CHAPTER IV

OBSERVATIONS
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I. *Schizothorax esocinus* HECKEL
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I. Schizothorax esocinus Heckel

(I) Breeding season and testes:

Schizothorax esocinus (Plate I) belongs to the family Cyprinidae sub-family Cyprininae. It is one of the endemic fresh water fishes of Kashmir. The pair of testes lies on either side of the swim-bladder and are somewhat light pinkish in color in early breeding season. They become whitish in color in late breeding season due to the accumulation of abundant spermatozoa. In order to establish the breeding period, following stages were observed during the annual gonodial cycle in Schizothorax esocinus:

(a) Stage I - July.
(b) Stage II - August - September.
(c) Stage III - October - November.
(d) Diapause - December - January - February.
(e) Stage IV - March.
(f) Stage V - April - May.
(g) Stage VI - June (Spent).

Collection of the material was done during the months
March to April. The material collected during the months November to February did not give better cytological results. The best time for the collection of material is the Pre-breeding period during which we have been able to get maximum number of dividing cells.

(2) **Spermatogonial mitosis:**

Spermatogonial mitosis has been investigated in the maturing testes of *Schizothorax esocinus*. It has been observed that the spermatogonia of early generation has got a large resting nucleus about 9 to 11 micra in size, with a large plasmosome and a few chromatin bodies scattered throughout the nucleus (Plate II Fig. I).

As the function of mitosis is to make two cells where there is only one cell before, ensuring at the same time that each cell has an identical set of chromosomes to that of parent cell. In order to fulfill both these conditions the following processes are involved:

(a) Duplication of the chromosomes.

(b) The "Condensation" of chromosomes accompanied by the "Disappearance" of the nucleolus."

(c) The arrangement of the chromosomes at the equator of the spindle.

(d) Division of the cytoplasm along the equator of the spindle.

(e) Reconstitution of complete nuclei, each with a full set of chromosomes.

The stages of mitosis are:
(a) Prophase  (b) Metaphase  (e) Anaphase  (d) Telophase.

(a) Prophase

The beginning of the prophase (late interphase) is marked by the structure of the nucleus which is quite characteristic for the chromomata becomes arranged in spirals. The most conspicuous feature of prophase is the "Condensation" of the chromosomes. During prophase the chromosomes pass through an optimal point at which their spiral condition is most evident. There is no space between the chromosomes in the early prophase. In the late prophase the chromatin threads which become progressively thicker and condensed are found scattered in the entire nucleus (Plate 0 Fig. A).

The second major problem of prophase is the establishment of the poles for the forthcoming division. The other conspicuous events in the description of prophase are the disappearance of the nucleolus and the disintegration of nuclear membrane. All these changes generally take place at the end of prophase. By the gradual thickening and contraction the net work breaks and thick threads are formed which assume the definite outline of the spermatogonial chromosomes. For a long time they are united by delicate filaments. These cross filaments between the chromosomes disappear after some time. Before their arrangement on the spindle the chromosomes come to lie below the nuclear membrane for a short period. By the time the chromosomes are arranged around
the equator of the spindle, they have reached their shortest and thickest form and get more darkly stained (Plate O Fig. B).

In the formation of the spindle the centrosomes appear to play an essential part. In early prophase it liberates two small parts of the divided centrosome called as centrioles which move slowly apart from each other. Between these two centrioles a system of radiating fibres 'an aster' develops. A portion of each aster extends to the equitorial plane, forming the complete spindle. The two centrioles occupy positions at opposite poles of the spindle. The 'asters' along with the spindle appear to persist throughout the process of mitosis up to early telophase.

(b) Metaphase

The term "mitotic apparatus" had been casually used by WILSON (1925) and it was defined both by MAZIA and DAN (1952) as "the ensemble of structures constituting the 'chromatic' and 'achromatic' figures in the classical description of mitosis". A convenient starting point for a sketch of the general scheme of mitosis is not its beginning but the climax of the metaphase, when the mitotic apparatus is completed and is about to perform the acts of chromosome movement that gives it meaning.

The spindle is formed across the cytoplasm of spermatogonium between the two centrospheres which have now moved to the opposite poles of the cell. Each chromosome gets attached to the spindle thread at the equator of the spindle through the
centromere after which the movements begins. Each half chromosome is called a chromatid and the position of the attachment to the spindle varies from one chromosome to another being some times at one end or at middle depending upon the position of the centromere in the chromosome.

The chromosomes now become shorter and thicker due to the coiling of strands (DNA) and the addition of heavily staining envelope the matrix (RNA). The nuclear membrane now disintegrates releasing the chromosomes along the spindle.

In the polar view of the spermatogonial metaphase in Schizothorax esocinus (Plate II Fig.2)(Pl.V. Microphoto. 2)82 chromosomes are seen. They appear radially arranged. All the chromosomes are rod shaped and are of telomitic nature. The chromosomes of a complement vary from 2 - 4 micra in length. The spindle attachment of each chromatid divide and the two chromatids now become separate chromosomes. It is at this stage that 2n number is counted. The number being constant for mitosis of the same individual as well as the individuals of the same species.

(c) Anaphase

At anaphase (Plate O. Fig. C)there is separation of two chromatids of each chromosome, each of which now acts as a separate chromosome, the two now move apart towards opposite poles of the spindle. OESTERGREN (1950,51) believes that the anaphase chromatids are attracted to the poles. Although the exact mechanism of this movement is not known, the movement of chromosomes appears to be directed by its spindle attachment,
It has been shown by experiments in which chromosomes have been broken by exposures to X rays or ultraviolet rays where upon the broken pieces without a spindle attachment do not move to the poles and only attached pieces move.

(d) Telephase

In telophase each group of chromosomes on reaching a pole of the spindle forms a compact mass, each mass being enclosed by a newly formed nuclear membrane (Plate C Fig. D). At this stage nucleoli appear and the chromosomes become less and less distinct. Finally spindle disappears and division of the cytoplasm takes place, with each new nucleus enclosed in its own part of the cytoplasm of the original spermatogonial cells.
3. Spermatogonial meiosis

Spermatogonial meiosis has been studied in spermatogenesis of the 12 species of fishes. In the production of sperm cells the early cells in the immature testes divide repeatedly by mitosis giving rise to primary spermatocytes. Each of these primary spermatocytes divide into secondary spermatocytes each of which in turn divide into two spermatids. The spermatid grows a tail or undergoes transformation without further division to become a sperm. The division of the early cells (spermatogonial) of the testes is by mitosis, as also in the formation of primary spermatocytes. While meiosis occurs only at the production of secondary spermatocytes from primary spermatocytes in which the diploid chromosome number is reduced to the haploid number.

Meiosis has a great role in evolution. During meiosis I pairing of maternal chromosomes (homologous chromosomes) is followed by the crossing over. It causes the formation of new combinations. These recombinations lead to produce variations which are directly responsible for evolution.

Meiosis idly consists of two divisions of the nucleus with only one reduplication of the chromosomes, at the first division. The two nuclear divisions are closely co-ordinated to each other. First division (Meiosis I) results in the reduction of chromosome number to half and the second division (Meiosis II) is responsible for the mitotic division of already reduced (halved) number of chromosomes.

It is a mechanism by which the diploid number of chromosomes
produced by fertilization is halved so that the sexual cells possess the haploid number. Fertilization and meiosis are compensating events; a failure of one or the other causes a break-down in the orderly system of reproduction.

Meiosis is regarded as precocious division, the reason being that when the prophase of meiosis starts the chromosomes have not reached the stage at which they split into two chromatids, and they appear therefore as single threads. So after the completion of meiotic divisions the resulting spermatocyte cells pass into resting phase (Plate IV MP I). In this condition the chromosomes are found as thin extended threads (chromonemata) and they do not undergo any visible change. The nuclei of such cells contain a light-stained plasmosome and a clear net-work of chromatin threads.

In meiosis the sequence of steps are similar to those in mitosis. Prophase, however, is longer in duration and profoundly modified in character, and five separate prophase stages are recognizable viz. (a) Leptotene, (b) Zygotene, (c) Pachytene, (d) Diplotene and (e) Diakinesis.

(1.) Prophase

The onset of meiotic prophase is marked by an increase in nuclear volume followed by gradual modification of nuclear structure. The first steps in the transition from premeiotic interphase to meiotic prophase occurs only in early leptotene stage. As the chromosomes become more condensed they appear as coiled threads.

The early leptotene spiral is lost as the chromosomes unravel to form the greatly extended and uncoiled threads found at early leptotene (Pl. II Fig.4). In later stages the reticular arrangement
gets disorganised and individual threads are distinguishable at leptotene, (Pl. II Fig. 5), these leptotene threads become thicker and they start to polarize at a small area nearest the centrosome adjacent to nuclear membrane (Pl. II Fig. 6). Finally the polarization gets complete resulting in the formation of light bouquet stage in to the interior of nucleus (Pl. III Fig. 7). SCHARADE (1953) emphasizes that the bouquet formation indicates an interaction between chromosome ends (telomeres) and a centrosome bringing about a chromosome movement wholly independent of the spindle. The threads of the bouquet are considerably thicker than the leptotene threads and reduced in number.

(b) The bouquet stage is believed to be the Zygotene. Although actual synapsis (pairing) was not observed due to the extreme thinness of threads. The bouquet disorganizes and nucleus enters in to pachytene stage.

(c) During the pachytene stage the chromosomes show unravelling and scatter in the entire nucleus. The threads are thicker and shorter at this stage, than the preceding ones and bear heterochromatic rounded ends (Pl. III Fig. 8). At this stage it is very difficult to determine the exact number of chromosomes owing to the overcrowding.

As a result of synapsis, the apparent number of chromosomes has been reduced to half, if there were 2n chromosomes in leptotene there will be (n) associations of two chromosomes at the beginning of the pachytene. These associations of pairs of chromosomes are called bivalents. Each pachytene bivalent appears to be made up of strands between which a split is visible.
Each paired chromosome, except in the region of centromere splits longitudinally into two sister chromatids. Thus each bivalent now consists of four chromatids (tetrads). Thus doubling in the number of chromatids is the result of doubling of DNA content which occurred much earlier. This may be followed by a process of exchange of certain parts between homologous chromosomes and not between sister chromatids.

WHITE (1963) states "The crossing over takes place in such a way that on one side of the chiasma (point of attachment of 4 chromatids) a paternal chromatid is paired with a paternal and maternal with a maternal, while on the other side a paternal is paired with maternal, and maternal with paternal. As a result of crossing over the chromatids involved in it contained genetic material of both maternal and paternal origin. However, this crossing over could not be observed in the present fishes."

(c) Diploctene (Diffuse): Pachytene is followed by a diffuse stage where irregular faintly stained chromatin bodies are observed, scattered uniformly in the nucleus (Pl. III Fig. 9). This diffuse stage is however, merely an interruption in the course of meiosis apparently caused by the unusual metabolic conditions such as extended growth of cytoplasm and no wise is an essential feature. It can be said conclusively in the absence of atypical diploctene stage of normal meiosis passes away in diffuse condition. Usually during diploctene stage one pair of sister chromatids begins to separate from other pair, but the separation is normally prevented at the places where crossing over occurs viz. chiasmata,
at these points chromatids may break and inter-change resulting in recombination, after which the chromatids become free from each other.

(d) Diakinesis approaches with the disappearance of nucleolus and the appearance of typical tetrads (bivalents) like figures in the nucleus of primary spermatocytes. These tetrads are of various shapes (Pl.III Fig.10) on account of continuous contractions and coiling they become more compact and come to lie below the nuclear membrane. The nuclear membrane now begins to disappear.

With the commencement of the prometaphase stage the bivalents begin to migrate towards the centre of the nucleus and acquire smooth outline with the dissolution of the nuclear membrane the dikinetic stage is ended and the compact bivalents move on the spindle at metaphase.

After the nuclear membrane has disappeared, a bipolar spindle is formed on which the bivalents move and become oriented on the equator of the spindle. The time between disintegration of nuclear membrane and the establishment of bivalents on the spindle is called prometaphase.

(ii) Metaphase

At full metaphase the two homologous centromeres of each tetrad or bivalent lie in the longitudinal axis of the spindle on opposite sides of the equitorial plate. Polar view of the metaphase I (Pl.III Fig.II-12) (Pl.V WP.3) discloses only the distribution of the bivalents on the spindle plate and reveal neither the number of chiasmata nor the shapes of the bivalents. In these chromosomes
complements 41 elements have been counted without any doubt. At the side view of the metaphase the mode of orientation of tetrads on the equator of the spindle shows that they are with telomitic fibre attachment. They are dumb-bell shaped thus revealing their bivalent nature (Pl. III Fig. 13) (Pl. VI MP.4).

