera* (Hewitt and John 1971)) where similar segments are noticed. A region in chromosome 9 of *Stethophyma lineatum,* appears as a large heterochromatic block at diplotene, but has been shown to be composed of several condensed regions separated by short euchromatic regions at pachytene (Fontana and Vickery 1974). Internal segmentation however has not been clearly revealed in the preparations of *Xenocatantops humilis* but their presence is quite obvious.

In *Chorthippus parallelus* supernumerary segments are known on three smallest chromosomes M6, M7 and S8 (Hewitt and John 1968; Westerman 1970). The segment system of *parallelus* would require three independent translocations in this one species. Thus the segment system of *X. humilis* like that of *C. parallelus,* and of *C. jucundus* (John 1973) are most easily accommodated on the assumption that the segments in question have arisen by the duplication of existing material rather than by translocations. Interstitial segments presumably also have an equivalent mode of origin since mechanical improbability of the 3 break insertions needed to produce interstitial and proximal extra segments. While heterochromatic material is normally not found at ends or interstitial regions, the duplicated material must have assumed a heterochromatic state at or after its production inactivating and nullifying the detrimental effects of superfluous unpaired material in meiosis. The heterochromatinization of aneuploid elements is a common event in grasshoppers (Hewitt and John 1968) and segmental duplications would be expected to behave in an equivalent manner. Thus the origin of these extra heterochromatic segments is by repeated duplication of existing material, the DNA of which may therefore be largely repetitive.

The supernumerary chromosomes are much smaller than the segments, consequently simple translocation can not be responsible for the formation of the segments in this species. Chances of translocation further appear to be remote since the varied configurations of these unequal bivalents indicate some interstitial location of supernumerary segments. This coupled with the fact that B-chromosomes show no tendency to pair, when present simultaneously. Thus, supernumeraries and supernumerary segments heteropycnotic during meiotic prophase had evolved independently in the *X. humilis.* Bs not represented in all the meiocytes of a cyst indicated their non disjunction in early spermatogonial divisions. This view is supported further by Hewitt's (1979) statement that "The incorporation of all or a part of a B- chromosome seem most unlikely in several species with extra segments where no B- chromosome systems have been recorded e. g., *Chorthippus, Kosciuscola* and *Stethophyma.* Conversely *Myrmeleotettix* and *Locusta* have B-chromosome polymorphism but there are no examples of extra segment in either"

The segments have been identified as heterochromatic in early prophase of meiosis. So, it has to be distinguished whether it is constitutive heterochromatin or facultative heterochromatinization has led to their present state. Following the convention first proposed by Brown (1966), the constitutive heterochromatin retains its heterochromatic state in all cell types and at all stages in a particular species and so forms a permanent structural feature of a given chromosome pair. The latter varies in its state in different cell types at different developmental stages or even from one homologue to another. The classification however fails to accommodate the frequent occurrence of supernumerary heterochromatic material which may be present in some members of a population but absent in others.

In this species structural heterozygote (unequals) and basic homozygote have been found. Whereas, structural homozygote (where extra heterochromatic segments are to be found on both members of homologues) have not been recognized (may be present in the population and that might also not be lethal). In this polymorphism we have already regarded the larger chromosome as carrying duplication. Another possibility may also exist where the short ones may have a deletion which is true in an evolutionary sense usually can not be determined. The most we could do here is to indicate that the extra segment is interstitial or terminal. Hewitt (1979) tabulated 88 cases in the Acridoidea in which only 3 involve large members of the complement, the rest involve the smaller chromosomes of which three can be unequal in the same species occasionally. The case of *Xenocatantops humilis* is also comparable with an endemic species in Australia *viz*., *Cryptobothrus crysophorus* (John and King 1977 a & b) where 4th, 5th 6th, 8th and 9th (medium sized) and 10th and 11th smallest bivalents have been found with supernumerary segments. Based on the distinct heterochromatin differences they have established two chromosome races the 'Northern' and 'Southern' in the species. While dealing with the range and patterns of 'C'banding they have also found that structurally distinct forms of constitutive heterochromatin have been masqueraded under the apparently uniform guise of heteropycnosis. Further, the range and patterns of C- banding demonstrated by the S_{10} and S_{11} autosomes in the Northern populations indicate that the build up of supernumerary heterochromatin may well have involved a step-wise process of accretion. Moreover, it has certainly involved the simultaneous addition of C-band positive and C-band negative material both of which may appear as heteropycnotic. In the species *Xenocatantops humilis* a limited number of individuals and populations have been represented, even provide an irregularity in the uniform appearance of the so called heteropycnotic nature of the added segments (Fig. 33) and that might also be correlated with the step-wise process of accretion. This evidence provides yet another example of heterochromatin heterogeneity. Direction of the evolutionary change in this species caused through polymorphism can be traced only when individuals representing through out its distributional range are thoroughly C-banded during mitotic as well as meiotic progressions.

The concept that specialized forms may have a lower DNA content than related contemporary forms, and specialization inevitably involves a loss of parts or functions (Hinegardner 1976), has been compounded by the fact that there is no single relationship between morphological change and either chromosome change or DNA changes. Jones and Rees (1982) on supernumerary heterochromatic segments had stated that like genes, they exhibit polymorphism but their consequences can not be identified simply by examining the exophenotype. This is evident in the present case.

The findings can be summarized as (i) the segments are heteropycnotic in nature in early stages of meiosis and (ii) it effects chiasma localization where it has gone towards more proximal region or it has led to a chiasma within the short arm of the euchromatic segment because, bivalents without segment had formed exclusively interstitial localization (Fig. 30). John (1981) after analyzing the effects of heterochromatic blocks on chiasma localization in *Cryptobothus chrysophorus* and *Atractomorpha similis* concluded that the presence of heterochromatic blocks is associated with a pronounced modification in chiasma distribution, thus providing an alternation in the progeny genotype. Such genotypes released in nature may have superior adaptability to different niches within the general environment occupied by the species. This is considered as an area effect speciation.

Influence of supernumerary heterochromatin on recombination is useful for the conservation of advantageous combination of traits and hence beneficial to a population. It also illustrates how heterochromatin as an originally neutral or parasitic element has outwitted selection by adapting a function.

To exist in nature, species has to continuously adapt to the environmental changes. The occurrence of supernumerary segments (in polymorphic state) is a mechanism of genotype evolution in *X. humilis*. The condition of continuous environmental changes and continuous evolution is known as the "Red queen effect", after the character in Lewis Carroll's story *Through the looking glass*, who said, "You always have to run faster to stay in place'.. (refer Bernstein, R. and Bernstein, S. 1988, page 149).

SUMMARY

Chromosomal study of *Xenocatantops humilis* (Serville) (Orthoptera : Acrididae : Catantopinae) revealed 23 acrocentric chromosomes in males and 24 in females. Sex-chromosome was found to be the 6th largest element in the complement. The chromosomes had a gradual gradation in their size. Meiotic behaviour of the two types of supernumerary chromosomes and two pairs of unequal bivalents were studied. The bivalents had shown more or less random distribution of their chiasmata. Behaviour of gaps, constrictions, NORs, sub-chromatid like visible structure, megameric chromosomes, and differential spiralization of the supernumerary segments were also studied.

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REFERENCES

Bajer, A. 1965. Sub-chromatid structure of chromosomes in the living state. *Chromosoma,* **17** : 291-302.

Becak, M. L. 1974. Chromosome structure; half-chromatids in chloroform treated metaphases. *Experientia,* **30** : 1138-1140.

Bernstein, R. and Bernstein, S. (ed.) 1988. Schaum's solved problems series-Biology. McGraw-Hill Book Company. Printed in Singapur : 1-406.

Brown, S. W. 1966. Heterochromatin. *Science,* **151** : 417-425.

Busch, H. and Smetana, K.(ed.) 1970. The Nucleolus. Chapter III Nucleolar Organizers, Nucleolar Size, Shape, and Number. *Academic Press,* New York : 116-159.

Carothers, E. E. 1931. The maturation divisions and segregation of heteromorphic homologous chromosomes in Acrididae (Orthoptera). *Biol. Bull. Woods Hole,* **41** : 324-349.

Darlington, C. D. and La Cour, L. F. 1940. Nucleic acid starvation of chromosomes in *Trillium. J. Genet.,* **40** : 185-213.

