PART - I

OBSERVATIONS
Chapter - III

GENERAL COURSE OF MITOSIS AND MEIOSIS
Continuation of any species from one generation to the next is governed and ensured by two processes - fertilization and cell division - the latter being characteristic of all cellular organisms. An understanding of the mechanism of cell divisions i.e. mitosis and meiosis is very basic for the interpretation of chromosomal evolution in any organism. Even normal meiosis varies to some extent in details of its various stages and processes from one group of organisms to another. Thus, when interpreting the chromosomal evolution of a population, species or a group, it is first of all necessary to know the details of a particular type of meiosis which is present.

In general, the nuclear phenomena of meiosis seem to be similar in the two sexes of a species, but the cytoplasmic processes are, of course, profoundly different. For technical reasons spermatogenesis is much easier to study than oogenesis. In the present studies, meiotic stages are studied only in the males.

The overall process of cell division is basically similar in all the eukaryotic cells although modifications do occur reflecting some degree of evolutionary divergence.

In the Pentatomoid bugs also the process of cell division is similar to that observed in the other animal
groups, of course with slight variations. During the present project mitosis and meiosis have been worked out in the male tissues of as many as 42 species of bugs belonging to the superfamily Pentatomoidea. However, only mitosis could be traced out in the female gonads of some species. In general it is similar in all the species studied with only minor differences existing between them. In the following account a general view of mitosis and meiosis is given and the details of peculiarities and deviations from general scheme, however, will be dealt with specially while describing individual species.

**Spermatogonial Mitosis**

Before the formation of the premeiotic cell, the primary spermatogonium divides many a time and finally undergoes considerable enlargement.

**Spermatogonium**

The spermatogonium (Fig. 1) is spherical in form and large in size with a vesicular nucleus endowed with their metabolic machinery set up for division. It is distinguishable from the rest of the cells in its constant shape and large size. Uniform distribution of the chromatin granules staining intensely with basic stains
is a characteristic feature of the nucleus at this stage. Here the nucleolus cannot be traced out easily. However, in some cases a positively stained spherical structure is conspicuous by its presence. This interphase nucleus is observed quite often because of its long duration, perhaps preparing for the division.

**Prophase (Figs. 2 and 3)**

The appearance of fine thread-like structures that fill up the entire nuclear cavity marks the beginning of prophase. The chromosomes become visibly distinct being lightly stained in the early prophase (Fig. 2), but they become increasingly stainable as prophase proceeds (Fig. 3) due to their contraction which gives a more compact surface for the attachment of chromatin dyes. With the progress of the prophase, the chromosomes become shorter, thicker and more distinct still remaining uncountable elements. The nuclear membrane disappears and the chromosomes are released in the cytoplasm towards the late prophase stage.

**Premetaphase (Figs. 17, 36, 51, 73 and 77)**

Prophase ends with a relatively brief period called premetaphase. This stage includes the establishment of spindle fibres and the movement of chromosomes towards the equitorial plate, which mark this stage a dynamic period. The process of 'orientation' or 'congression'
of chromosomes to the equator is irregular. The holocentric chromosomes, the material under study, show that the whole chromosome is presumably active in 'congression'. The diploid chromosome number specific for each species is countable at this stage. Thus, figure 17 shows a diploid count of 10 chromosomes for Coptosoma nepalense.


At this stage the chromosomes have attained the maximum degree of condensation. It is essentially a period of stasis during which the orientations, relative positions and dimensions of the chromosomes do not undergo any change. With regard to the spatial arrangement of chromosomes, as the chromosomes are holocentric, they are entirely enclosed in the spindle at metaphase and their arrangement is fairly random without any particular tendency to any kind of orientation. Large chromosomes may occupy the centre of the metaphase plate surrounded by smaller ones (Fig. 52A). This stage is the most favourable one for the study of number, size and morphology of the chromosomes. It reveals the diploid chromosome number specific for the species.

The chromosomes in all the species studied during the present investigations appear to be rod-shaped
without exhibiting any constriction along their whole length. Thus, they can be considered as holocentric/polycentric. The chromosomes are grouped arbitrarily into three groups based on their size as long chromosome, medium-sized and small chromosomes. Usually, the homologous chromosomes show a particular tendency to lie close together (Figs. 23A, 28A and 52A). As the sex-chromosomes do not show any heteropycnotic differential behaviour at this stage, it was difficult to determine them at this stage. However, the smallest element in the complement without its homologue has been designated as the Y-chromosome in all the species studied herein.