(iii) Anaphase

Metaphase is followed by anaphase of the primary spermatocytes. All the bivalents get separated synchronously in to two equal halves (Pl. VI MP.5) and the chromosomes move towards the poles with the result haploid number of chromosomes moves towards the poles. The secondary spermatocytes are thus formed from primary spermatocytes, when the reduction division takes place. Anaphase is of short duration and at this stage the chromosomes usually get clumped due to overcrowing which renders the exact counting impossible.

(iv) Telophase

Once the dyads reach the spindle poles they form at each pole a telophasic nucleus with a nuclear membrane. The telophase nucleus thus contains only half the number of chromosomes obtained in the primary spermatocytes. After the partition of primary spermatocyte cell in to two secondary spermatocyte cell in to two secondary spermatocytes each containing one telophase nucleus. The secondary spermatocyte stage is reached. Each one of these cells with a haploid number of chromosomes is thus secondary spermatocyte. The actual reduction division thus lying in between primary
and secondary spermatocyte stages.

In the secondary spermatocyte metaphase at polar view (Pl. VI Fig. 15) again 41 spherical univalents have been counted. The size of the plate as well as of individual chromosomes is almost half of the bivalents seen at first metaphase. At the secondary anaphase, all the chromosomes divide mitotically and move towards the opposite poles (Plt IV Fig. 16). These chromosomes differ from the first (primary spermatocytes) anaphase chromosomes in being small and rounded where as in the later ones are thicker. In the secondary telophase only two big chromatin clumps lying on opposite poles of spindle, are seen (Pl. IV Fig. 16) therefore no detailed study of this stage could not be made.
(4) Chromosome number, shape and size

Schizothorax esocinus HECKEL exhibits 32 acrocentric \((2n)\) elements (Pl. II Fig. 2) at the polar view of the spermatogonial metaphase. All the chromosomes are small rod shaped structures. The size of the chromosomes varies from 1 - 3 micra. They do not seem to vary much in shape and size.

41 elements have been counted at metaphase I (Pl. III Fig. II-I2) all the chromosomes are somewhat spherical in shape. (Pl. V MP. 3). In meiotic metaphase second again 41 elements have been counted (Pl. IV Fig. I5). The size of the secondary spermatocyte metaphase chromosomes is almost half of the size of plate as well as that of individual chromosome than that of first spermatocyte metaphase plate.
Z. Orenus plegiotormus (BECKL.).
2. Oreinus plagiotomus (HECKEL).

(1) Breeding season and testes:

Oreinus plagiotomus (HECKEL) (Pl. I) belongs to the family Cyprinidae, Schizothoracinae. It is one of the endemic fishes of Kashmir. The testes are large elongated structures lying ventro-laterally on either side of swim-bladder. The testes grow in size very much during the breeding season due to the presence of abundant spermatozoa.

The seasonal gonadal changes of this fish resemble those of Schizothorax caprota. Observations on the annual gonadal cycle in Oreinus plagiotomus are described under following heads:

(a) Stage I - July - August.
(b) Stage II - September to October.
(c) Stage III - October to November.
(d) Diapause - December, January and February.
(e) Stage IV - March.
(f) Stage V - April.
(g) Stage VI - May (spawning).

The best time for the collecting of the material was early breeding season i.e. March, April. During this period we have been able to get the maximum number of division figures.
(2) Spermatogonial mitosis

The study of the spermatogonial cells in *Orsinus* reveals that the size of early generation is larger than the later ones. The size of the early spermatogonial cells varies from 10 to 12 micra with a large nucleolus (plasmosome) and small patches of chromatin which are darkly stained and are scattered throughout the periphery of the nucleus. Such nuclei are called Resting nuclei (Pl. VII Fig. 17). The resting nucleus or the interphase nucleus does not undergo any visible change involved in mitosis.

(a) Prophase

In spermatogonial mitosis karyokinetic figures are almost the same as in *Scocimus* therefore have not been figured. The nuclei of spermatogonial prophase show long fine darkly stained chromatin threads. The chromosomes are thin and long in form of net-work. It is very difficult to perceive the ends of individual chromosomes. If the term "Prophase" is to be defined at all for purposes other than the arbitrary scoring for mitotic stages, it may be viewed as including all the visible events in the mobilization of mitotic apparatus. As the most important aspect of prophase is the "condensation" of the chromosomes. This is followed by progressive spiralization. The functional significance of condensation is clear enough, it solves in a straightforward way the problem of effecting mitotic movement of chromosomes clearly with minimum resistance and the risk of entanglement. The establishment of the poles is one of the last events of prophase. By the gradual thickening and contraction the
network breaks into small threads which finally assume the form of spermatogonial chromosomes. At the end of prophase the nuclear membrane disappears and the nucleolus also disintegrates.

The process of mitosis itself can be divided into two stages: (i) the establishment of the mitotic apparatus and (ii) the transportation of the chromosomes at the poles. The mitotic apparatus or the spindle is formed across the cytoplasm of spermatagonium between the two centro-spheres. The chromosomes which lie scattered throughout the nucleus move towards the centre and get oriented on the plane which is circumscribed by the equator of the spindle known as equatorial plate.

(b) Metaphase

At the end of the prophase the chromosomes have reached their maximum degree of condensation and have acquired smooth outline. Each element consists of two chromatids lying parallel. In the polar view of the spermatogonial metaphase 76 chromosomes are seen radially arranged (Pl.VII Fig.18). All the chromosomes are small rod shaped and are acrocentric in nature. The chromosomes of the complement vary from 2 to 4 micra in length. The spindle attachment of each chromatid divide and the two chromatids now become separate chromosomes. It is at this stage that the complement is counted. The number being constant for mitosis of the same individual as well as the individuals of the same species. In the later generation the chromosomes do not have any morphological distinction and are smaller that indicates that condensation increases with successive divisions.
(c) **Anaphase**

During anaphase the centromeres part from one another and drag the chromatids (which are attached to them) towards the poles. Although the actual mechanism of movement of chromosomes towards the poles is not known; (but some observations suggest that an active substance possibly proteolytic enzyme is liberated by the centromeres at this stage) but the precise nature of the forces involved in the earlier phase of anaphase separation is much more uncertain.

(d) **Telophase**

In telophase each group of chromosomes on reaching a pole of the spindle forms a compact mass, each mass being enclosed by a newly formed nuclear membrane. The spindle disappears and each set of chromosome develops its own nucleolus and the division of cytoplasm takes place, with each new nucleus enclosed in its own part of the cytoplasm of the original spermatogonial cell.
(3) Spermatogonial meiosis

The meiosis in *Graefia plagiostomus* has been studied under the following heads:

(a) Prophase (b) Metaphase (c) Anaphase (d) Telophase.

After the completion of mitotic division the resulting spermatocyte cells pass into the resting phase. The nucleus is bounded by a nuclear membrane. The nuclei of such cells contain a light stained plasmosome and clear nucleoplasm with few chromatin bodies. In this resting nucleus no visible change has been observed (Pl. VII Fig. 19) (Pl. IX MP. 6).

(a) Prophase

(i) Leptotene  (ii) Zygotene  (iii) Pachytene  
(iv) Diplotene  (v) Diakinesis.

(i) Leptotene

With the disintegration of the plasmosome a very fine reticulum of chromatin threads appear in the whole nucleus. This stage corresponds to early leptotene (Plt. VII Fig 20). The onset of meiotic prophase is marked by an increase in nuclear volume followed by gradual modifications in nuclear structure. The first steps in the transition from premeiotic interphase to meiotic prophase occurs in the early leptotene stage. In the latter stages the reticular arrangement of threads get disorganised and individual threads are distinguishable at leptotene.

(ii) Zygotene

The leptotene threads start to polarize at a small area of nuclear membrane (Pl. VII Fig. 21). Finally the polarization gets complete resulting in the formation of bouquet at one pole.
of the nucleus (Pl. VII Fig. 22). The threads of the bouquet are thicker than the leptotene threads and also reduced in number. In the bouquet the intermingling loops of chromosomes are visible (Pl. VIII Fig. 23) (Pl. X MP. 7). Synapsis can not be definitely made out. Bouquet stage is concluded as Zygotene.

(iii) Pachytene

In this stage the chromosome appear as short thick threads with heterochromatic knobbed ends (Pl. VIII Fig. 24). These threads are bivalents and represent the haploid number, but the counting is impossible. This stage is of longer duration than the other stages of prophase. Pachytene stage is followed by diffuse stage.

(iv) Diploëten (Diffuse stage)

In diffuse stage the staining capacity is lost and only irregularly scattered, small chromatin patches are visible. The diffuse stage is however not an important stage it only interrupts in the normal course of meiosis. Diploëten has not been observed clearly, it seems that diploëten passes away in diffuse stage (Pl. VIII Fig. 25).

(v) Diakinesis

At diakinesis the chromosomes become shorter and deep staining bodies and they are in the form of typical bivalents (tetrads) which are of various shapes (Pl. VIII Fig. 26). These bivalents move to the periphery of the nucleus, where they lie close to the nuclear membrane and widely separated from one another. Before the formation of the spindle, with the disappearance
of nuclear membrane the diakinetic stage is ended and the compact bivalents move to the spindle at metaphase.

A bipolar spindle is formed on which the chromosome move and become oriented. The time between the disintegration of the nuclear membrane and establishment of the bivalents on the spindle is called prometaphase.

(b) Metaphase

During metaphase the two homologous centromeres of bivalent lie on longitudinal axis of the spindle on opposite poles of the equatorial plate. Polar views of the metaphase (Pl.VIII Fig. 27-28)(Pl.X MP18)(Pl.XI MP.9) discloses only 38 elements. The arrangement of the chromosomes is radial. They are all spherical or some what elliptical in shape. In the side view all the dumb-bell shaped bivalents lie with their longitudinal axis parallel to that of spindle (Pl.VIII Fig. 29).

(c) Anaphase

During anaphase I separation of each tetrad into two dyad chromosomes takes place (Pl.IX Fig. 30) as two co-oriented centric regions begin moving to opposite poles of the spindle. The chromosomes of lagging or precocious nature have not been observed. The bivalents are elliptical in shape (Pl.IX Fig 30).

(d) Telophase

As the dyads reach the poles of the spindle they form a telophasic nucleus (Pl.IX Fig. 31) with a nuclear membrane. The chromosomes accumulate to form big chromatin mass rendering the detailed study impossible. The telophase nucleus contains
only half the number of chromosomes obtained in the primary spermatocytes.

The nucleus undergoes short interphase before passing in to the second meiotic division. The partition of primary spermatocyte cell in to two secondary spermatocytes, each contains one telophasic nucleus. Thus the secondary spermatocyte stage is reached with the haploid number of chromosomes. The actual reduction division lies between the primary and secondary spermatocyte stages as usual.

In the secondary spermatocyte metaphase at polar view (Pl. IX Fig. 32) shows 36 rounded chromosomes. The staining capacity of the secondary metaphase is less than that of first metaphase. The size of the chromosomes is also less than that of primary metaphase. At the secondary enaphase all the chromosomes divide normally and pass to the opposite poles. In the second telophase only two chromatin clumps lying on opposite poles of the spindle are seen (Pl. IX Fig. 33).
§ 4) Chromosome number, shape and size.

Oreinus plagiostomus (HECKEL) exhibits 76 acrocentric elements (Pl. VII Fig. 18) at the polar view of the spermatogonial metaphase. All the chromosomes are rod shaped structures. The size of the chromosomes varies from 3 to 5 micra.

In the polar view of the spermatogonial metaphase 1,38 elements (Pl. VIII Fig. 27-28) (Pl. X MP. 8) have been observed. Similarly 38 univalents have been counted in the secondary spermatocyte metaphase plate (Pl. IX Fig. 32). The size of the chromosomes is approximately half of the size of the bivalents seen at metaphase one.
3. *Cyprinus carpio communis* LINN
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(I) **Breeding season and testes**

*Cyprinus carpio communis* (Pl.XII) belongs to the family *Cyprinidae*. The testes are paired structures and are straight strap shaped in early breeding season but with further maturity testes become lobulated. The breeding season of this fish has been studied under the following heads:

(a) Stage I  -  September - October.
(b) Stage II  -  November - December.
(c) Diapause  -  January - February.
(d) Stage III  -  March.
(e) Stage IV  -  April - May.
(f) Stage V  -  June - July.