Davidson, E. H. and Hough, B. R. 1971. Genetic information in oocyte RNA. *J. molec. Biol.,* **56** : 491-506.

Dirsh, V. M. 1975. Classification of the Acridomorphoid insects. E. W. Classey Ltd. Faringdon, London : 1-170.

Dyer, A. F. 1963. Allocyclic segments of chromosomes and the structural heterozygosity that they reveal. *Chromosoma,* **13** : 545-576.

Egozcue, J. 1973. Banding patterns of uncoiled mammalian chromosomes. *Nucleus,* Calcutta, **16** : 53-56.

Fontana, P. G. and Vickery, V R. 1974. Heterochromatin content and chiasma distribution in the megameric chromosomes of *Stethophyma gracile* and *S. lineatum* (Orthoptera : Acrididae). *Chromosoma,* **46** : 375-395.

Gimenez-Martin, G. 1968. Structural levels in chromosomes under the light microscope. *Nucleus,* Calcutta (Seminar on chromosome, 1968) : 52-63

Hewitt, G. M. 1973. Evolution and maintenance of B-chromosomes. *Chromosomes Today,* **4** : 351-369.

Hewitt, G. M. 1979. Animal Cytogenetics, Vol. 3, Insecta 1. Orthoptera. Gebrüder Borntraeger, Berlin Stuttgart : 1-170.

Hewitt, G. M. and John, B. 1968. Parallel polymorphism for supernumerary segments in *Chorthippus parallelus* (Zetterstedt) I. British populations. *Chromosoma,* **25** : 319-342.

Hewitt, G. M. and John, B. 1971. The cytogenetic systems of grasshoppers and locusts I. *Chortoicetes terminifera. Chromosoma,* **34** : 302-323.

Hinegardner, R. 1976. Evolution of genome size. IN : Molecular Evolution. *Sinaur Assoc. Inc.* Massachusetts : 179-199.

John, B. 1973. The cytogenetic systems of grasshoppers and locusts II. The origin and evolution of supernumerary segments. *Chromosoma,* **44** : 123-146.

John, B. 1981. Heterochromatin variation in natural populations. *Chromosomes Today,* **7** : 128-137.

John, B. and King, M. 1977a. Heterochromatin variation in *Cryptobothrus chrysophorus* I. Chromosome differentiation in natural populations. *Chromosoma,* **64** : 219-239.

John, B. and King, M. 1977b. Heterochromatin variation in *Cryptobothrus chrysophorus* II. Patterns of C-banding. *Chromosoma,* **65** : 59-79.

Jones, R. N. and Rees, H. 1982. B Chromosomes. *Academic Press,* London, New York, Paris : 1-266.

King, R. C. and Stansfield, W. D. 1990. A dictionary of genetics (4th edition). *Oxford University Press* : 1-406.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas,* Lund, **52** : 201-220.

Mickey, G. H. 1946. The presence of multiple strands in chromosomes of *Romalea* (Orthoptera). *American Naturalist,* **80** : 446-452.

Moorhead, P. S., Nichols, W. W., Perkins, F. T. and Hayflick, L. 1974. Chatham workshop conference on karyological monitoring of normal cell populations. *J. boil. Standard,* **2** : 95-101.

Nur, U. 1961. Meiotic behaviour of an unequal bivalent in the grasshopper *Calliptamus palaestinensis* Bdhr. *Chromosoma,* **12** : 272-279.

Rieger, R., Michaelis, A. and Green, M. M. 1976. Glossary of genetics and cytogenetics: classical and molecular. *Springer-Verlag,* Berlin Heidelberg, New York : 1-647.

Satya Prakash, K. L., Hsu, T. C. and Pathak, S. 1981. Chromatid lesions and chromatid core morphology. *Cytogenet. Cell Genet.,* **30** : 248-252.

Schinzel, A. and Schmid, W. 1976. Lymphocyte chromosome studies in humans exposed to chemical mutagens: the validity of the method in 67 patients under cytostatic therapy. *Mut. Res.,* **40** : 139-166.

Schulz-Schaeffer, J. 1980. Cytogenetics. Plants, Animals, Humans. *Springer-Verlag,* New York, Hiedelberg, Berlin : 1-496.

Shaw, D. D. 1970. Supernumerary segment system of *Stethophyma* I. Structural basis. *Chromosoma,* **30** : 326-343.

Shaw, D. D. 1971. The supernumerary segment system of *Stethophyma* II. Heterochromatin polymorphism and chiasma variation. *Chromosoma,* **34** : 19-39.

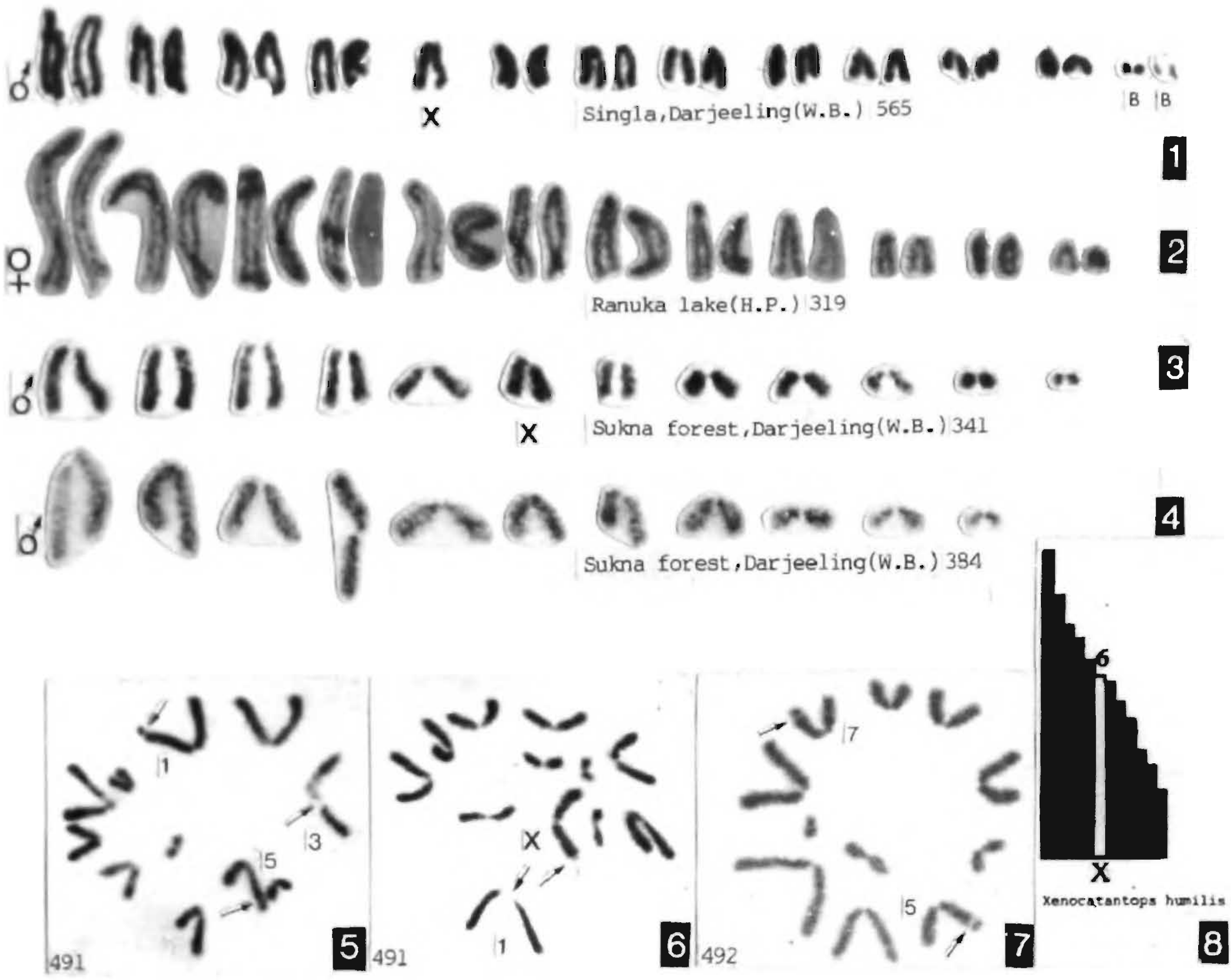
Vosa, C. G. 1968. A method to reveal sub-chromatids in somatic chromosomes. *Caryologia,* **21** : 381-383.