**Anaphase/Telophase (Figs. 38, 79 and 19)**

The spermatogonial anaphase (Figs. 38 and 79) begins when the sister-chromatids separate from one another and start moving towards the opposite poles of the spindle. The characteristic feature of the Heteropteran mitotic anaphase is that they will move to the pole with their long axis parallel to the equatorial plate demonstrating the holokinetic/polycentric nature of the chromosomes. This stage has, however, been met with very rarely and whenever observed is at an early stage where the chromatids are seen lying parallel to each other without showing any connection between them.

Telophase (Fig. 19) is the period of regrouping
of the chromosomes into a nuclear structure within a membrane. The chromosomes after reaching the poles of the spindle undergo progressive decondensation whereby the individual chromosomes lose their identity and again enter the interphase condition.

The primary spermatogonium after undergoing a definite number of mitotic divisions, enters the growth phase so as to become the premeiotic interphase cell.

**Meiosis**

In most of the animals, meiosis occurs just prior to fertilization and results in the formation of sex cells the sperm and egg. It is a special cycle of cell division which consists essentially of two nuclear divisions meiosis-I and meiosis-II where the latter follows the former in a rapid sequence. As it halves the number of chromosomes, it is considered as the antithesis of fertilization. The meiotic division, though a continuous one has been divided into various stages and substages that possess certain characteristics permitting an easy recognition. Prophase of the first meiotic division is usually very protracted and involves a complicated sequence of events which are genetically significant. For this reason prophase-I is divided into different substages as leptotene, zygotene, pachytene,
diplotene and diakinesis.

**Premeiotic Interphase Cell/Primary Spermatocyte (Figs. 5A and 5B)**

A primary spermatocyte (Fig. 5A) is marked with the presence of an increased nuclear volume and is similar to the primary spermatogonium, except for its larger size with decreased amount of cytoplasm. The chromatin material is in the form of uniformly distributed granules. However, in some cases small patches of condensed chromatin are present here and there (Fig. 5A). The sex-chromosomes are positively heteropycnotic and may be fused to form a single mass (Fig. 5A) or may lie separately (Fig. 5B). The premeiotic interphase stage is of short duration and enters the leptotene soon.

**Leptotene (Fig. 6)**

The onset of meiotic prophase (Fig. 6) is marked by an extensive increase in the nuclear volume followed by a gradual modification of the nuclear structure. The chromosomes at an early leptotene are unpaired and are at their maximum extension. They are randomly distributed filling up the entire nuclear space. The coils of the chromonema called the chromomeres distributed throughout the entire length of the chromosome give a beaded appearance for the chromosomes. The chromomeres are constant in number, size and position. Thus, the fine
granular autosomal threads cross and intercross each other. The sex-chromosomes anyhow are positively heteropyenotic and may lie close together forming a sex vesicle/mass. The nucleolus which stains lightly lies slightly away from the sex vesicle in most of the cases.

**Zygotene (Fig. 7)**

The end of leptotene finds the chromonemata shorter in length and wider in diameter. The most characteristic feature being observable at this stage is that the homologous chromosomes become closely approximated side by side along their entire length for synapsis (Fig. 7). This results in the reduction in the number of chromosomal threads observed at leptotene. The ends of the chromosomes get oriented to one side and give rise to the 'bouquet' stage as is observed in *Coptosoma indicum* (Fig. 7). The positively heteropyenotic sex-chromosomes lie very close to each other at the base of the bouquet.

**Pachytene (Figs. 8, 16, 20, 24, 33, 40 and 193)**

This stage is comparatively of long duration and is otherwise designated as 'stable stage'. It begins when zygotene pairing ceases. The bivalents continue to shorten and thicken staining more uniformly than those of zygonemes. The bouquet orientation is retained and is more pronounced at the early pachytene stages (Figs. 16 and 40). The repulsive force developed at this stage
result in the disruption of the bouquet arrangement at late pachytene stages (Figs. 8, 20, 24, 33 and 193). The sex-chromosomes are positively heteropycnotic and lie at the base of the bouquet in the form of a sex vesicle/sex mass. The number of bivalents can be counted at pachytene stage. Likewise, 5 autosomal bivalents are countable in Coptosoma decodemountatum (Fig. 16), Coptosoma noulhieri (Fig. 24) and Poecilocoris hardwickii (Fig. 40).