*Cyprinus carpio communis* spawns in July. Collection of the material was done during the months April and May. The material collected during these months proved useful for our studies.
(2) **Spermatogonial mitosis**

Spermatogonial mitosis has been studied in the maturing testes. Study of the germ cells reveals that the size of early generation is larger than the later ones. The size of the early spermatogonial cells varies from 11 to 12 micra, whereas the size of the later generation varies from 7 to 8 micra. The nuclei of such cells contain large nucleolus and darkly stained chromatin (Pl. XIII Fig. 34). Whereas the nuclei of later generation has a small nucleolus lying in the homogeneous nuclear sap. The interphase nucleus or the resting nucleus does not undergo any change.

The nucleus of the spermatogonial prophase shows long thin darkly stained chromatin. The most peculiar thing about the prophase is that the chromosomes undergo considerable "condensation" and undergo coiling upon greatly extended chromosomes of interphase nucleus. The condensation of the chromosomes helps in the movement towards poles. By gradual thickening and contraction the chromosomes break in to small threads. These chromosomes are found scattered in the entire nucleus (Pl. XIII Fig. 35). These chromosomes appear different from that of *Cyprinus carpio specularis*.

The poles are established at the end of prophase for the forthcoming division. The poles are established by the separation of the centres. As the centres move apart a set of fibrous connections designated as the central spindle is observed. All these changes take place in the later part of the prophase.

The collapse or the dispersion of the nucleolus during
prophase is one of the most characteristic events of the mitotic cycle. The nuclear membrane also breaks down and central spindle is formed. The central spindle or mitotic apparatus is a dual structure; (1) a system of pole-to-pole connections, the central spindle, (2) a system of chromosome-to-pole connections, which is established at the end of prophase.

(b) **Metaphase**

At metaphase the (i) movement of chromosomes takes place typically to a point midway between the poles, (ii) the alignment of the chromosomes on a well defined equatorial plate, often with a rather consistent arrangement of individual chromosomes on the plate. The equatorial position refers only to the spindle axis and its polar ends. It has been observed in the polar view of the metaphase plate that all chromosomes are arranged radially. The size of the chromosomes varies from 1 to 2 microns. From the study of several clear metaphase plates the author has counted 104 chromosomes which constitutes the diploid complement of Cyprinus carpio communis (Pl. XLI Fig. 36)(Pl. XVI Fig. II). Almost all the chromosomes are rod shaped. In the later generation the chromosomes do not show any morphological distinction in size, and are smaller showing thereby that condensation increases with successive divisions.

(c) **Anaphase**

At anaphase there is pulling and pushing. The centromeres part from one another and drag the chromatids towards poles. The "parting" of sister chromosomes is a distinct event, marking the transition from metaphase to anaphase.
(d) **Telophase**

In telophase each group of chromosomes on reaching the pole of the spindle it forms a compact mass. Each mass being enclosed by a newly formed nuclear membrane. The spindle disappears and each set of chromosomes develops its own nucleolus and the division of cytoplasm takes place with each new nucleus enclosed in its own part of cytoplasm.
(3) **Spermatogonial meiosis**

Meiosis has been studied under following heads:

(a) Prophase (b) Metaphase (c) Anaphase (d) Telophase.

(a) **Prophase**

Prophase is a division of longer duration and is divided into following steps:

(i) Leptotene (ii) Zygotene (iii) Pachytene (iv) Diplotene (v) Dikinesis.

After the completion of the mitotic divisions the resulting spermatocyte cells pass into resting phase often termed as interphase nucleus. The interphase nucleus contains light stained plasmosome and clear nucleoplasm with few chromatin bodies (Pl.XVI Pl.12).

(i) **Leptotene**

The plasmosome disintegrates and a very fine network of chromatin appears in the whole nucleus. This stage corresponds to early leptotene (Pl.XIII Fig.37).

In the latter stages the net-work arrangements of threads get disorganized and individual threads are distinguished at leptotene. The chromosomes are very elongated and slender and have a polarized orientation (Pl.XIV Fig.38-39) with all their ends directed towards one small area on one side of the nucleus resulting in the formation of the bouquet (Pl.XIV Fig.40).

(ii) **Zygotene**

The bouquet stage is concluded as the zygotene stage. The threads of bouquet are very much thicker than the early leptotene
threads and are also reduced in number. Synapsis has not been observed due to the extreme thinness of chromosomes.

(iii) **Pachytene**

Gradually the bouquet disorganises and the nucleus enters the pachytene stage, a stage of long duration. The chromosomes start unravelling and scatter throughout the nucleus. The threads are shorter and thicker at this stage and bear heteropygmentic knobbed ends (Pl.XIV Fig.41). The exact number could not be counted as the chromosomes show over crowding.

(iv) **Diffuse stage (Diplotene)**

Pachytene is followed by a diffuse stage where irregularly staining chromatin bodies are observed scattered in the nucleus (Pl.XIV Fig.42). Such nuclei of diffuse condition are found lying in the same lobe of testes where its adjacent stages are seen i.e. pachytene, diakinesis and some times first spermatocyte metaphases. So it can be said conclusively in the absence of atypical diplotene stage of normal meiosis, diplotene passes away in diffuse condition.

(v) **Diakinesis**

Diakinesis approaches with the appearance of typical tetrad like figures in the nucleus. These tetrads are of various shapes viz. V's, dumb-bells and spheres (Pl.XIV Fig.43). On account of continuous contraction, they become more compact and get scattered in the whole nucleus. With the commencement of pro-metaphase stage the bivalents acquire a smooth outline. The nuclear membrane dissolves gradually with the formation of spindle and the chromosomes start to orientate on the spindle
(Pl.XVI MP.13).

(b) Metaphase

In the polar view of the metaphase plate chromosomes are
seen arranged radially, 52 elements have been counted without
any doubt (Pl.XV Fig.44-45)(Pl.XVII MP.14). The chromosomes
of the complement are of same size and are all rod or ovoid
shaped. All the chromosomes have got smooth outline and by now
they have reached their maximum degree of condensation. The
bivalents of the spindle can be seen clearly in side or lateral
view of spindle (Pl.XV Fig.46). They are all dumb-bell shaped
thus revealing their bivalent nature.

(c) Anaphase

Separation of each tetrad in to two dyad chromosomes takes
place at first anaphase as the two co-oriented centric regions
begin moving to opposite poles (Pl.XIII Fig.47)(Pl.XVIII MP.15).
Anaphase first is of very short duration and usually chromosomes
get clumped due to overcrowding.

(d) Telophase

As the dyads reach the spindle poles they form at each
pole a telophase nucleus with a nuclear membrane (Pl.XV Fig.48)
and then interkinesis intervenes where the nuclei remain almost
empty with a few diffuse chromatin bodies.

In the secondary spermatocyte metaphase at polar view
(Pl.XV Fig.49) again 52 ovoid shaped univalents have been counted.
The size of the plate as well as of individual chromosome is
half of the bivalents seen at first metaphase. At second anaphase
all the chromosomes divide normally and pass to the opposite poles
These chromosomes differ from first anaphase in being small
whereas the later ones are thick. In second telophase the chromosomes are seen only in the form of two clumps lying on opposite poles of the spindle (Pl. XV Fig. 50) hence no details can be given of this stage.
(4) **Chromosome number, shape and size.**

The number 104 (Pl.XIII Fig.36)(Pl.XVI M.P.II) constitutes the diploid set of chromosomes in *Cyprinus carpio communis* LINN as in *C.c. specularis*. All the chromosomes are rod or oval shaped. The size of the chromosomes varies from one to two micra.

The primary as well as the secondary metaphase plates reveal 52 elements (Pl.XV Fig.44-45-49)(Pl.XVII M.P.14) being the haploid number. They are somewhat spherical in shape. The size of the secondary metaphase chromosomes is almost half of the size of chromosomes of primary metaphase chromosomes.
Cyprinus carpio specularis Linn.
4. *Cyprinus carpio specularis* LINN

(I) Breeding season and testes:

*Cyprinus carpio specularis* LINN belongs to the family *Cyprinidae* and subfamily *Cyprininae* (Pl.XIX). In this cyprinid the testes are thick and whitish structures lying ventro laterally to the air bladder. The testes become lobulated during the breeding season and get enlarged due to the accumulation of abundant spermatozoa. The seasonal changes in the testes of *Cyprinus carpio specularis* resemble those of *Cyprinus carpio communis*. The collection of the material was done during the months March, April and May as during this period we have been able to get most of the dividing cells.
(2) Spermatogonial mitosis

Spermatogonial mitosis has been studied in *Gyprinus carpio specularis* in the maturing tests. The essential steps of mitosis are:

(a) Duplication of the chromosomes.

(b) The "condensation" of the chromosomes accompanied by the "disappearance of the nucleolus".

(c) The arrangement of the chromosomes at the equator of the spindle.

(d) Division of the cytoplasm along the equator of the spindle.

(e) Reconstitution of complete nuclei each with a full set of chromosomes and nucleolus.

The study of the spermatogonial cells reveals that the nucleus of early generation possess a large plasmosome. The size of the nucleus varies from 9 to 10 micra (Pl. XX Fig. 51). Small patches of Gomori's stained chromatin are scattered throughout the nucleus.

(1) Prophase

The Karyokinetic figures are almost same as that of *Schizothorax esocinus* therefore have not been drawn. The onset of prophase is marked by reticular structure spread throughout the nucleus. The meshes of the net-work are gradually reduced in to spiral threads by condensation and contraction. The most essential feature of the prophase is the "condensation" of the chromosomes, which takes place generally the imposition of several orders of spiralization or coiling upon greatly extended
interphase chromosome threads.

By gradual contraction and condensation the reticular net-work breaks in to small threads which finally assume the definite outline of chromosome. For a long time they are linked by delicate filaments which disappear with further condensation. In mid prophase the volume of nucleolus increases, both the nucleoli and chromosomes are seen clearly in the late prophase. The nucleolus and nuclear membrane disappear which is one of the most characteristic observed events of mitotic cycle. By the end of prophase the poles are established. All these steps lead to the formation of mitotic apparatus.

(ii) Metaphase

After the long and involved stages of preparation, the chromosomes begin to move in relation to the poles and mitosis is underway. The first movements culminate in the establishment of the chromosomes in their metaphase position. (a) the movement of the chromosomes is typically to a point midway between the poles (b) the arrangement of the chromosomes on a well defined equitorial plate (c) most important fact is that the sister chromosomes are invariably attached to different poles at the termination of the process.

In the polar view of the spermatogonial metaphase (Pl.XX Fig.52) 104 chromosomes are seen arranged radially. All the chromosomes are long and rod shaped. The number 104 constitutes diploid number of Cyprinus carpio specularis. The chromosomes of the complement vary from 3 to 4 micra in length. Both the ends of the chromosomes are rounded. The rod shaped chromosomes
are originally threads formed by the transformation of the net-work by thickening and contraction.

(iii) Anaphase

The "parting" of the sister chromosomes is a distinct event marking the transition from metaphase to anaphase. The sister chromatids move towards the opposite poles.

(iv) Telophase

Each group of chromosomes on reaching respective pole of the spindle forms a compact mass, each mass being enclosed by a newly formed nuclear membrane. At this stage nucleoli appear and the chromosomes become less and less distinct. Finally spindle disappears and division of the cytoplasm takes place, with each new nucleus enclosed in its own part of the cytoplasm of the original spermatogonial cell.
(3) **Spermatogonial meiosis**: Meiosis is the anti-thesis of fertilization. The diploid complement is reduced to haploid number. In the course of meiosis there are two divisions of nucleus, of course chromosomes divide only once. There is considerable increase in the nucleus in growth period. All the nuclei measured at different stages that is, from resting nucleus to diakinesis show a very little size variation. The average nuclear size varies from 10 to 11 micra. The increase in nuclear volume is followed by gradual modifications in nuclear structure. The resting nucleus appears quite homogeneous except for a centrally placed spherical nucleolus with few faint and extremely fine chromatin threads in the form of net-work. (Pl. XX Fig. 51)

Meiosis has been studied under following heads:
(a) Prophase (b) Metaphase (c) Promaphase (d) Telophase

(a) **Prophase**

It is a stage of longer duration and five separate stages are recognizable: (i) Leptotene (ii) Zygotene (iii) Pachytene (iv) Diplotene (v) Diakinesis.