Wenrich, D. H. 1916. The spermatogenesis of *Phrynotettix magnus* with special reference to synapsis and individuality of chromosomes. *Bull. Mus. Com. Zool.* Harvard, **60** : 57-135.

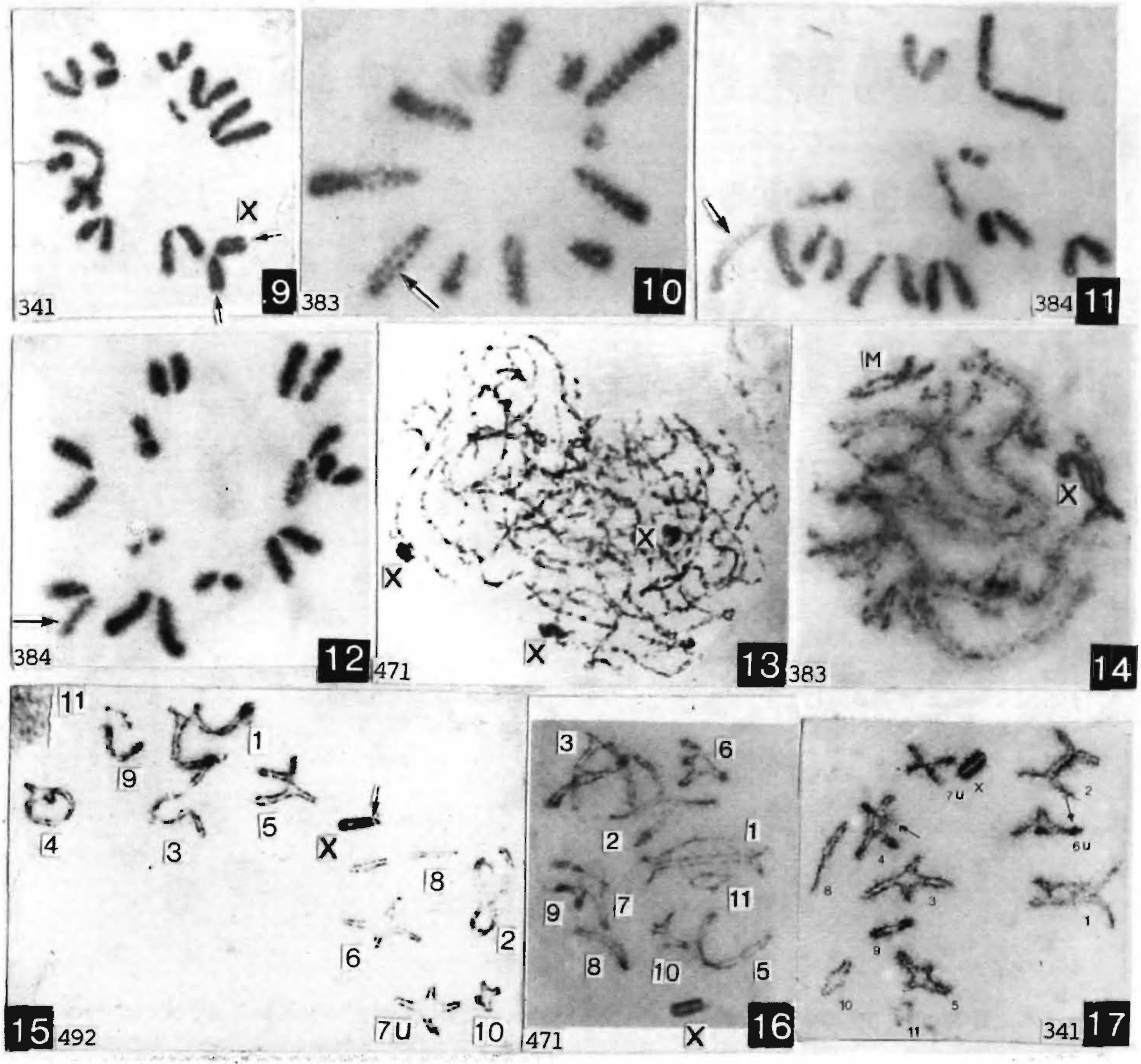
Westerman, M. 1970. Parallel polymorphism for supernumerary segments in *Chorthippus parallelus* V. A new polymorphism in Europe. *Heredity,* **25** : 662-667.

White, M. J. D. 1973. Animal Cytology and Evolution. Cambridge University Press, London : 1-961.

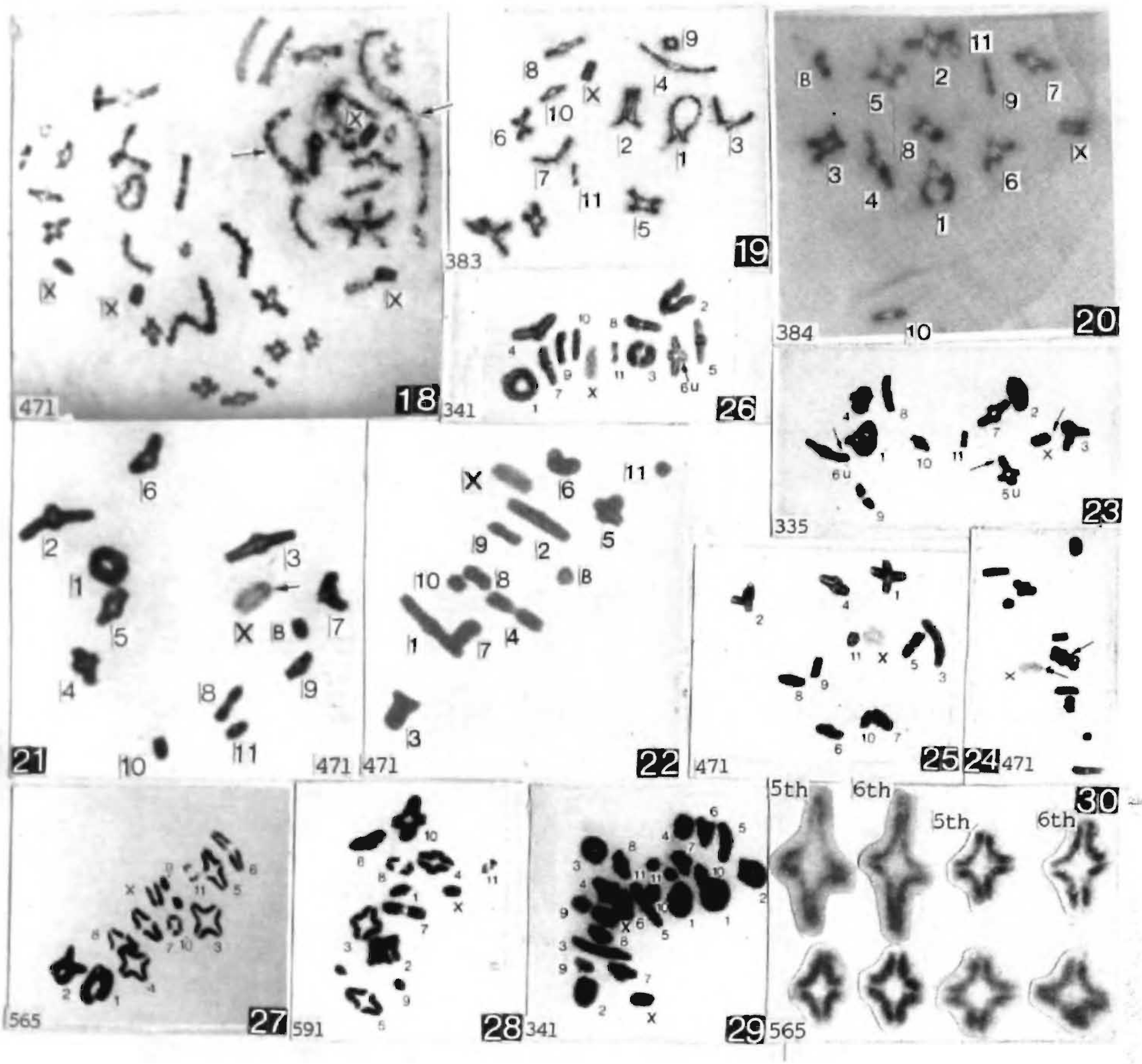
Wolf, S. 1969. Strandedness of chromosomes. *Int. Rev. Cytol.,* **25** : 279-296.