Diploptene (Figs. 9, 25, 29, 45, 53, 81, 115, 121, 147, 148 and 187)

The longitudinal duality of the chromosomes become clearly evident at diploptene. The chromosomes are actively shortening and their coiled nature becomes distinct (Figs. 25, 29 and 45). The repulsive force developed between the homologues has resulted in their separation. In most of the diploptene stages, it is observed that the chiasmata are almost at the terminal points and the bivalents are held only at one point showing an end-to-end association. In some of the bivalents - the terminalization of the chiasmata is over at an early stage since the homologues are seen lying far apart from each other at this stage (Fig. 9). Ring bivalents showing two terminal chiasmata are observed in a few cases like Coptosoma sianicum (Fig. 29), Halys magnus (Fig. 81) and Carbula aliena (Fig. 121). The sex-chromosomes are still darkly stained
and may form a single sex-mass as in *Coptosoma indicum* (Fig. 9) or may lie separately as in *Coptosoma nouvelhieri* (Fig. 25) and *O. siamicum* (Fig. 29).

**Diakinesis** (Figs. 10, 30, 46, 54, 65, 75, 76, 82, 89, 94, 99, 100, 101, 105, 106, 111, 116, 127, 128, 131, 137, 149, 173, 188 and 194)

Diplotene proceeds to diakinesis where the chromosomes get more condensed and darkly stained. The chiasmata become completely terminalized and the homologues of the bivalents lie in an end-to-end fashion. The sex-chromosomes lie separately and exhibit clearly their chromatid separation in almost all the cases. In some cases like *Plautia viridicollis*, it is observed that the sex-chromosomes reveal negative heteropycnosis at diakinesis (Fig. 137).

**Metaphase-I** (Figs. 11, 21, 26, 34, 41, 47, 48, 55, 59, 66, 83, 90, 95, 96, 102, 107, 112, 117, 118, 123, 129, 132, 133, 138, 139, 142, 143, 150, 156, 158, 159, 166, 169, 170, 174, 177, 178, 182, 183A, B and 195)

The nuclear membrane disappears completely. The bivalents move to the equator and become oriented in the longitudinal axis of the spindle, on opposite side of the equatorial plate. The bivalents undergo much condensation. They stain uniformly and appear as dumb-bells (Figs. 11, 21, 26, 34 and 66). There is no characteristic pattern of spatial distribution in most
of the species as the autosomes form an irregular ring with the sex-chromosomes in the centre (Figs. 11, 26 and 34). The sex-chromosomes, X and Y orient themselves separately with each chromatid of the chromosome facing to the opposite poles of the spindle. Thus at metaphase-I there are present 6 elements (4 autosomal bivalents + X and Y) in *Coptosoma nepalense* (Fig. 21), 7 elements (5 autosomal bivalents + X and Y) in *Coptosoma indicum* (Fig. 11) and 8 elements (6 autosomal bivalents + X and Y) in most of the pentatomids studied here and 9 elements (7 autosomal bivalents + X and Y) in *Acanthosoma* sp. (Fig. 183).

**Anaphase-I/Telophase-I** (Figs. 12, 42, 84, 151; 85 and 134)

Separation of each tetrad into two dyad chromosomes takes place at anaphase-I. The two disjoining dyads are connected by interchromosomal fibres as seen in figures 12, 42, 84 and 151. The first meiotic division is reductional for the autosomes and equational for the sex-chromosomes. The dyads move to the opposite poles parallel to the spindle and behave as if they are attached by the spindle at the end facing the poles. The movement of chromosomes is synchronous, mostly.

During telophase-I (Figs. 85 and 134) the dyads reach the spindle poles and slowly lose their identity. Nuclear membrane is reconstructed at late telophase.
No interkinetic stage has been observed as the two meiotic divisions are continuing in a rapid sequence. It seems that the dyads of anaphase-I reappear during the second meiotic division without any pronounced delay.

Metaphase-II (Figs. 13, 22, 27, 31, 35, 43, 49, 56, 57, 60, 61, 67A, 67B, 86, 91, 97, 103, 113, 119, 124, 125, 135, 140, 144, 152, 160, 167, 171, 175, 179, 189 and 196)

The metaphase-II stage represents the haploid set of autosomes including both the sex-chromosomes (X and Y). The second metaphase of almost all the species studied show a characteristic spatial arrangement of chromosomes; the autosomes form a ring enclosing a sex-pseudobivalent. The association of the sex-chromosomes is called the 'touch and go' pairing. Chromatid separation is clear in the case of autosomes (Fig. 31).

Anaphase-II/Telophase-II (Figs. 87, 14, 92 and 108)

During anaphase-II (Fig. 87) the chromatids of autosomes separate and move to the opposite poles of the spindle. Of the sex-chromosomes, the X moves to one pole and Y to the opposite poles of the spindle. So second meiotic division is equational for the autosomes and reductional for the sex-chromosomes. The movement of the chromosomes is synchronous. Here also as in the case
of anaphase-I the kinetic activity is restricted to one end of the chromosomes (Fig. 87). Two types of daughter cells are formed at telophase-II (Figs. 14, 92 and 108); some with X-chromosome and others with Y-chromosome.