The first steps in transition from premeiotic interphase to meiotic prophase occurs in the preleptotene stage. Preleptotene (Pl. XX Fig. 53) is marked by dissolution of the plasmosome and appearance of very fine reticulum of chromatin threads which spread throughout the nucleus. At the end of the leptotene (Pl. XXI Fig. 54) the chromonemata shortens in length and widens in diameter. The leptotene threads start polarizing at a small point of the nucleus (Pl. XXI Fig. 55). A large bouquet is seen
on one side of the nucleus in which anastomosing loops of chromatin threads are visible (Pl.XXI Fig.56).

(i) **Zygote**

The bouquet stage has been concluded as zygote although actual synapsis could not be made out clearly. But the number of threads and their thickness gives an idea that they are paired. The bouquet stage shows the thickened chromosomes arranged at one pole of the nucleus.

(iii) **Pachytene**

This stage is of long duration, the paired homologus, consisting of four chromatids and known as tetrads or bivalents with heterochromatic knobbed ends become both shorter and thicker by progressive increase in the diameter of their coils. The exact counting of the chromosomes at this stage is impossible (Pl.XX Fig.57).

(iv) **Diffuse stage**

In diffuse stage the bivalents loose their staining capacity and are irregularly scattered in the nucleus (Pl.XXI Fig.53).

(v) **Diakinesis**

At diakinesis the chromosomes reappear in the form of typical bivalents which become more contracted and darkly staining (Pl.XXI Fig.59). The bivalents show a tendency to move out to the inner surface of the nuclear membrane during diakinesis. The typical bivalents are of various shapes viz rods, crosses and dots. At late diakinesis almost all chromosomes come to lie below the nuclear membrane just before the formation of spindle. The nuclear membrane disappears and
initiates the formation of first metaphase of meiosis. A bipolar spindle is formed on to which the bivalents move and become oriented. The time between the disintegration of the nuclear membrane and establishment of bivalents on the spindle is called prometaphase.

(b) Metaphase

At full metaphase (Pl.XXI Fig.60)(Pl.XXII Fig.61) polar view discloses 62 rod shaped elements. All of which are approximately of the same length. These can be seen clearly in side or lateral view of the spindle (Pl.XXII Fig.62) where the two homologous centromeres of each tetrad or bivalent lie on the longitudinal axis of the spindle on opposite sides of the equatorial plate (co-orientation). When equilibrium is reached the two centric regions are at equal distance from the spindle plate. Microphotograph 17 Platte XXIV also discloses 52 chromosomes.

(c) Anaphase

At first anaphase of the first meiotic division (Pl.XXII Fig.63) (Pl.XXIV MP 18) whole centromere moves in the direction of nearest pole so the centromeres drag after them the chromatids which are attached to them. No chromosome of lagging or precocious nature has been observed.

(d) Telophase

By first telophase the chromosomes get completely over crowded in way fused with eachother forming two chromatin clumps at the two poles (Pl.XXII Fig.64).

The secondary spermatocyte metaphase plate shows 52 rod shaped elements (Pl.XXII Fig.65). The size of the secondary
spermatocyte chromosomes is less than the primary spermatocyte chromosomes. The staining capacity of the chromosomes at this stage is also less than that of the chromosomes of first metaphase.

At second anaphase the division of the chromosomes takes place normally and daughter cells pass to the opposite poles. No succession or procession of any chromosome takes place, nor abnormal behaviour of any chromosome has been observed in anaphase.

During second meiotic telophase (Pl.XXII Fig.66) the chromosomes are seen only in the form of two chromatin clumps on each pole of the spindle.
(4) **Chromosome number, shape and size.**

*Cyprinus carpio* sp. *speculatia* LMK exhibits 104 chromosomes. All the chromosomes are rod shaped elements (*Pl. XXI Fig. 58*) as seen at the polar view of the spermatogonial metaphase. The size of the chromosomes varies from 3 to 4 micra. They do not seem to vary much in shape and size.

52 elements have been counted at metaphase first (*Pl. XXXI Fig. 60*) (*Pl. XXXIV Fig. 17*). All the chromosomes are spherical or small rod shaped. In meiotic metaphase second again 52 elements have been counted (*Pl. XXXII Fig. 65*). But the size of the secondary spermatocyte chromosomes as well as of piote is half of the size of chromosomes seen at first spermatocyte metaphase.
S. Labor dero (HECKEL)
5. *Labeo dero* (HECKEL).

(I) **Breeding season and testes:**

*Labeo dero* belongs to the family *Cyprinidae* subfamily *Cyprininae* (Pl. XXV). It is one of the fresh water fishes of Kashmir. The testes are paired lying on either side of the swim-bladder. The seasonal gonidial stages of *Labeo dero* are as follows:

(a) Stage I - August.
(b) Stage II - September - October.
(c) Diapause - November - February.
(d) Stage III - March - April.
(e) Stage IV - May.
(f) Stage V - June
(g) Stage VI - July

*Labeo dero* spawns in July. The collection of the material was done during the months March, April and May. As material collected during these months proved useful.
(3) Spermatogonial mitosis:

The study of the spermatogonial cells reveals that not all the nuclei in the cells of the same individual are of the same size. The earlier spermatogonial cells possess a large nucleolus about 6 to 7 micra in size with a big plasmosome in the centre (Pl.XXVI Fig.67). At the periphery of the nucleus small patches of granules are seen scattered. The nucleus also shows a fine network of chromatin. This type of resting nucleus does not undergo any visible change in mitosis. But the chromosomes are not distinguishable in interphase.

(a) Prophase

In early prophase the appearance of nucleus is quite distinct. There are no spaces betwenn the chromosomes in early prophase. The chromosomes undergo "Condensation" which is just a sort of coiling upon interphase chromosome threads. The spaces between the chromosomes become evident at opposite ends of nucleus which form the opposite poles. By gradual condensation and contraction the reticulum breaks in to thick threads which take the definite outline of the spermatogonial chromosomes. Thus the establishment of the poles is one of the first and last events of prophase. The nucleolus and nuclear membrane disappears which generally take place at the end of prophase. The disappearance of nuclear membrane coincides with the formation of spindle. Once the spindle is formed the chromosomes move to a region midway between the poles called equator of the spindle. The fibres of the spindle, however, are really fine tubules.
(b) **Metaphase**

In the polar view of spermatogonial metaphase 56 chromosomes are seen arranged radially. All the chromosomes are rod shaped telomites (Pl. XVI Fig. 68) (Pl. XVIII Fig. 19). The chromosomes of the complement vary from 2 to 4 micro in length.

(c) **Anaphase**

During which the chromosomes divide normally and move to the opposite poles. Termination of the anaphase movement occurs when the chromosomes form densely packed groups at the two poles each group is thus a telephasic nucleus.
(3) Spermatogonial meiosis

There is a considerable increase in the size of nucleus than that of mitosis during this growth period. All the nuclei measured at different stages show a very little size variation. The average nuclear size is about 5 to 6 micra. The resting nucleus appears quite homogeneous except a centrally placed nucleolus with few faintly stained and extremely fine chromatin threads (Pl.XXVI Fig.69) contrary to the resting stage in spermatogonial mitosis where peripheral chromatin bodies are conspicuous.

(a) Prophase

(i) Leptotene

Preleptotene is marked by the dissolution of plasmosome and the appearance of very fine reticulum of chromatin threads which spreads throughout the nucleus ( Pl.XXVI Fig.70). The chromosomes are usually long and slender in leptotene stage. All the chromosomes have polarized orientation with all their ends directed towards one small area on the side of the nucleus (Pl.XXVI Fig.71-72) so that a large bouquet is seen at one end of the nucleus.

(ii) Zygotene

The bouquet is considered as zygotene stage. The intermingling loops of chromatin threads are clearly visible in this bouquet ( Pl.XXVII Fig.73). Synapsis can not be definitely made out but the number of threads and their thickness gives an idea that they are paired. Following the bouquet is pachytene stage.

(iii) Pachytene

At pachytene there appears short and thick threads with
heterochromatic rounded ends (Pl.XXVII Fig.74). These threads are obviously bivalents and represent the haploid number, but the exact counting of the chromosomes is impossible. This stage is of longer duration than the other stages of prophase.

(iv) Diffuse stage

Pachytene is followed by diffuse stage (Pl.XXVII Fig.75) in which the bivalents lose their shape and staining capacity. Diplotene is found to be completely abolished.

(v) Diakinesis

The chromosomes reappear in the form of typical bivalents which are of various shapes with terminalized chiasmata, V's, rods and crosso (Pl.XXVII Fig.76). At late diakinesis the almost all the chromosomes come to rest just below the nuclear membrane. At late diakinesis the chromosomes are more contracted and deep staining bodies. The nuclear membrane disappears and a bipolar spindle is formed on which the bivalents move and become oriented. The time between disintegration of the nuclear membrane and establishment of the bivalents on the spindle is prometaphase.

(b) Metaphase

At full metaphase the bivalents lie in the longitudinal axis of the spindle on opposite sides of the equatorial plate. The two centric regions are equidistant from the spindle plate. The chromosomes by now have reached their maximum degree of condensation and the outline appears to be smooth.

In the polar view of the first spermatocyte metaphase (Pl.XXVII Fig.77-78) (Pl.XXIX MP.20) shows only the 27 elements.
The polar view shows only the distribution of the bivalents on the spindle plate and does not reveal the shape of the bivalents. These can be seen in side or lateral view of the spindle (Pl. XXVII Fig. 79). In the side view all the dumb-bell shaped bivalents lie with their longitudinal axis parallel to that of the spindle.

(e) Anaphase

At first anaphase (Pl. XXVIII Fig. 80)(Pl. XXIX Fig. 21) all the paired elements separate normally and travel to the opposite poles of the spindle. No chromosomes of lagging or precocious nature has been observed. The bivalents are dumb-bell shaped.

(d) Telophase

By first meiotic telophase the bivalents get completely fused with each other forming a big chromatin mass on each pole thus rendering their detailed study impossible (Pl. XXVIII Fig. 81).

The secondary spermatocyte metaphase plate reveals 27 rounded univalents (Pl. XXVIII Fig. 82). The staining capacity of the chromosomes at this stage is less than that of the first spermatocyte metaphase plate. The size of the chromosomes is also less than the first metaphase chromosomes. At the second anaphase the division of the chromosomes takes place normally and daughter chromosomes pass to the opposite poles. During telophase second only two chromatin clumps are seen at opposite poles (Pl. XXVIII Fig. 83). No abnormal behaviour of any chromosome has been observed during the whole meiotic cycle.
(4) Chromosome number, shape and size

The diploid number of Labeo dero (HECKEL) is 54 (PI.XXVI Fig. 68) (Pl. XXVII MP. 19). The chromosomes of the complement vary from 2 to 4 micro in size. Most of the chromosomes are rod shaped only few are dot or ovoid in shape.

The primary spermatocyte metaphase has 27 elements. All the chromosomes are spherical in shape (Pl. XXVII Fig. 77-78) (Pl. XXIX MB. 20). In secondary spermatocyte metaphase plates again 27 elements have been counted. The size of the secondary spermatocyte metaphase chromosomes is smaller than that of primary spermatocyte metaphase chromosomes.
6. Genus Cumachelus latius diplochilus (HISC.)
6. *Crossochilus latius diplochilus* (HECKEL)

(I) Breeding season and testes:

*Crossochilus latius diplochilus* (Pl. XXX) belongs to the family Cyprinidae. It is a small fish, the testes are small ribbon shaped lying on either side of the swim-bladder and extends up to the anterior end of the abdominal cavity. The breeding season of this fish has been studied under the following heads:

(a) Stage I — September - October.
(b) Stage II — November
(c) Diapause — December - January - February.
(d) Stage III — March - April.
(e) Stage IV — May.
(f) Stage V — June - July.
(g) Stage VI — August (spent)

*Crossochilus latius diplochilus* spawns in July so the collection of the material has been done during the months May, and June.
(2) Spermatogonial mitosis:

The study of spermatogonial cells reveals that the nucleus of early generations are larger in size than the later ones. The size of the nucleus varies from 9 to 10 micra. The nuclei of early generations contain a large plasmosome and few chromatin bodies scattered throughout the nucleus (Pl. XXXI Fig. 84). Where as the nucleus of the later generations has a small nucleolus. The nuclei of spermatogonial prophase exhibits nuclear framework which transforms into thick threads by gradual thickening and condensation.