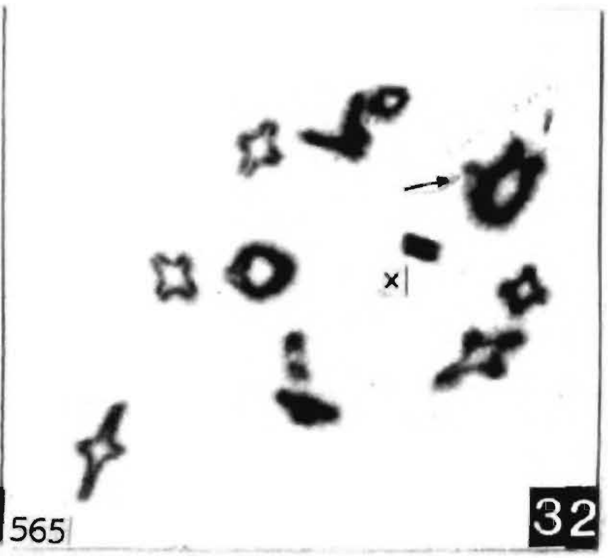
Figs. 1-8. **1.** Spermatogonial metaphase karyotype with a pair of minute supernumerary chromosomes (mini Bs); **2.** Karyotype from hepatic caeca of a female individual; **3 & 4.** Metaphase II karyotypes. The X is distinct among the autosomes; **5, 6 & 7.** Chromosomes in metaphase II cells. In figs. 5 and 7 arrow indicate gaps/unstained regions on the single chromatids. In fig.6, arrow on the X points to a satellite region at the terminal end of one chromatid and centromeric constriction on the largest chromosome; **8.** Idiogram; the sex chromosome is the 6th largest in the species.

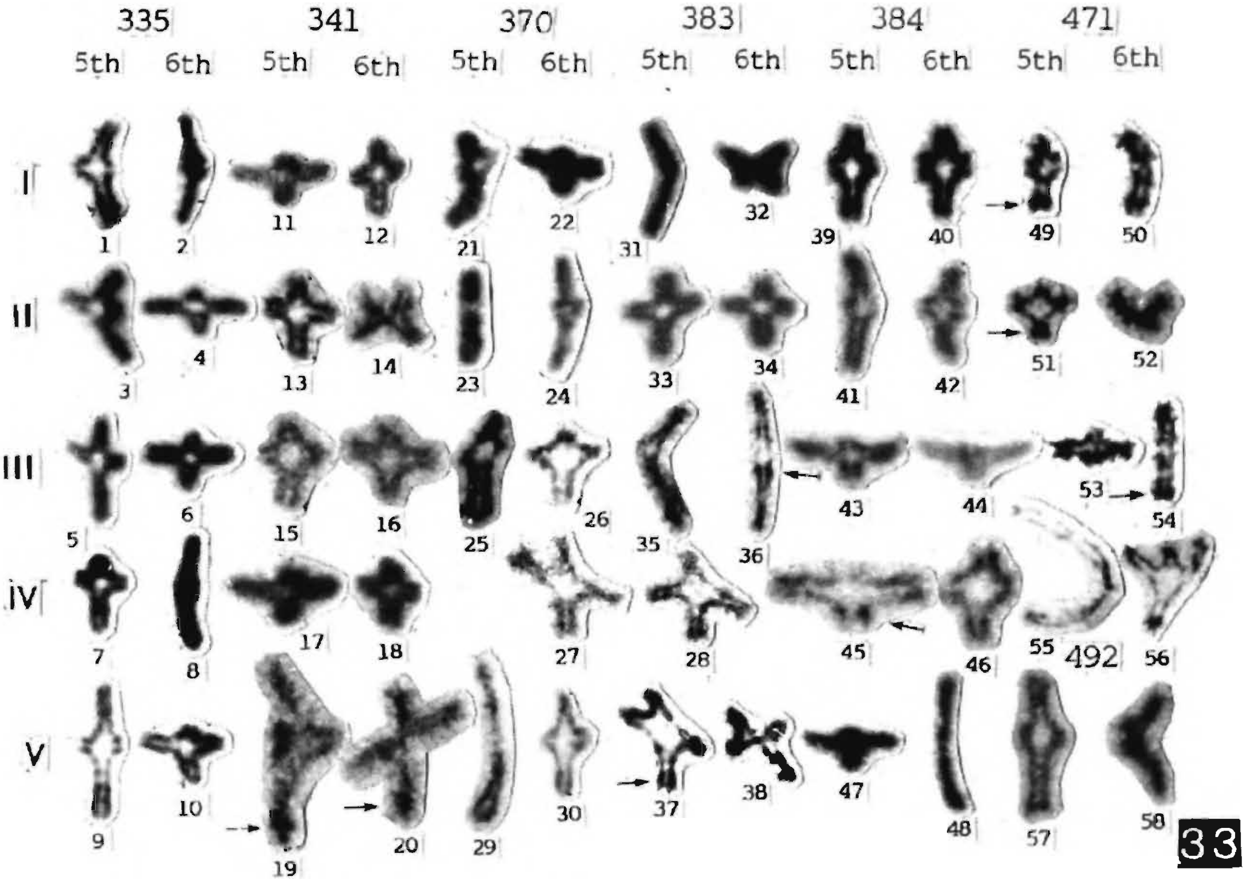


Figs. 9-17. **9 & 10.** Chromosomes at metaphase II and anaphase II stages. Sub-chromatids of the X and of an autosome are visible (arrows); **11 & 12.** Metaphase II chromosomes. Arrows point at undercondensed single chromatids of two different chromosomes; **13.** Tetraploid cell at zygotene stage showing homologue pairing. Both the Xs are equally condensed and are lying apart. In the adjoining cell at the same stage, the X is having similar appearance lying at the periphery; **14.** A pachytene stage. Megameric bivalent (M) is precociously condensed with diplotene configuration. The positively heteropycnotic distal half is prominently depicted. The course of chromatids of the X can be followed; **15, 16 & 17.** Early diakinetic stage, where chiasma has assumed definitive "cross" shape. The 9$^{\bullet}$ megameric bivalent is visible with prominent distal heteropycnotic segment. In fig. 15, a gap is located close to the terminal-end of the X (arrow). In fig. 17, arrow on the 4$^{\bullet}$ bivalent indicates associated nucleolus. Hairy appearance of the bivalents is also evident.



Figs. 18-30. **18.** A tetraploid cell at diplotene stage showing multivalents (arrows). Both the Xs have been equally stained. In the adjoining two diploid cells no multivalent has been formed; **19 & 20.** Cells at diakinesis. In fig.19, the smallest element (11*) is unequal along with the 5* and 6*. In fig. 20, one supernumerary bivalent (B) of the magnitude of 10* regular element is distinct; **21-26.** Metaphase I of different individuals in polar (Figs. 21 and 25) and side views (Figs. 22, 23, 24 and 26). At this stage characteristic feature of the allocyclic X is worth noting. In Fig. 23, the X is equally stained (isopycnotic)as compared to the bivalents. With this state of condensation, negative heteropycnosis of this element (which is cellular in origin) begins which can be arranged in a gradual order as depicted in the figures 22→21→26→24→25. In figs. 24 and 25, positively heteropycnotic centromeric region as a minute dot (arrow) remains visible indicating certain feature of the constitutive heterochromatin; **27 & 28.** With the onset of anaphase I, the X has become isopycnotic as compared to bivalents. Observe quite early separation of the 9* megameric element in both the cells in side view; **29.** A tetraploid cell at metaphase I. Both the Xs are isopycnotic. No multivalent has been formed; **30.** The 5* and 6* normal (homomorphic) bivalents of an individual from Singla, Darjeeling. In other individuals both the bivalents were unequal.





Figs. 31-33. **31.** A diakinetic cell of an individual from Sukna forest Darjeeling. The largest bivalent (No. 1) is revealing allocycly (showing differences in the degree of coiling). The bivalent at this allocyclic state is negatively heteropycnotic. This is a rare phenomenon involving an autosomal bivalent of the complement. Its one end is however positively heteropycnotic equivalent to other bivalents. This is unique since allocycly is recorded with sex-chromosomes, B-chromosomes and sex-limited chromosomes of certain Diptera; **32.** A diakinetic cell of an individual from Singla in district Darjeeling. Arrow indicates a supernumerary bivalent, designated as mini Bs.; **33.** Meiotic configurations of 58 bivalents, all monochiasmate at different stages belonging to 7 individuals. The additional chromatin of each one is facing downwords for uniformity in presentation apart from those where chiasma is formed within the additional segment or just beyond it. Each bivalent has been numbered so that similar configurations can be grouped. Arrows indicate the allocyclic additional segment (positively heteropycnotic).