(a) Prophase

The major problem of prophase is the establishment of poles for the forthcoming division. The other conspicuous events in the prophase are the disappearance of nucleolus and dissolution of nuclear membrane. The changes take place at the end of prophase. The poles are established by the separation of centres. As centres move apart a set of fibrous connections designated as the central spindle is observed. The chromosomes orientate themselves on the equator. Then the chromosomes begin to move in relation of the poles and mitosis is under way.

(b) Metaphase

The chromosomes arrange themselves in a radial manner on the metaphase plate. The size of the chromosomes varies from 1 to 2 micra. The counting of the many clear metaphase plates reveals the diploid number as 48 (Pl. XXXI Fig. 85)(Pl. XXXIII MP22). With 12 metacentric chromosomes having median or
submedian centromere. The other 36 elements are acrocentric.

On account of extremely small size of the chromosomes we cannot designate the various chromosomes as V's. The metacentric chromosomes lie scattered among the other acrocentrics without any definite pattern. The acrocentrics are rod shaped. Some are so small that they appear as dots.

(c) **Anaphase**

All the chromosomes divide synchronously at anaphase and move along the parallel axis of spindle with their pointed attached ends pole-words thus indicating their acrocentric nature.

(d) **Telophase**

At telophase only two chromatin clumps are seen on either side of the spindle. The karyokinetic figures of mitosis resemble those of *Schizothorax esocinus* therefore have not been figured.
(3) Spermatogonial meiosis:

After the completion of mitotic divisions, the resulting spermatocyte cells pass into the resting or interphase nucleus. During this stage no visible change takes place, and the chromosomes do not stain well. The nuclei of resting cells contain a plasmosome and clear nucleoplasm with few chromatin bodies (Pl. Xxi Fig. 86)(Pl. XXXIV MP. 23).

(a) Prophase

(1) Leptotene

Gradually the plasmosome disintegrates and a very fine reticulum of chromatin threads appears in the whole nucleus. This stage corresponds to early leptotene (Pl. XXXI Fig. 87). The leptotene initiates the meiosis. The chromosomes are longer and thinner than in mitosis. Leptotene is usually of very short duration. In latter stages the reticular arrangement disorganized and individual threads are distinguishable at late leptotene (Pl. XXXI Fig. 88). The free ends of the leptotene chromosomes are attracted to one side of the nucleus with the body of the chromosomes extending in a loop into the interior of the nucleus thus they form a bouquet like structure at one end of nucleus (Pl. XXXI Fig. 89).

(ii) Zygotene

The threads of bouquet are thicker than the leptotene chromosomes and also halved in number. Thus it can be concluded that this stage corresponds to Zygotene as actual pairing (Pl. XXXII Fig. 90) could not be observed on account of extreme thinness of chromosomes. The bouquet formation and synapsis at
only one pole of the nucleus has been explained due to the
centrosome lying at the end. The reason for synapsis before
division of centrosome and formation of spindle, is the fact
that if the spindle where formed before synapsis the chromosomes
( which have to be halved) could not freely pair up when once
attached to the spindle fibres. Bouquet stage possibly also
helps in exchange of chromosomal material ( crossing over) which
' could not be observed due to the small size of the nucleus.

(iii) Pachytene

The bouquet disorganises and nucleus enters into
pachytene stage. This stage is of longer duration. The threads
are thicker and shorter at this stage than the preceding ones
and bear heteropycnotic rounded ends ( Pl.XXXII Fig.91). The
exact number of the threads is very difficult to determine
owing to their overcrowding. Pachytene is followed by diffuse
stage.

(iv) Diffuse stage

During diffuse stage irregular faintly staining
bodies are observed scattered uniformly in the nucleus
( Pl.XXXII Fig.92). It seems that diplotene passes away in
diffuse stage.

(v) Diakinesis

Diakinesis approaches with the appearance of typical
tetrad like figures in the nucleus. These tetrads are of various
shapes viz. V's, dumb-bells and spheres ( Pl. XXXII Fig.93). On
account of continuous contraction, they become more compact and
and get scattered throughout the nucleus. The nuclear membrane gradually dissolves with the formation of spindle. A bipolar spindle is formed on to which the bivalents move and become oriented. The homologous centromeres of each bivalent lie in the longitudinal axis of the spindle on opposite sides of equatorial plate.

(b) Metaphase

Well preserved metaphase plate in polar view reveals 24 elements. The chromosomes of the complement are of variable shape and size, six of which are larger and metacentric (Pl. XXXII Fig. 94-95)(Pl. XXXIV MP. 24). These occur regularly in all metaphase plates. Remaining are small dots with circular outline. At the side view of metaphase the tetrads on the equator are dumb-bell shaped thus revealing their bivalent nature (Pl. XXXII Fig. 96).

(c) Anaphase

During first spermatocyte anaphase all the bivalents get separated synchronously in to two equal halves(Pl. XXXIII Fig. 97)(Pl. XXXV MP. 25) and move towards poles.

(d) Telophase

After anaphase one, telophase one starts during which the chromosomes form two clumps on each pole. The chromosomes get over-crowded (Pl. XXXIII Fig. 98)(Pl. XXXV MP. 26) with the result exact counting is rendered impossible. After first spermatocyte telophase a long interkinesis intervenes where the nuclei remain almost empty with a few diffuse chromatin bodies.

In the secondary spermatocyte metaphase at polar view
(Pl. XXXIII Fig. 99) again 24 univalents have been counted. Metacentrics appear slightly elongated which can be easily distinguished from acrocentrics. The size of the plate as well as the size of individual chromosome is about half of the bivalents seen at first metaphase.

At second anaphase all the chromosomes divide synchronously and move to opposite poles. These chromosomes differ from first anaphase chromosomes in being small rounded bodies as latter are thicker. Second telophases are seen only in the form of two big chromatin clumps lying on the opposite poles of spindle (Pl. XXXIII Fig. 100) hence no details of the chromosomes has been observed.
(4) **Chromosome number, shape and size:**

The diploid number of the *Specie Calatius diplochilus* (HECKEL) is 48. Out of which 12 are metacentrics having median or submedian centromere, the other 36 are acrocentric (Pl. XXXI Fig. 85)(Pl. XXXIII MP. 22). We can not categorise them as V's as they are extremely small. The acrocentric are typical rods some of which are so small that they appear mere dots.

The primary spermatocyte metaphase has 24 elements in it, 6 are larger than the others (Pl.XXXII Fig.94-95)(Pl.XXXIV MP. 24). In secondary spermatocyte metaphase plates the metacentrics appear slightly elongated and can be easily distinguished from acrocentrics (Pl.XXXIII Fig.99).

The differentiation between acrocentric and metacentric chromosomes in the stage: (1) Metaphase of mitosis;(2)First meiotic metaphase;(3)Second meiotic metaphase has been shown in this fish for the first time. The importance of this lies in the fact that in most Teleosts acrocentric and metacentric can not be distinguished on account of their small size.
7. Salmo trutta fario  LINN
7. Salmo trutta fario LINN

(i) Breeding season and testes:

Salmo trutta fario belongs to the family Salmonidae and sub-family Salmoineae (Pl.XXVI). In this salmonid the pair of testes lies on either side of the swim-bladder. The testes are white large structures running throughout the entire length of the abdominal cavity. The breeding season of this fish has been studied under the following heads:

(a) Stage I - March.
(b) Stage II - April - May.
(c) Stage III - June - July.
(d) Stage IV - August - September.
(e) Stage V - October - November.
(f) Stage VI - December - January.

Salmo trutta fario spawns in October and November. The collection of material was done during the months September and October.
(2) **Spermatogonial mitosis:**

The study of the spermatogonial cells reveals that their early generations possess a large nucleus 10 to 12 micra. While the size of the later generations varies from 7 to 8 micra. The early spermatogonial cells possess a large plasmosome (nucleolus) (Pl. XIX-VII Fig. IOI) and a fine network of chromatin. In the entire nucleus small patches of granules interconnected by strands or fibres of Gomori's stained chromatin are seen. The nucleus of the spermatogonial cell has got a well defined nuclear membrane. During the resting stage of the nucleus the interphase, the nuclear division does not take place. The nuclear structures of the resting nucleus do not take up the stain.

(a) **Prophase**

The onset of prophase is marked by the appearance of fine reticulum which spreads throughout the nucleus. The beginning of the prophase is not very well defined but it terminates when the chromosomes begin their movement towards their metaphase position. At prophase there is the condensation of chromosomes which undergo coiling upon greatly extended interphase threads. The condensation helps in the movement of chromosomes without any risk of entanglement. By the gradual thickening and contraction the reticulum breaks and the thick threads are formed which finally assume the definitive outline of spermatogonial chromosomes. After the thick threads are formed, poles are established for the forthcoming division. During the prophase in *Salmo trutta fario* the nucleolus
diminishes in size and finally disappears towards the end of the stage. The nuclear membrane also disappears in late prophase and metaphase begins. With the disappearance of nuclear membrane a new structure appears in the cytoplasm, the spindle, once the spindle is formed the chromosomes, which lie scattered throughout the nuclear region, move to the central, thickest region of this spindle and come to lie in a plane across the equator. By the time the chromosomes are arranged around the equator of the spindle they have reached their shortest and thickest form and are darkly stained.

(b) Metaphase

In the polar view of spermatogonial metaphase (Pl. XXXVII Fig. 102) (Pl. XL MP. 27) 80 chromosomes are seen radially arranged. The diploid complex is composed of various kinds of telomitic chromosomes. At least 28 or more in number are metacentric and the remaining ones are telomites. In the polar view the bivalents assume rounded or somewhat elongated/recurved metacentric chromosomes. The chromosomes of the complement vary from 2 to 4 micra in length.

(c) Anaphase

At anaphase all the chromosomes divide normally and move to the opposite poles with their ends pole wards, thus manifesting their acrocentric nature.

(d) Telophase

Termination of anaphase movement occurs when the chromosomes form densely packed telophasic mass on opposite poles of the spindle.
(3) **Spermatogonial meiosis**

There is a considerable increase in the size of the nucleus during the growth period. All the nuclei measured at different stages i.e. from resting nucleus to diakinesis show a little size variation. The average nuclear size is 5 to 6 micra. The resting nucleus appears quite homogeneous except for a centrally placed spherical nucleolus with few faint and extremely fine chromatin threads and some chromatin masses (Pl.XXXVII Fig.103 A)(Pl.XL MP.28).

(a) **Prophase**

(1) **Leptotene**

Preleptotene stage is marked by the dissolution of the plasmosome and appearance of very fine reticulum of chromatin threads which spread throughout nucleus (Pl.XXXVIII Fig.103 B). Leptotene is usually passed through in a very short time. The chromosomes in leptotene stages are very elongated and slender. The threads continue to undergo contraction and they have a polarized orientation with all their ends directed towards one small area on one side of the nucleus (Pl.XXXVIII Fig.104-105). It is the characteristic feature of the ends or telomeres.

(ii) **Zygotene**

This polarization of free ends results in the formation of a light bouquet. In which the loops of chromatin threads are visible (Pl.XXXVIII Fig.106). This stage is concluded as the zygotene although synapsis could not be definitely made out. But the number of threads and their longitudinal thickness gives an idea that these are paired.
(iii) Pachytene

Following the bouquet, at pachytene (Pl.XXXVIII Fig.107) there appear short and thick threads with heterochromatic rounded ends. The threads are obviously bivalents and represent haploid number but their counting is not possible. This stage is of longer duration than the other prophase stages. Diplotene is completely abolished, even diffuse stage could not be observed in this fish.

(iv) Diakinesis

At diakinesis (Pl.XXXVIII Fig.108) the chromosomes reappear in the form of typical bivalents. The chromosomes become shorter and contracted deep staining bodies. The bivalents are of various shapes viz V's, rods and crosses. At diakinesis only few chromosomes migrate to the periphery of the nucleus where they lie close to nuclear membrane and widely separated from one another. With the dissolution of nuclear membrane the diakinesis stage ends and the compact bivalents next move on to the spindle at metaphase.

After the nuclear membrane has disappeared, a bipolar spindle is formed on to which the bivalents move and become oriented.

(b) Metaphase

At full metaphase the bivalents lie in the longitudinal axis of spindle on opposite sides of equatorial plate. In the polar views of first spermatocyte metaphases 40 elements have been counted (Pl.XXXIX Fig.IIO-III)(Pl.XLI MP 29)
In the side view of spindle all the chromosomes are seen as elliptical structures thus revealing their bivalent nature. The arrangement of the chromosomes is radial. In the side view all the elliptical bivalents lie with their longitudinal axis parallel to that of the spindle (PI.XXXIX Fig.II2)(Pl.XLII MP.31).