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SUPERNUMERARY CHROMOSOMES OF *PATANGA SUCCINCTA* (JOHANSSON) (ORTHOPTERA : ACRIDIDAE : CYRTACANTHACRIDINAE)

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Supernumerary chromosomes

The effects of supernumerary chromosomes upon the distribution of chiasmata during meiosis are well known (Jones and Rees 1982). This could well be of adaptive significance in generating novel and potentially superior genotypes. Hewitt and John (1967) had already argued that B-chromosomes and supernumerary segments as well, supplement in a most dramatic fashion the control of determinants located in A-chromosomes.

Thus, supernumerary chromosomes have potential in changing the progeny genotype which is vital for species evolution.

INTRODUCTION

Supernumerary or B-chromosomes are additions to the normal karyotype and not homologous, or only partly homologous, to members of the regular set. They are present in some individuals of a population but not in all. A species which show supernumeraries in certain geographic areas may lack them in other geographic areas.

The B-chromosomes have been a source of embarrassment to the geneticists ever since they were first distinguished and named (Longley, 1927). The reason for this is simple; the rules which govern the behavior of the supernumerary elements are not the rules which operate for the standard members of the complement.

These chromosomes represent one of the many sources of numerical chromosome variation. It is therefore necessary to take a special care when assigning the supernumerary status to

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a given extra chromosome since, polysomics of A- chromosomes may be identified wrongly as supernumeraries.

White (1954) had divided animal B-chromosomes in to two groups :

a) Those which are mitotically stable, where all the cells of the individual have the same number of supernumeraries and

b) Those which are mitotically unstable, where different cells of the same individual having different number of supernumeraries.

The supernumeraries of the present species belong to the second group. Their characterization as extra elements is based on their lack of pairing with the standard chromosomes and also based on their distinction from polysomics, by not participating in the formation of multivalents in the cells. After the karyological analysis of the conventionally stained complements of different individuals and after cytological observations on the prophase of meiosis, three types of supernumeraries have been recognized depicting intriguing polymorphism in the species.

MATERIALS AND METHODS

Two males and two females comprised the material for the present investigation. The insects were collected from Chail, Saproon and Masobra in Himachal Pradesh. The species is commonly known as Bombay locust and is a pest.

Individuals Studied		**Site of Collection**
Code number	**Sex**	
409	Male	Chail
425	Female	Chail
447	Male	Saproon
456	Female	Masobra

Technique used in Chromosome Preparation

For arresting cells at metaphases, selected male and female individuals were injected with colchicine at a concentration of 0.05% and with an amount of 0.03 to 0.04 ml in each case. Thereafter they were kept alive for at least 4 hrs. in insect cages. Chromosomal preparations were made from the cells of testes and hepatic caeca. The tissues were dissected out and cleaned in 0.67% sodium chloride solution. The testes were pretreated (hypotonic treatment) in 0.9% sodium citrate solution for 45 minutes and hepatic caeca for 1 hr. in the same solution, diced and fixed in freshly prepared methanol-acetic acid (3 : 1) with three changes of 15 minutes. A slightly more hypotonic treatment and fixation was preferred for the somatic cells. In the field the tissues were fixed in small tubes, brought to laboratory and stored at 4°C. For preparation of the slides, the fixed tissues were transferred to 50% acetic acid till it became soft. The materials were then teased and squashed in one or two drops of 50% acetic acid. The slides were stored in vapour of 50% acetic acid overnight in cold. Next morning the slides were brought at room temperature and immersed in a solution of 3 : 1 methanol-acetic acid for an hour. The cover slips were removed with the sharp edge of a blade, while immersed in the medium and dried at room temperature in a dust free chamber. Staining was done in 2% Giemsa [E.Merck (India) Private Ltd.] prepared in phosphate buffer (pH 6.8). After differentiating in phosphate buffer (pH 6.8), slides were rapidly air dried under a lamp, cleaned in xylene and mounted in DPX.

C-banding

C-banding was carried-out following the method by Sumner (1972) with some minor modifications. The air-dried slides were treated with 0.2 N Hydrochloric acid for 30-60 minutes at room temperature, rinsed in distilled water and dried. The slides were then dipped in a freshly prepared 5% aqueous solution of Barium hydroxide octahydrate kept at 50°C for 1-5 minutes. The duration of this treatment depended on the age of the slides, i.e., the slides which were stored after squash preparation needed longer treatment. After thorough rinsing with several changes in distilled water, the slides were incubated for an hour at 60°C in 2 X SSC (0.3 M sodium chloride and 0.03 M tri-sodium citrate at pH 7), rinsed in distilled water and then dried. The slides were then stained for 30 to 90 minutes in Giemsa stain (2.5 ml of stock solution added to 50 ml of buffer at pH 6.8), rinsed briefly in distilled water, blotted, allowed to dry thoroughly, then soaked in xylene and finally mounted in DPX.

Photomicrography

Well spread and well stained mitotic and meiotic stages were photomicrographed with a Leitz Ortholux microscope. Filters with different combinations were used to obtain the best possible contrast. For photographs black and white film *viz*., N.P. 22(120 ASA) were mostly used. The negatives were printed on sterling SW glossy 3 and glossy 4 papers of Allied Photographics India Ltd. Fine grain film and paper developers of Agfa-gevaert (A 901 and A 902) and Kodak (D 76 and Kodak 163) were used in developing negatives and for positive prints. Digital cameras, scanners and printers have been used for quick and more precise results.

Karyotyping and construction of idiograms

The diploid number (2n) was determined by the most predominant number observed in the individual. The cut out of the individual chromosomes which appeared similar in morphology and staining intensity were paired to construct karyotypes. The karyotypes were used in the morphometric measurements. Their mean values so obtained were used in calculating the relative length of the chromosomes in percent of the total haploid length. Chromosome morphology and nomenclature was based on the system of Levan *et al.* (1964).

OBSERVATIONS

Karyological Details

Diploid Number, Chromosome Morphology and Sex Chromosome Constitution

2n = 23 in males and 24 in females with XO(♂) / XX(♀) sex chromosomes. All the chromosomes were acrocentrics (Figs. 1-4). The X was the 2^{nd} largest in the complement with 12.90% relative length. The last three pairs were distinctly small in the complement (Fig. 5).

Karyotype

19 conventionally stained karyotypes from 4 individuals were analysed. 4 C-band plates were studied from 1 individual. 2 individuals were studied for meiotic progression and analysis of their 26 meiotic karyotypes was done.

Relative length (RL) in percentage

1	2	3	4	5	6	7	8	9	10	11	X
15.40	11.54	10.57	9.35	8.59	7.95	7.46	6.77	3.81	3.24	2.55	12.90

Gaps / Constrictions

A gap was present in the distal region of one homolog of the 3^{rd} pair of chromosome from hepatic caeca of a female individual collected from Masobra (Fig. 4a). Another gap was present in the proximal region of one homolog of the 2^{nd} pair of a male hopper from Chail (Fig. 2a arrow). Both the gaps were inconsistent in nature.

Idiogram

In idiogram, the X was the 2^{nd} largest member. The last 3 were distinct as small pairs. The other chromosomes could not be grouped into any size class because of the gradual gradation of size (Fig. 5).

Supernumerary Chromosomes

Supernumerary or B chromosomes were found in all the 4 individuals studied from 3 different locations of Himachal Pradesh. 1 male and 1 female grasshoppers were collected from Chail, 1 male from Saproon and 1 female from Masobra. Morphology and cytological behaviours were studied after karyotyping of spermatogonial metaphase chromosomes, meiotic bivalents and meiotic metaphase II chromosomes of the males and hepatic caeca chromosomes of the females.

Supernumeraries of two different sizes were present in hepatic caeca of a female grasshopper (No. 425 H) collected from Chail (Fig. 1b). It was found in 2 out of 6 karyotypes of the individual studied. The size of one of the supernumerary was similar to the 9th pair of the complement, whereas other was smaller. Both the Bs were placed between 9th and 10th autosomal pairs. No supernumerary was present in the karyotype No.1a of this individual.

In spermatogonal metaphase complement of a male individual (409 T) of the same locality Chail, 3 supernumeraries of two different sizes were present (Fig. 2a). They were found in 4 out of 5 spermatogonial metaphases analysed. Of these 3 elements, two were paired and placed between 9th and 10th autosomal pairs. The 3rd unpaired element was found to be more stained in this spermatogonial cell than the smaller one of the karyotype No. 1b. In another karyotype of this individual (Fig. 2b), no supernumerary element was present. Thus, supernumeraries of at least 2 different sizes were located in the two karyotypes of the two individuals (male and female) belonging to same locality.

The 3rd type of supernumerary was located in 1 out of 9 metaphase karyotypes of hepatic caeca of a female individual (456 H), collected from Masobra. This was the smallest element in the complement (Fig. 4b). A supernumerary of this size was also located in a C-band metaphase II karyotype of an individual (447 T), collected from Saproon. This was acrocentric with a minute centromeric C-band (Fig. 3). This might be the same element present in the Masobra female.

In addition to these supernumeraries of varying sizes present in different combinations, their occurrence and behaviour was also investigated in the meiotic stages of the male individual (409 T).

In 5 out of 8 metaphase II karyotypes, 13 chromosomes were present including the X. One extra element was found to be the 8th largest element in them. In addition to this supernumerary, in 2 karyotypes with 14 elements, another one was located at 11th position. In one karyotype with the X chromosome no supernumerary was present. Thus the minute supernumerary of the magnitude of the last pair was not found in these karyotypes.

Study of 24 cells at diplotene, diakinesis and metaphase I stages added further information.

One large supernumerary was distinct on its bivalent configuration in 5 cells (Figs. 6, 7, 9, 11 and 12). In these cells this was found in varying configurations and has been marked as Bs. In one cell at pachytene stage (Fig. 6) this was associated with the similarly condensed X at the periphery of the cell (arrow). The second supernumerary was distinct on its univalent nature in 4 cells and marked as B (Figs. 8, 10, 11 and 12). Staining intensity of this univalent was comparable with the regular bivalents. The third one was a minute univalent found only in figs. 8 and 10 (arrow). This also depicted comparable staining intensity with the regular bivalents in these cells. When the meiotic karyotypes of 24 cells were analysed, the supernumerary bivalent was of the size of the 8th regular bivalent and the larger univalent was bigger than the 9th bivalent. The minute one was found to be the smallest in the karyotypes. Thus the supernumeraries of the meiotic cells and of metaphase II karyotypes were similar but the smallest one of the two meiotic cells (Figs. 8 and 10) was not represented in any of the metaphase II karyotypes. This minute element (B) could be compared with the minute chromosomes of 2 karyotypes of a male (447 T) and a female (456 H) representing two different localities, Saproon and Masobra (Figs. 3 and 4b).

The supernumerary pair of the karyotype No. 2a was the same forming bivalent configuration in the meiotic cells, while its unpaired smaller one was represented in only 4 meiotic cells and in 2 metaphase II karyotypes (Figs. 8, 10, 11 and 12). Both the supernumeraries were also represented in the karyotype of the hepatic caeca of the female individual (425 H, fig. 1b) as unequal pair (BB). In fig. 13, the homologues of the supernumerary bivalent segregated to opposite poles like autosomes while the univalent moved to one pole and the movement being random relative to the X chromosome.

Chiasma Distribution

For analysis of chiasma distribution, each bivalent was arbitrarily divided into 3 equal segments as proximal (P), interstitial (I) and distal (D). The chiasmata were randomly distributed in all the bivalents except the 10th bivalent which always had distally located chiasma.

Bivalents	1	2	3	4	5	6	7	8	9	10	11
Chiasma, Location & Number	P 02	P 02		P 03	P 02	P 06	P 03	P 01			
	P,I 01	P,I 02	P,I 02	P,I 01			PD 01	PD 02			
	P,I,D, 07	P,I,D, 01					I 10	I 15	I 10	I 02	I 17
	P,D 07	P,D 05	P,D 11	P,D 02	P,D 04	P,D 03	2I 01				
	I 01	I 04	I 05	I 11	I 16	I 11	I,D 01	I,D 01			
	2I 01	2I 02					D 10	D 07	D 16	D 24	D 09
	I,D, 07	I,D, 09	I,D, 07	I,D, 04	I,D, 01	I,D, 03					
		D 01	D 01	D 05	D 03	D 03					
Total	P=17	P=10	P=13	P=6	P=6	P=9	P=4	P=3			
	I=18	I=20	I=14	I=16	I=17	I=14	I=13	I=16	I=10	I=02	I=07
	D=21	D=16	D=19	D=11	D=8	D=9	D=12	D=10	D=16	D=24	D=09
	36	46	46	33	31	32	29	29	26	26	26

A total of 380 chiasmata (68 Proximal, 157 Interstial and 155 Distal) were scored from the 286 bivalents studied. A rare event of interlocking of two bivalents was observed in one of the pachytene cells (Fig. 8 arrow).

C-band

C-band staining of spermatogonial metaphase, meiotic bivalents and metaphase II revealed centromeric band in all the chromosomes. Distal band is also very distinct on the 4th, 6th and 7th chromosomes in the metaphase II (Fig. 4). The smallest element in the plate is a supernumerary chromosome which also depicted a minute centromeric band.

DISCUSSION

In *Patanga succincta* transmission of Bs through the male side has been deduced from the study of mitotic and meiotic metaphase I and II cells. The 3 types of supernumeraries located in this species have been found mitotically unstable, because all of them have not been represented in the karyotypes and meiotic cells.

The number of B chromosomes varied in somatic tissue as a result of non disjunction during mitosis. Bs were eliminated from some cells and accumulated in others (Karyotypes 1a, 1b and 4a, 4b). This was an important finding on intra individual variation in the number of Bs in the cells of gastric caeca. This depicted that Bs even in bivalent configuration in meiotic cells are mitotically unstable. Others in univalent configuration are randomly distributed to daughter cells leading to their absence in the progeny genotype. In early spermatogonial divisions (mitotic divisions), this homologue might not divide equationally. Apart from mitotic division, accumulation of Bs in gametes due to preferential segregation at meiosis is also evident in the meiotic cells (Figs. 6-13). One supernumerary is so small in the individuals that it is persisting mostly on its functional centromeric region (Karyotypes 3 and 4b).

Chiasmate association of heterochromatic B bivalents have also been reported by Sannomiya (1962) in *Patanga japonica*; Rees and Jamieson (1954) in *Locusta migratoria;* John and Hewitt (1965a,1965b) in *Myrmeleotettix maculatus*; Westerman and Fontana (1973) in *Phaulacridium marginale*; John and Freeman (1974) *in Phaulacridium vittatum* and in *Eyprepocnemis plorans* by Henriques Gill *et al.* (1982). In these species workers have considered them as regular supernumeraries.

Intra individual variations in the number of supernumeraries in different populations of *Atractomorpha bedeli* in Japan have been investigated by Sannomiya (1973). Such variation in the number of B-chromosomes have also been reported in *Camnula pellucida* (Carroll, 1920; Nur, 1969), *Calliptamus palaestinensis* (Nur, 1963), *Locusta migratoria* (Itoh, 1934; Rees and Jamieson, 1954; Nur, 1969; Kayano, 1971), and *Patanga japonica* (Sannomiya,1962). In these species due to mitotic non disjunction of Bs, the mean number of Bs per cell is higher than the original number of B's in the fertilized egg from which the individual developed.

Absence or variation in the number of Bs in the somatic and meiotic cells of this species is explainable on the "preferential non–disjunction" of B's in mitoses in germ line, and in somatic tissues both occurring independently at an early stage of development. Preferential non–disjunction is a hypothesis presented by Nur (1963) to explain the variation in number of B's resulting into accumulation of B's. This species has tendency of getting eliminated Bs from somatic tissues but maintains them in the germ track.

While discussing supernumeraries, we have to trace their origin also. Amos and Dover (1981) had hypothesized that supernumeraries may arise as trisomic fragments from certain chromosomes of the standard complement (A chromosomes) and then undergo differentiation and heterochromatinisation to maintain the disomic gene dosage. In *P. succincta* we have not found multivalent configurations, indicating that they (trisomic fragments) have become modified in structure to such an extent that prevented their homologous association with A chromosome ancestors. In the present species with a variety of supernumerary, there is a probability that each one may have distinct origins from different members of the complement, and or one type may be a secondary deletion derivative of another.