There is an essential difference between meiotic and mitotic metaphase at the first meiotic metaphase each bivalent possess two independent and undivided centromeres, which are arranged above and below the equator and not parallel to it as in mitosis. The different pairs of homologue chromosomes align themselves on the equator of the cell in an independent fashion. These alignments occur with equal frequency and indicate that non homologous chromosomes are independent of each other in their arrangement during metaphase of the first meiotic division. This is significant difference from metaphase of mitosis.

(c) Anaphase

At first anaphase all the paired elements separate normally and travel to the opposite poles of the spindle (Pl.XXXIX Fig.II3)(Pl.XLII MP.32). No chromosome of lagging or precocious nature has been observed. The univalents are elliptical in shape.

(d) Telophase

By telophase first the chromosomes get completely fused with each other forming big chromatin mass on each pole thus rendering their study impossible.
The secondary spermatocyte metaphase plates show 40 rounded elements (Pl. XXXIX Fig. II4). The staining capacity of the chromosomes at this stage is less than that of the chromosomes of the first metaphase and the size of the chromosomes as well as that of the plate is almost half of the size seen in first metaphase. At second anaphase the division of chromosomes takes place normally and daughter chromosomes pass to the opposite poles. No abnormal behaviour of any chromosome has been observed during the whole meiotic cycle. At second telophase two chromatin clumps have been observed on the opposite poles of the spindle (Pl. XXXIX Fig. II5)
(4) **Chromosome number, shape and size**

A thorough study of the spermatogonial mitosis (Pl. XXVII Fig. 102) reveals that the diploid complex of *Salmo trutta fario* is composed of 80 chromosomes. More than 28 chromosomes are atelomitic and rest are telomites. The size of the chromosomes varies from 2 to 4 micra. Spermatogonial meiosis first (Pl. XXXIX Fig. 110-III) and meiosis second (Pl. XXXIX Fig. 114) also reveals 40 chromosomes. In the polar view of the primary and secondary spermatocyte metaphase plates the chromosomes assume circular or somewhat elongated outline.
8. Salvelinus fontenalis [LINN]
8. Salvelinus fontinalis LINN

(1) Breeding season and testes:
In this salmonid (Pl.XLIII) the testes are huge pinkish structures lying ventro-laterally to the air bladder. During the breeding season the testes become huge whitish structures due to the presence of abundant spermatozoa. The collection of the material was done during the months September-October and November. The stage is observed during the breeding season resemble those of Salmo trutta fario.
(2) Spermatogonial mitosis:

The size of the early germ cells is larger than the size of the germ cells of the later generations. The size of the early generations varies from 8 to 10 micra whereas in latter generations it varies from 5 to 6 micra. The nuclei of early generations have got a well-defined nuclear membrane and they contain a large pleasmosome on one side of nucleus (Pl. XLIV Fig. 116). Small patches of chromatin are seen scattered throughout the nucleus and some are arranged in a row on the inner side of nuclear membrane. The nuclei of later generations have got a small nucleolus lying in the homogeneous nuclear sap. During the resting stage the nucleus does not show any activity.

Nuclear division takes place by mitosis, the essential steps of which are: (a) The duplication of the chromosome substance; (b) the condensation of the chromosomes, accompanied by the "disappearance" of the nucleolus; (c) movement of the sister chromosomes to the opposite poles; (d) division of the cytoplasm accompanied by the constitution of two complete nuclei, each with a full set of chromosomes and a nucleolus. The daughter cells are equivalent in all respects.

(a) Prophase

The onset of prophase is marked by the appearance of long fine chromatin threads. During prophase the "condensation" of the chromosomes takes place and extreme coiling and thickening on greatly extended interphase chromosome threads has been observed.
The poles are established at the end of prophase for the fourth coming division. In late prophase the nucleolus disappears and nuclear membrane also disintegrates and metaphase begins. The disappearance of the nuclear membrane coincide with the the formation of spindle. The fibres of the spindle however are really fine tubules. Once the spindle is formed the chromosomes move through the cytoplasm to it, and become fastened by their centromeres to a region midway between the poles at the equator of the spindle.

(b) Metaphase

From the polar view of metaphase (Pl. XLIV Fig. 117) 100 chromosomes are seen arranged in a radial manner. All the chromosomes are almost of the same shape. The size of the chromosomes in early spermatogonial metaphase plates varies from 1 to 2 micra. The diploid complement counted on many metaphase plates is 100. In the later generations the chromosomes do not have any obvious morphological distinction in size and are smaller showing thereby condensation increases with successive divisions.

(c) Anaphase

Later pulling and pushing movements comprise anaphase. At anaphase all the chromosomes divide normally and move along the parallel axis of the spindle. Termination of the anaphase movement occurs when the chromosomes form a densely packed telophasic nuclei at the two poles. (Various stages of spermatogonial mitosis have not been drawn as they resemble those of Sphizothorax esocinus.)
(3) Spermatogonial meiosis:

After the completion of the mitotic divisions the resulting spermatocyte cell passes into resting phase (Pl.XLV Fig.123) (Pl.XLVIII MP.33).

(a) Prophase

(i) Leptotene

The onset of meiotic prophase is marked by an increase in nuclear volume and followed by modifications in the nuclear structure. The first steps in the transition from premeiotic interphase to meiotic prophase occurs in the proleptotene stage. With the disintegration of the plasmosome a very fine reticulum of chromatin threads appears in the whole nucleus. This stage corresponds to early leptotene (Pl.XLIV Fig.II8). In later stages the reticular arrangement of threads get disorganised and individual threads are distinguishable at late leptotene (Pl.XLIV Fig.II9). The chromosomes present in the diploid number, are thinner and longer than in mitosis.

(ii) Zygotene

The free ends of the leptotene chromosomes are attracted to one side of the nucleus nearest the centrosome, with the body of chromosome extending in a loop into the interior of the nucleus (Pl.XLV Fig.I20). Finally the polarization gets complete resulting in the formation of light bouquet (Pl.XLV Fig.I21) as the threads of the bouquet are considerably thicker than the leptotene threads and are also reduced in number. It is reasonably concluded that this stage is zygotene, although the actual pairing could not be observed owing to the extreme thinness of threads. The bouquet disorganises and
the nucleus enters pachytene.

(iii) Pachytene

Chromosomes unravel at this stage and scatter in the whole nucleus. The threads are thicker and shorter at this stage than the preceding ones and bear heteropycnotic rounded ends (Pl.XLV Fig.122).

It has not been possible to trace out the diffuse stage in *Salvelinus fontinalis* so it cannot be said as to how the diplotene stage occurs in case of Salmonidae. As the same stage has not been even located in *Salmo trutta fario*.

(iv) Diakinesis

Diakinesis approaches with the appearance of typical tetrad like figures in the nucleus. These tetrads are of various shapes i.e. V's, rods, spheres and dumb-bells (Pl.XLVI Fig.124). On account of continuous contraction they become more compact and darkly staining and get arranged just below the nuclear membrane. With the commencement of prometaphase stage the bivalents begin to migrate towards the centre of the nucleus and acquire smooth outline. With the dissolution of the nuclear membrane the daikinetic stage is ended and the compact bivalents will next move on to the spindle at metaphase.

After the disappearance of the nuclear membrane, a bipolar spindle is formed on to which the bivalents move and become oriented. The time between the disintegration of the nuclear membrane and the establishment of bivalents on the spindle is prometaphase. The chromosomes come on the equator of the spindle and in the polar view they are found arranged radially.
(b) **Metaphase**

In well preserved metaphase plates 50 chromosomes have been counted without any doubt (Pl.XLVI Fig.125-126)(Pl.XLIX Mp.35-36). The chromosomes of the complement are of variable sizes. In some of the early metaphase plates, a few tetrads appear - V shaped such shapes have been observed in some of the metaphase plates which are possibly owing to the differentiation in degree of contraction of individual chromosomes. In the side view of the spindle the bivalents are seen in the form of dumb-bells (Pl.XLVII Fig.127)(Pl.XLVIII MP.34).

4c) **Anaphase**

During first spermatocyte anaphase all the bivalents get separated synchronously into two equal halves and travel to the opposite poles (Pl.XLVII Fig.128)(Pl.L MP.37). The univalents are elliptical in shape.

(d) **Telophase**

By first telophase the chromosome get completely fused with each other and form large chromatin mass rendering their detailed study impossible (Pl.XLVII Fig.129).

After first spermatocyte telophase a long interkinesis intervenes where the nuclei remain almost empty with few diffuse chromatin bodies. In the secondary spermatocyte metaphase at polar view (Pl.XLVII Fig.130) again 50 spherical univalents have been counted. The size of the metaphase plate as well as that of individual chromosome is almost half of the plate as well as the bivalents seen in the first metaphase. At second anaphase all the chromosomes divide normally and move to the opposite poles. Second telophases are seen only in the form of two big chromatin slumps lying on the opposite poles of the spindle (Pl.XLVII Fig.131) hence no details can be given of this stage.
(4) Chromosome number, shape and size

A thorough study of the spermatogonial mitosis and meiosis reveals that the diploid complement of *Salvelinus fontinalis* is composed of 100 acrocentric elements (Pl.XLIV Fig.II7). All the chromosomes are spherical in shape. The size of the chromosomes varies from 1 to 2 micra.

Metaphase also reveals 50 chromosomes (Pl.XLVI Fig.I25) (Pl.XLIX MP.35-36) all of which are somewhat spherical in shape. Metaphase second (Pl.XLVII Fig.I30) also gives the same result that 50 chromosomes forms the haploid number of *Salvelinus fontinalis*. 
9. Nemachilus kashmiriensis  HORA
9. *Nemachilus kashmirensis* HORA

(I) Breeding season and testes

*Nemachilus kashmirensis* (Pl. LI) belongs to the family Cobitidae. It is a small fish, the pair of testes extend up to the anterior end of the abdominal cavity. The testes are thin strap shaped. The breeding season of this fish has been studied under the following heads:

(a) Stage I - June - July.
(b) Stage II - August - September.
(c) Stage III - October - November.
(d) Dispause - (December, January and February)
(e) Stage IV - March.
(f) Stage V - April.
(g) Stage VI - May (spent).

*Nemachilus kashmirensis* spawns in April as the collection of the material was done during the month of March.
(2) Spermatogonial mitosis

The size of the germ cells in early generations is somewhat smaller than the other species studied by the author. The early spermatogonial cells vary in size from 5 to 6 micra. The nuclei of early generations contain a large plasmosome and chromatin network (Pl.LII Fig.132). While as the nucleus of the latter generations contains a small nucleolus lying in the homogeneous nuclear sap. Small patches of chromatin are scattered in the net work of resting nucleus.

(a) Prophase

The karyokinetic figures of mitosis resemble those of Schizoneura exocinus therefore have not been figured. The prophase is marked by the appearance of fine reticulum, spreads throughout the nucleus. The condensation of the chromosomes takes place which is viewed as the imposition of the several orders of coiling upon greatly extended interphase chromosome threads. Second major problem of prophase is the establishment of poles for the forthcoming division. By gradual contraction and thickening the reticulum breaks in to thick threads which finally assume the definite out line of spermatogonial chromosomes. Gradually at the end of the prophase the nucleolus and the nuclear membrane disappear. By the end of the prophase the mitotic apparatus is formed. The chromosomes orient themselves on the equator of the spindle.

(b) Metaphase

In the polar view of the spermatogonial metaphase (Pl.LII Fig.133) Pl.LV MP.39) 52 rod shaped chromosomes have been
counted without any doubt. The chromosomes of the complement vary from 2 to 3 micra in else.

(c) **Anaphase**

At anaphase all the chromosomes divide normally and move to the opposite poles directing their ends pole wards thus indicating their acrocentric nature.
(3) Spermatogonial Meiosis

There is a considerable increase in the size of the nucleus during the growth period. All the nuclei measured at different stages, that is, from resting nucleus to diakinesis show a very little size variation. The average nuclear size varies from 4 to 5 micra.

The resting nucleus appears (Pl.LII Fig.134)(Pl.LIV MP 38) quite homogeneous except for a centerally placed ablong nucleolus with few faint and extremely fine chromatin threads and with the disintegration of the nucleolus a very fine reticulum of chromatin threads appears in the whole nucleus.

(a) Prophase

(i) Leptotene

The appearance of reticular net work in the nucleus initiates the formation of early leptotene (Pl.LII Fig.135). In latter stages the reticular arrangement of threads gets disorganized and individual threads are distinguishable at late leptotene (Pl.LII Fig.136). The chromosomes present in diploid number are thinner and longer than mitosis. The chromosomes start to polarize at a small area of nuclear membrane (Pl.LII Fig.137).

(ii) Zygotene

The body of the leptotene chromosomes extends in to a loop in to the interior of nucleus. Finally the polarisation gets complete resulting in the formation of a Bouquet (Pl.LIII Fig.138). The threads of the bouquet are considerably thicker than the leptotene threads and also reduced in number. So this stage is concluded as zygotene. Although actual pairing could not be observed.
(iii) **Pachytene**

The bouquet disorganises and nucleus enters into pachytene stage (Pl. LIII Fig. 139) by unravelling of chromosome threads which scatter in the whole nucleus. The threads are thicker and shorter at this stage than the proceeding ones and bear heterophycomotic knobbed ends. The exact number of the chromosomes is very difficult to determine owing to their overcrowding at this stage.

(iv) **Diffuse stage**

Pachytene is followed by diffuse stage where few faintly staining chromatin bodies are scattered throughout the nucleus (Pl. LIII Fig. 140). Such nuclei of diffuse condition are seen lying in the same lobes of the testes where its adjacent stages are seen i.e pachytene, diakinesis and sometimes first spermatocyte metaphase.

So it can be said that deplotene of normal meiosis passes away in diffuse condition.

(v) **Diakinesis**

Diakinesis approaches with the appearance of atypical tetrad like figures in the nucleus. These tetrads are of various shapes (Pl. LIII Fig. 141). The bivalents become considerably contracted. Finally the bivalents acquire smooth outlines. The nuclear membrane dissolves gradually with the formation of spindle. The breakdown of nuclear membrane and appearance of the spindle terminates prophase and initiates first metaphase of meiosis.

At first spermatocyte metaphase the bivalents orient themselves on the spindle and lie on either side of equatorial plate which is equidistant on either side from plate.
(b) **Metaphase**

In well preserved metaphase plates the chromosomes are arranged radially. In these chromosome complements 26 elements have been counted without any doubt (Pl. LIII Fig. 142-143) (Pl. LV MP. 40) (Pl. LVI MP. 41). Polar view of the metaphase one discloses only the distribution of the bivalents on the spindle plate. The shape of the bivalents can only be seen in the lateral view or the side views of the spindle. They are dumb-bell shaped this revealing their bivalent nature.

(c) **Anaphase**

During first spermatocyte anaphase all the bivalents get separated synchronously into two equal halves (Pl. LIV Fig. 145) (Pl. LVI MP. 42). Anaphase is of very short duration and chromosomes usually get clumped due to overcrowding. After first spermatocyte telophase (Plate. LIV Fig. 146) a long interkinesis intervenes where nucleus remains almost empty with a few diffuse chromatin bodies.

In the secondary spermatocyte metaphases of polar view (Pl. LIV Fig. 147) again 26 spherical univalents have been counted. The size of the chromosomes and that of plate is almost half of the bivalents seen at first spermatocyte metaphase. At second anaphase all the chromosomes divide synchronously and move to the opposite poles. These chromosomes differ from first anaphase chromosomes in being smaller rounded bodies whereas the later are spherical and thick. Second telophase (Pl. LIV Fig. 148) is seen only in the two big chromatin clumps on opposite poles of spindle. Hence no details can be given of this stage.
(4) Chromosome number, shape and size

*Nomachilus kashmirensis* exhibits 52 (Pl. LII Fig. 133) (Pl. LV MP. 39) acrocentric chromosomes at the polar view of the spermatogonial mitotic metaphase. They do not seem to vary much in shape and size. The size varies from 1 to 2 micra.

At the both meiotic metaphases 26 rounded elements (Pl. LIII Fig. 142) (Pl. LV MP. 40) (Pl. LVI MP. 41) can be counted. The shape of the chromosomes is somewhat spherical.
10. Puntius conchonius HAM. BUCH
10. *Puntius conchonius* HAM. BUCH

(I) **Breeding season and testes**

*Puntius conchonius* (PL. LI) belongs to the family Cyprinidae. It is a small fish. The testes are small thin strap shaped lying in the abdominal cavity. The breeding season of this fish has been studied under the following heads:

(a) **Stage I** - July to August.
(b) **Stage II** - September to October.
(c) **Stage III** - November to December.
(d) **Diaspase** - January to February.
(e) **Stage IV** - March.
(f) **Stage V** - April.
(g) **Stage VI** - May (spawn)

The fish spawns in May so the collection of the material was done during March and April.
(8) Spermatogonial mitosis

In spermatogonial mitosis karyokinetic figures are almost the same as in other fishes therefore have not been figured. Study of the spermatogonial cells reveals that their early generations possess a large nucleus about 10 to 12 micra in size. In the resting nucleus very little definable structures except a large plasmosome and a fine net work of chromatin in which small patches of chromatin are attached are seen (Pl.LVII Fig.149). The chromosomes therefore are not individually distinguishable in interphase. The resting nuclei do not undergo any visible change involved in mitosis. They remain unaltered for long periods of time and they do not obviously change their shape or appearance.

(a) Prophase

The resting nucleus enters prophase when the chromosomes become distinctly visible as long thin threads. The increasing visibility of the chromosomes is caused by the gradual thickening and condensation. The chromosomes become shorter and thicker. This process continues throughout the prophase. During the prophase the nucleoli which are initially prominent, now diminish in size towards the end of stage and finally disappear. The nuclear membrane also disintegrates in late prophase and metaphase begins.

The disappearance of the nuclear membrane coincides with the appearance of the bipolar spindle. The fibres of the spindle are very fine tubules. Once the spindle is formed, the chromosomes move through the cytoplasm to it, and orient between the two poles on the equator of the spindle. In the side view of the spindle the
chromosomes are seen as dumb-bells.

(b) Metaphase

Polar view of the metaphase plates reveals 56 chromosomes. The number 56 constitutes the diploid set of chromosomes. The chromosomes of the complement vary from 1 to 2 micra in length (Pl. LVII Fig. 150) (Pl. LIX MP 42). Most of the chromosomes are dot shaped and only few are rod shaped. They are radially seen arranged on the metaphase plates.

(c) Anaphase

Anaphase follows metaphase in the mitotic cycle. All the chromosomes divide normally and move apart from each other and initiate slow movement that takes the sister chromosomes to opposite poles. Termination of the anaphase movement occurs when the chromosomes form a densely packed group at two poles called telophase.
Spermatogenial meiosis

There is a considerable increase in the size of the nucleus during the growth period. All the nuclei measured from resting stage up to the diakinesis show little size variation. The average size of the nucleus is 3 to 4 micra. The resting nucleus appears quite homogeneous except for a centrally placed nucleolus and few faintly stained chromatin threads and masses scattered throughout the nucleus (Pl.LVII Fig.161).

(a) Prophase

(i) Leptotene

The prophase of meiosis falls into several more or less distinct stages. When the chromosomes first appear they resemble those of early prophase of mitosis in being long thin threads. But in meiosis they appear as single threads. Preleptotene is marked by the dissolution of plasmosome and the appearance of very fine reticulum of chromatin threads which spread throughout the nucleus as broken pieces (Pl.LVII Fig.158). The chromosomes at early leptotene are at their maximum extension. But by late leptotene the chromosomes of uniform diameter is thrown into large number of coils of small size, which continue to undergo contraction and become completely polarized (Pl.LVII Fig.153-154). So that the chromosomes may be clumped in a dense tangle of threads to one side of the nucleus called the bouquet stage.

(ii) Zygotene

The bouquet formation indicates an interaction between chromosome ends. In this bouquet (Pl.LVIII Fig.156) loops of chromatin threads are easily visible. Synapsis can not be clearly made out but the number of threads and their size gives an idea that these are paired.
(iii) Pachytene

Following the bouquet at pachytene thick and short threads with heterophycnotic knobbed ends are visible (Pl. LVIII Fig. 156). The threads are obviously bivalents and represent the haploid number but their counting is not possible. This stage lasts for a longer time in contrast to other prophase stages. Diplotene is completely abolished only diffuse stage could be observed (Pl. LVIII Fig. 157).

(iv) Diakinesis

At diakinesis the chromosomes become shorter and at late diakinesis the chromosomes are contracted, deep staining bodies. The chromosomes acquire various shapes viz. spheres, dumb-bells etc. On account of continuous contraction they become more compact. They are scattered throughout the nucleus. The breakdown of the nuclear membrane and the appearance of the spindle terminate prophase and initiate the first metaphase of meiosis. A bipolar spindle is formed on to which the bivalents move and become oriented. At full metaphase the two centroomers of each bivalent lie in the longitudinal axis of spindle on opposite side of the equatorial plate.

(b) Metaphase

Polar view of the metaphase one reveals that the chromosomes are arranged radially but we can not see the shape of the bivalents. These can be seen clearly in the side view of the spindle (Pl. LVIII Fig. 160) which discloses that they are dumb-bell shaped. In these chromosome complements 26 elements have been counted without any doubt (Pl. LVIII Fig. 158-159)(Pl. LX MP. 43). The chromosomes
of the complement are spherical and ovoid shaped.

(c) Anaphase

Separation of each tetrad into two dyad chromosomes takes place at anaphase one as the two co-oriented centric regions begin moving to opposite poles (Pl. LI X Fig. I 6 1)(Pl. LX MP. 44). Anaphase is of very short duration and the chromosomes usually get clumped due to overcrowding with the effect that their exact counting is rendered impossible. After first spermatocyte telophase (Pl. LXI Fig. I 6 2) a long interkinesis intervenes where the nuclei remain almost empty with a few diffuse chromatin bodies.

In the secondary spermatocyte metaphases of polar view (Pl. LI X Fig. I 6 3) again 28 dots shaped univalents have been counted. The size of the spermatogenousial chromosomes as well as that of metaphase plate is almost half of the size the chromosomes and metaphase plate at first metaphase. At anaphase all the chromosomes divide normally and move to the opposite poles (Pl. LI X Fig. I 6 4). The chromosomes differ from first anaphase chromosomes in being smaller and rounded bodies. Where as the later ones are thick and long. Second telophases are seen only in two big chromatin clumps lying on opposite poles of the spindle (Pl. LI X Fig. I 6 4). No abnormal behaviour of any chromosome has been observed in the whole cycle of meiosis and mitosis.
(4) **Chromosome number, shape and size**

The number 56 constitutes the diploid set of chromosomes (Pl. LVII Fig. 150)(Pl. LIX MP. 42) in *Puntius conchonius*. All the chromosomes are acrocentric rod shaped and dot shaped. The primary (Pl. LVIII Fig. 158)(Pl. LX MP. 45) as well the secondary metaphase plate reveal the number 28, being the haploid number. The size of the chromosomes varies from 1 to 2 micra.
II. Gambusia affinis Holbrooki (BIARD AND GIRARD)
II. *Gambusia affinis Holbrooki* (BIARD and GIRARD)

(I) **Breeding season and testes**

*Gambusia affinis Holbrooki* (Pl.II) belongs to the family **Pseudiliidae**. It is a very small fish. The pair of testes are very thin small ribbon shaped extending up to the anterior end of the abdominal cavity.

*Gambusia affinis* gives birth to the young ones twice a year so the following stages have been observed in the gonidal cycle:

I. (a) Stage I - October - November.
(b) Stage II - Diapause (December, January and February).
(c) Stage III - March - April.
(d) Stage IV - May.
(e) Stage V - June (spawn).

II. (a) Stage I and II - July.
(b) Stage III and IV - August.
(e) Stage V and spawn - September.

So the material was collected during April, May and August.
(2) Spermatogonial mitosis

The size of the germ cells is small. The early spermatogonial cells are larger than the later generations. The size of the nucleus of early generations varies from 4 to 6 micra. The nuclei of early generations contain a large plasmosome and masses of chromatin arranged near the periphery of the nucleus (Pl.LXI Fig.165). Whereas the nucleus of later generations has a small plasmosome lying in the homogeneous nuclear sap.