Apart from behaviour and origin, the study of the effects of B-chromosomes has been a subject matter of a number of studies (refer Jones and Rees 1982). Here we are concerned with their effect on chiasma frequency because by posing this effect they are going to alter the recombination potential of the species.

An absence of effect has been reported by Stephens and Bregman (1972) and John and Freeman (1975). But John and Hewitt (1965), Hewitt (1976), Fletcher and Hewitt (1980) and Camacho *et al.* (1980) observed an increase in the mean chiasma frequency. This reflects that there is certain interaction between genotype and B-chromosomes, leading to contradictory results. Varied effect of B-chromosomes on chiasma frequency is therefore explainable in terms of variation in the genetic background among populations. Regarding role of particular B-chromosomes on recombination, Ward (1973) in maize, reported that heterochromatic regions of B-chromosome seem to be responsible for most of its effect on recombination. Thus genotype dependent effect of B-chromosomes on chiasma frequency suggests that this phenomenon can be of a widespread occurrence. Jones and Rees (1982) in an overall view stated that "at meiosis in most species they affect the distribution and number of chiasmata." By this, they changes the pattern of recombination affecting the release of heritable variation to gametes and progeny.

The 3 types of supernumerary chromosomes of *Patanga succincta* as they are distributed in different numbers in the meiotic cells (Figs. 6-12) are interfering in the distribution and frequency of chiasmata, as supernumerary segments in *Xenocatantops humilis*, an Acridid grasshopper (refer Part-2). The genetic significance of the effect over recombination by these Bs is quite obvious since one in bivalent configuration is also found heteropycnotic in prophase of meiosis. Thus supernumerary element has potential in changing the progeny genotype of the species which is vital for species evolution.

SUMMARY

Three types of supernumerary chromosomes have been identified in 4 individuals of *Patanga succincta* (an Acridid grasshopper), collected from three different localities in Himachal Pradesh. All have been found mitotically unstable. Absence, or variation in number of Bs in the somatic and meiotic cells have been explained on the "preferential non-disjunction" of B's in mitosis in germ line and in somatic tissues, both occurring independently at an early stage of development. Their presence in meiotic cells as univalents and also forming bivalent configurations have been observed. This heteropycnotic supernumerary bivalent has been found to be affecting the chiasma frequency in those cells. The sex chromosome was the 2nd largest member in the complement. The last 3 pairs were distinct as the short elements.

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REFERENCES

Amos, A. and Dover, G. 1981. The distribution of repetitive DNA's between regular and supernumerary chromosomes in species of *Glossina* (Tsetse) : a two-step process in the origin of supernumeraries. *Chromosoma*, **81** : 673-690.

Camacho, J. P. M., Carballo, A. R. and Cabrero, J. 1980. The B-chromosome system of the grasshopper *Eyprepocnemis plorans* subsp. *plorans* (Chapopentier). *Chromosoma*, **80** : 163-176.

Carroll, M. 1920. An extra dyad and an extra tetrad in the spermatogenesis of *Camnula pellucida* (Orthoptera): Numerical variation in the chromosome complex within the individual. *J. Morph.*, **34** : 375-455.

Fletcher, H. L. and Hewitt, G. M. 1980. Effect of a 'B'chromosome on chiasma localization and frequency in male *Euchystira brachyptera*. *Heredity*, **44** : 341-347.

Henriques-Gil, N., Santos, J. L. and Giráldez, R. 1982. B-chromosome polymorphism and interchromosomal chiasma interference in *Eyprepocnemis plorans* (Acrididae, Orthoptera). *Chromosoma*, **85** : 349-359.

Hewitt, G. M. 1976. Meiotic drive for B-chromosomes in the primary oocytes of *Myrmeleotettix maculatus* (Orthoptera : Acrididae). *Chromosoma*, **56** : 381-391.

Hewitt, G. M. and John, B. 1967. The B chromosome system of *Myrmeleotettix maculatus* (Thunb.) III. The statistics. *Chromosoma*, **21** : 140-162.

Itoh, H. 1934. Chromosomal variation in the spermatogenesis of grasshopper, *Locusta danica* Linnaeus *Jap. J. Genet.*, **10** : 115-134.

John, B. and Freeman, M. 1974. B-chromosome behaviour in *Phaulacridium vittatum*. *Chromosoma*, **46** : 181-195.

John, B. and Freeman, M. 1975. The cytogenetic structure of Tasmanian populations of *Phaulacridium vittatum*. *Chromosoma*, **53** : 283-293.

John, B. and Hewitt, G. M. 1965a. The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.) I. The mechanics. *Chromosoma*, **16** : 548-578.

John, B. and Hewitt, G. M. 1965b. The B-chromosome system of *Myrmeleotettix maculatus* (Thunb.) II. The statics. *Chromosoma*, **17** : 121-138.

Jones, R. N. and Rees, H. 1982. B Chromosomes. *Academic Press,* London, New York, Paris : 1-266.

Kayano, H. 1971. Accumulation of B-chromosomes in the germ line of *Locusta migratoria*. *Heredity*, **27** : 119-123.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas,* Lund, **52** : 201-220.

Longley, A. E. 1927. Supernumerary chromosomes in Zea mays. *J. Agric. Res.* **35** : 769-784.

Nur, U. 1963. A mitotically unstable supernumerary chromosome with an accumulation mechanism in a grasshopper. *Chromosoma*, **14** : 407-422.

Nur, U. 1969. Mitotic instability leading to an accumulation of B chromosomes in grasshoppers. *Chromosoma,* **27** : 1-19.

Rees, H. and Jamieson, A. 1954. A supernumerary chromosome in *Locusta. Nature (Lond.)* **173** : 43-44.

Sannomiya, M. 1962. Intra-individual variation in number of A-and B-chromosomes in *Patanga japonica. Chromosome Information Service*, **3** : 30-32.

Sannomiya, M. 1973. Cytogenetic studies on natural populations of grasshoppers with special reference to B-chromosomes II. *Atractomorpha bedeli. Chromosoma*, **44** : 99-106.

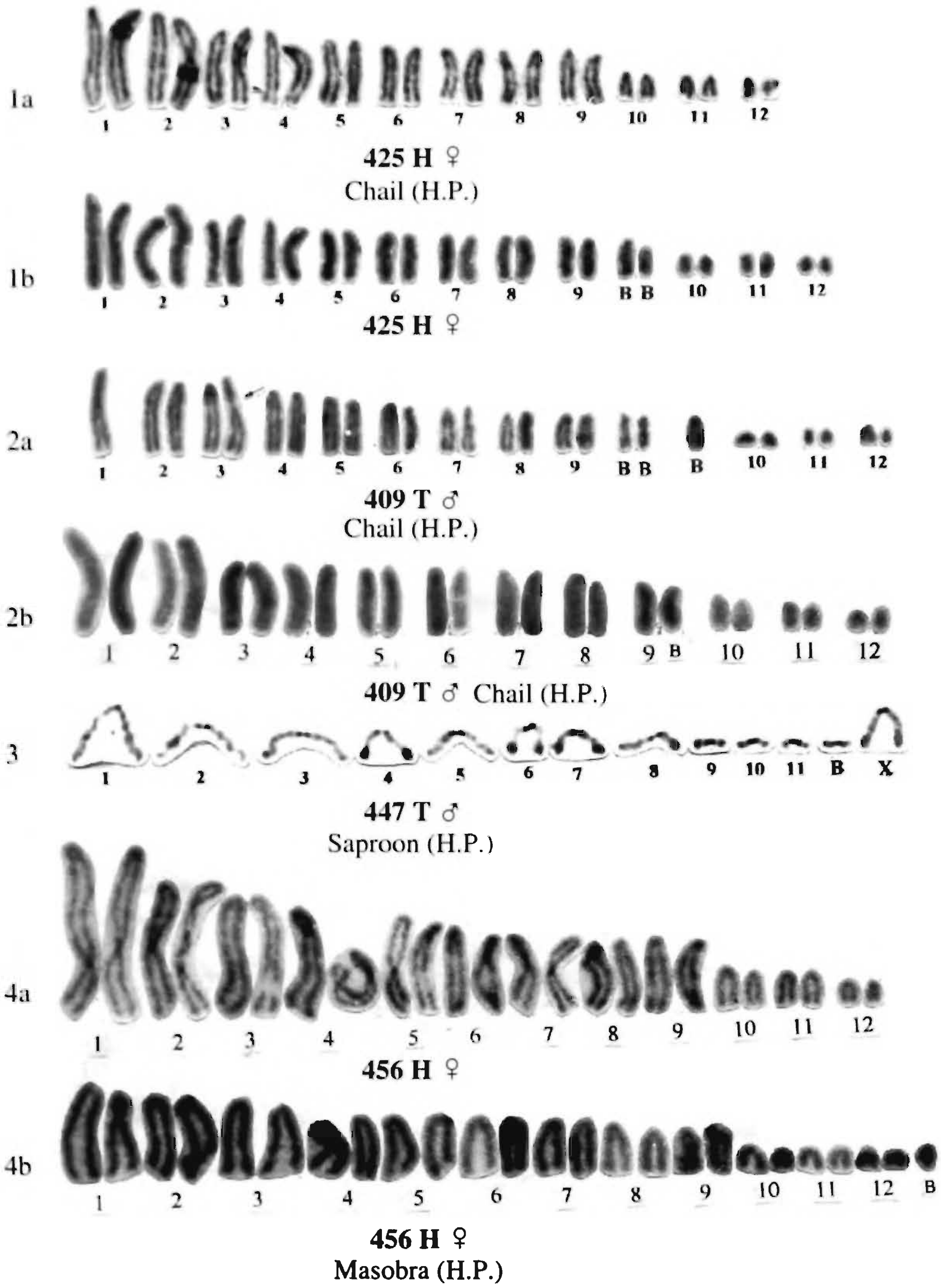
Stephens, R. T. and Bregman, A. A. 1972. The B-chromosome system of the grasshopper *Melanoplus femur-rubrum. Chromosoma*, **38** : 297-311.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Exptl. Cell Res.*, **75** : 304-306.