(a) Prophase

The nuclei of spermatogonial prophase exhibit long fine chromatin threads in the form of net work. Which become progressively thicker and condensed and found scattered in the whole nucleus. Poles are established for the coming division. Finally at the end of prophase the plasmosome and nuclear membrane disorganised and bipolar spindle is formed. The chromosomes orient themselves on the equatorial plate. The chromosomes vary in size from 1 to 2 micra.

(b) Metaphase

From the study of several clear complements, it has been found that the diploid number is 48 (Pl.LXI Fig.166)(Pl.LXIV MP 45). All the chromosomes are alike. Being in the form of rods and few U shaped. There is no regular pattern displayed by the chromosomes. In the latter generations chromosomes do not have obvious morphological distinction in size and are smaller showing there by that condensation increases with successive division. All the chromosomes divide synchronously at anaphase and move along the parallel axis of spindle towards the poles, reflecting their acrocentric nature.
(3) Spermatogonial meiosis

The general course of meiosis is typical. There is a considerable increase in the size of the nucleus during the growth period. All the nuclei measured at different stages show little size variation. The average nuclear size varies from 3 to 5 micra (Pl.LXI Fig.167).

The resting nucleus appears quite homogeneous except for a centrally placed spherical nucleolus and few chromatin bodies scattered throughout the nucleus (Pl.LXIV MP.46).

(a) Prophase

(1) Leptotene

The reticular net work of threads gets broken and nucleus shows distinguishable leptotene threads. In the early leptotene (Pl.LXI Fig.168) the chromosomes are very fine and thinner than mitosis. The leptotene chromosomes are attracted to the side of the nucleus (Pl.LXI Fig.169) with the body of the chromosome extending in a loop in to the interior of the nucleus, the bouquet stage.

(iii) Zygotene

The threads of the bouquet (Pl.LXII Fig.170-171) are considerably thicker than the leptotene threads and also reduced in number. This stage is concluded as zygotene. Although actual pairing could not be observed owing to the extreme thinness of chromosomes.

(iii) Pachytene

The bouquet disorganises and enters in to pachytene stage by unravelling of chromosomes (Pl.LXII Fig.172). The zygotene and pachytene reveal a gradual thickening of the paired elements which
which now take up a deep stain and have a positively heteropycnotic thickened ends. The exact number of chromosomes is difficult to determine owing to their overcrowding.

**-(iv) Diffuse stage**

The pachytene is followed by a diffuse stage (Pl.LXII Fig 173) during which the chromosomes lose their staining capacity so that ultimately nucleus reveals only one or two darkly staining bodies. The chromosomes as they appear out of the diffuse stage are of the form of typical bivalents.

**(v) Diakinesis**

By diakinesis (Pl.LXII Fig.174) the bivalents undergo linear contraction so that their size is reduced and they become thicker. Further contraction and thickening retain their original form.

At the same time they arrange themselves along the periphery of the nucleus immediately below the nuclear membrane. Just before the metaphase one the chromosomes take the rounded shape. The two homologues of the bivalents are seen to lie close together giving the appearance of a dumb-bell.

**(b) Metaphase**

At first spermatocyte metaphase all the chromosomes come on the equator of the spindle and in the polar view they reveal 24 elements, which lie in one plane (Pl.LXIII Fig.175-176) (Pl.LXV MP. 47). Most of the chromosomes are rod shaped and only few being spherical. The bivalents undergo co-orientation so that in the side view each is in the form of a dumb-bell lying with its long axis parallel to that of spindle (Pl.LXIII Fig.177).
(c) **Anaphase**

During first spermatocyte anaphase the elliptical chromosomes move to their respective poles without showing any abnormal behaviour (Pl. LXIII Fig. I78)(Pl. LXVI MP.48). Anaphase is of very short duration and chromosomes usually get clumped in telophase (Pl. LXIII Fig. I79) due to the over crowding with the result exact counting is impossible.

In the secondary spermatocyte metaphase of polar view (Pl. LXIV Fig. I80) again 24 rod shaped univalents have been counted. Which are however smaller in size than the chromosomes of the metaphase one. The chromosomes at this stage have a tendency to clump together to form darkly staining masses, each proceeding towards the respective poles of the spindle (Pl. LXIV Fig. I81-I82). The first division is reducational and second is equitorial for all the chromosomes. The second telophase shows only two chromatin clumps on each pole (Pl. LXIV Fig. I82)
(4) **Chromosome number, shape and size**

The study of the spermato gonial metaphase plate reveals that the diploid complement is composed of 48 acrocentric elements (Pl. LXI Fig. 166) (Pl. LXV MP. 48). The size of the chromosomes varies from 1 to 2 micra. Almost all the chromosomes are rod shaped.

First spermatocyte metaphase plate reveals 24 spherical univalents (Pl. LXV MP. 47) whereas the secondary spermatocyte metaphase plate also shows the 24 univalents (Pl. LXIV Fig. 180). All the chromosomes are rod shaped only few being rounded and are smaller in size than the first spermatocyte metaphase chromosomes.
12. Botia birdi  CHAUDHRY
II. Botia bardi

CHAUDHRY

(I) Breeding season and testes

*Botia bardi* belongs to the family Cobitidae (Pl. LI). The pair of testes are thin and small lying on either side of the swim-bladder. The breeding season of this fish has been studied under the following heads:

(a) Stage I - August - September.
(b) Stage II - October - November.
(c) Dispause - December - January - February.
(d) Stage III - March.
(e) Stage IV - April.
(f) Stage V - May.
(g) Stage VI - June.

This fish spawns in June so the collection of material was done during April and May.
(2) Spermatogonial mitosis

The study of the spermatogonial cells reveals that the size of the germ cells in small. It varies from 3 to 5 micra. The early spermatogonial cells are larger in size than the later ones. The nuclei of early generations contain a large nucleolus \textit{XXI} and chromatin (Pl.LXVII Fig.183). While the nucleus of the later generations has a small nucleolus lying in the homogeneous nuclear sap. The interphase nucleus, in addition to the nucleolus is uniformly filled with what appears to be fine granules, which are actually the irregularly disposed coiled chromonemata.

(a) Prophase

In the early prophase the appearance of nucleus is quite distinct. The chromonema spreads throughout the nucleus in the form of net work. There are no spaces between the chromosomes in early prophase, during the prophase the chromosomes pass through an optimal point of condensation. Finally the condensed and thickened chromonemata breaks in to chromosome threads which finally assume the regular shape of acrocentric threads. The nucleolus diminishes in size towards the end of prophase and disappears. The nuclear membrane also disintegrates in the late prophase, contraction of the chromosomes ceases and the metaphase begins.

The disappearance of nuclear membrane coincides with the appearance of new structure in the cytoplasm, the spindle. Once the spindle is formed the chromosomes move on to a region midway between the poles called equator of the spindle. The chromosomes vary in size ranging from 1 to 2 micra in early spermatogonial metaphase plates.
(b) **Metaphase**

From the study of the several clear complements, it has been found that diploid number is $6\ell$ (Pl.IX VII Fig.184)(Pl.LXX MP 49). All the chromosomes are rod shaped. In the later generations chromosomes do not have obvious morphological distinction in size and are smaller showing thereby condensation increases with successive divisions.

(c) **Anaphase**

Anaphase follows metaphase in the mitotic cycle. All the chromosomes divide normally and slow movements take the chromosomes to opposite poles. Termination of anaphase movement occurs when the chromosomes form a densely packed group at two poles.
(3) Spermatogonial meiosis

After the completion of mitotic division, the resulting spermatocyte cells pass into the resting phase. The nuclei of such cells contain a light stained plasmosome and clear nucleoplasm with few chromatin bodies (Pl.LXVII Fig.185) (Pl.LXX MP.50).

(a) Prophase

(i) Leptotene

The disintegration of plasmosomes causes the appearance of a fine reticulum of chromatin threads in the whole nucleus. This stage corresponds to early leptotene (Pl.LXVII Fig.186). The leptotene stage initiates the meiosis. The chromosome threads, present in the diploid number, are thinner and longer than in mitosis. In later stages the reticular arrangement of threads get disorganized and individual threads are distinguishable at leptotene. Where they start to polarize at a small area of nuclear membrane (Pl.LXVII Fig.187) with one end attached to nuclear membrane; thus they come to lie parallel to each other (Pl.LXVII Fig.188).

(ii) Zygotene

Later on, during the zygotene, the other end of the threads also bend down and touch the same area of nuclear membrane, thus giving rise to loop like structures (Pl.LXVIII Fig.189). Late zygotene is characterized by the presence of a compact bouquet; now they look thicker and smaller, showing that pairing has taken place. Although the actual pairing could not be observed owing to the extreme thinness of the chromosomes.

(iii) Pachytene

Pachytene is characterized by the disorganization of the
bouquet and the spreading out at random of all the threads in the entire nucleus. During this stage the chromosome threads are thicker and shorter than the proceeding ones and bear rounded ends (Pl. LXVIII Fig. I90). No heteropycnosis of entire chromosome has been observed in any of the fishes during these prophase stages. The exact number of threads is difficult to determine owing to the overcrowding.

(iv) Diffuse stage

The pachytene is followed by diffuse stage, during which the chromosome lose their staining capacity, so that ultimately the nucleus reveals only a number of faintly stained and irregular bodies scattered at random (Pl. LXVII Fig. I91).

(v) Diakinesis

The chromosomes as they immerge out of the diffuse stage are of typical bivalent nature. Some are spherical and some V shaped (Pl. LXVIII Fig. I92) and crosses. At late diakinesis these bivalents undergo further linear contraction so that they still get more reduced in size and acquire more or less round shape and they get arranged on the periphery of the nucleus immediately beneath the nuclear membrane. At prometaphase the bivalents move inwards and assume a rounded shape and begin to be arranged on the spindle.

The breakdown of nuclear membrane and the appearance of the spindle terminate prophase and initiate the first metaphase of meiosis. The bivalents move and become oriented. The time between disintegration of the nuclear membrane and the establishment of the bivalents on the spindle is prometaphase.
(b) **Metaphase**

At full metaphase the two homologous centromeres of each bivalent lie in the longitudinal axis of the spindle on opposite sides of the equatorial plate. Polar view of the metaphase first discloses 32 elements (Pl.LXVIII Fig.193)(Pl.LXXI MP 51). All the chromosomes are rounded in shape. Microphotograph 51 shows that the chromosomes are arranged on the periphery of the metaphase plate as seen in the polar view. At the side view of the metaphase the mode of orientation of the bivalents on the equator of the spindle shows that they are with telomitic fibre attachment. They are dumb-bell shaped thus revealing their bivalent nature (Pl.LXVIII Fig.195).

(c) **Anaphase**

During first spermatocyte anaphase all the bivalents get separated normally into two halves (Pl.LXIX Fig.196) when the anaphase movement begins it has been observed that one or more bivalents travels ahead of others towards poles (Pl.LXVIII Fig.195).

(d) **Telophase**

First anaphase is followed by first telophase (Pl.LXIX Fig.197) (Pl.LXXI MP.52) where the chromosomes form chromatin clumps on each pole. Microphotograph 52 reveals two telophasic nuclei at opposite poles which are yet linked by spindle 'fibres'.

The secondary spermatocyte metaphase at polar view (Pl.LXIX Fig.198) again 32 elements have been counted. At the stage chromosomes acquire more rounded shape. The size of the plate as well as of each individual chromosome is half of the size of plate and chromosomes seen in the first metaphase. At second anaphase all the
chromosomes divide synehrocously and move to the opposite poles. The chromosomes differ from first anaphase chromosomes in being small rounded bodies, whereas latter are thick and spherical. Second telophase is seen only in the form of two big chromatin clumps lying on opposite poles of the spindle (Pl.LXIX Fig.199). Hence no details can be given of this stage. No abnormal behaviour of any chromosome has been observed in the whole process of meiosis.
(4) **Chromosome number, shape and size**

The diploid set of *Botia birdi* is composed of 62 elements (Pl. LXVII Fig. 184)(Pl. LXX MP 49). All the chromosomes are alike and rod shaped with only slight variation in size. The size of the chromosomes varies from two to three microns.

32 elements have been observed in metaphase one (Pl. LXXI MP. 51) during this stage all the chromosomes have acquired rounded shape. Metaphase second (Pl. LXIX Fig. 198) also reveals 32 elements. The size of the chromosomes studied in metaphase second is about half of the size of chromosomes studied in metaphase one.

The coloured plate which forms the frontispiece shows a remarkable picture of the shape of bivalents seen as diakinesis in oogonial mitosis of *Botia birdi*. 