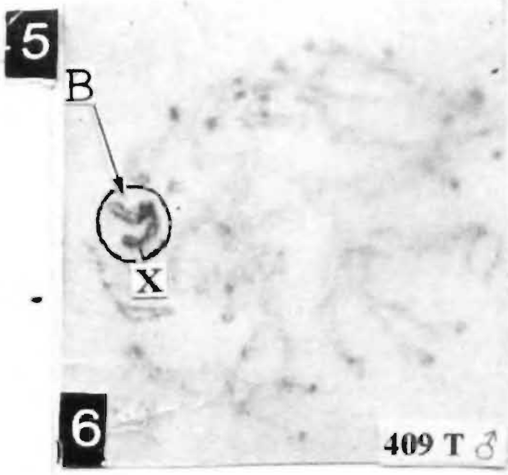
Ward, E. J. 1973. The heterochromatic B-chromosome of maize: the segments affecting recombination. *Chromasoma*, **43** : 177-186.

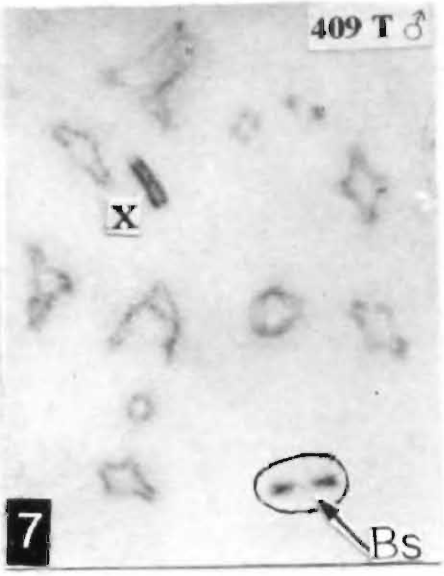
Westerman, M. and Fontana, P. G. 1973. Polymorphism for extra heterochromatin in *Phaulacridium marginale. Heredity*, **31** : 223-229.

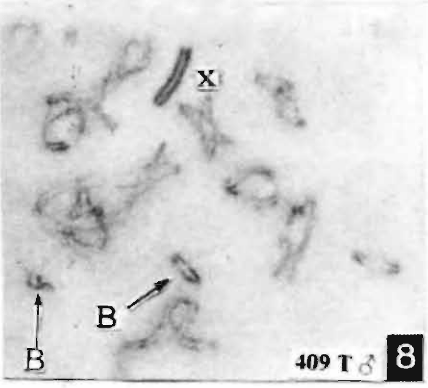
White, M. J. D. 1954. Animal cytology and evolution. (Second edition) Cambridge University Press.

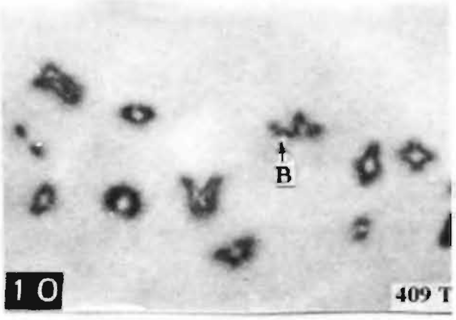


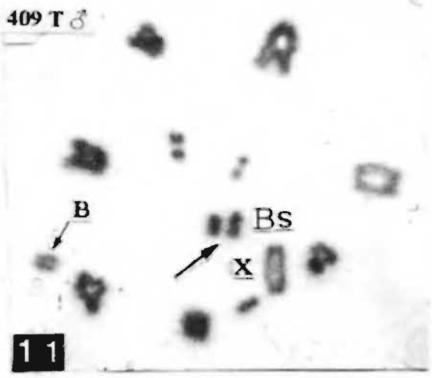
Figs. 1-4. **1a.** Karyotype of a female individual collected from Chail. No supernumerary chromosome is present; **1b.** Karyotype of the same individual. 2 supernumeraries (B) of different sizes are present; **2a.** Spermatogonial metaphase karyotype of an individual from the same locality. 3 supernumeraries (B) of 2 different sizes are present. Observe smaller unpaired supernumerary of this karyotype bigger than the smaller supernumerary of the female (Fig. 1b); **2b.** Karyotype of the same individual without supernumerary; **3.** C-band metaphase II karyotype of a male individual from Saproon.The smallest element is found to be a supernumerary (B) with a visible centromeric band; **4a.** Karyotype from the hepatic caeca of a female individual collected from Masobra; **4b.** Karyotype of the same individual, with a minute supernumerary (B).

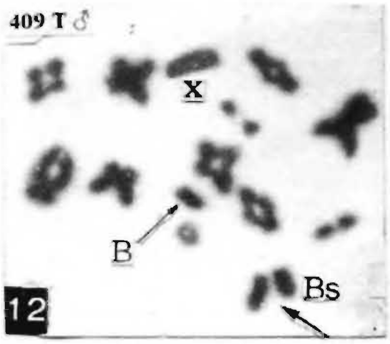


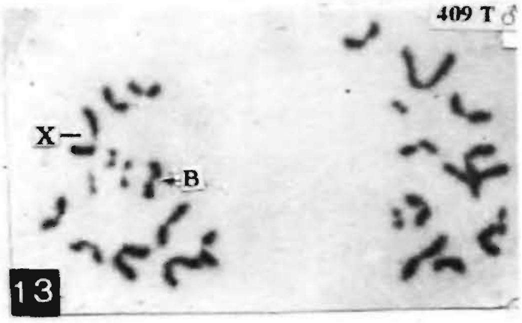












Figs. 5-13 **5.** Idiogram of *P. succincta;* **6 & 7.** Observe one precociously condensed supernumerary bivalent (Bs) in these pachytene and diplotene cells. In pachytene (Fig. 6), this is associated with the similarly condensed X chromosome. **8.** Observe interlocking of two bivalents. Also observe two extra elements in this diplotene cell; **9, 11 & 12.** Observe one supernumerary bivalent (Bs) in the cells. An univalent of smaller size noted in Figs. 11 and 12 (arrow) is not present in Fig. 9; **10.** In this cell, minute supernumerary univalent (arrow) of the size of the 11• pair is present; **13.** Observe 11A+X+B and 11A+2B chromosomes separated at two poles. This indicate equational separation of the B-bivalent and reductional separation of the single supernumerary and the X.