CHAPTER V

DISCUSSION
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Family Cyprinidae:

The karyotypes of seven Cyprinids, belonging to sub-family Schizothoracinae: Cyprininae have been worked out. The fishes are Schizothorax esocinus, Oreinus plagiosomus, Cyprinus carpio communis, Cyprinus carpio specularis, Labeo dero, Crossochilus latius diplochilus and Puntius conchonius.

The 2n complement of Schizothorax esocinus exhibits 82 spherical acrocentric chromosomes, which do not vary much in their length. The spermatogonial metaphase plate of Oreinus plagiosomus consists of (2n) 76 chromosomes. Both the fishes have been studied for the chromosomes for the first time from Kashmir. Cyprinus-carpio communis and Cyprinus carpio specularis are two allied species they have got the same chromosome number that is (2n)104 and (n) 52. The same number has been reported by early workers also. All the chromosomes are rod shaped and acrocentric.

Spermatogonial metaphase of Labeo dero consists of (2n)54 chromosomes. Both the primary and secondary spermatocyte metaphases possess (n) 27 spherical elements almost of the same size.

Crossochilus latius diplochilus shows (2n)48 chromosomes on the spermatogonial metaphase plate of mitosis, out of which 12 are metacentric with median or submedian centromeres; the other 36 are acrocentric. The metacentrics lie scattered amongst the other chromosomes without following any rigid pattern of distribution. It has been reported for the first time.

The diploid complement of Puntius conchonius is composed of 56 spherical or small rod shaped chromosomes. Both primary and
and secondary spermatocyte metaphases show (n) 28 chromosomes.

Beside the above mentioned seven species about 37 other Cyprinids are known to us cytologically. Whose male Karyotypes have been reported by various workers. In the family Cyprinidae MAKINO was the first to give an accurate accounts of chromosomes in two species Cyprinus carpio and Carassius auratus. Additionally BARIGOZZI (1937) made also a study of chromosomes of C. a. specularis. Then the chromosomes of six Cyprinids have been reported by MAKINO (1939, 41). 15 species have been described by NOGUSA (1943, 51, 60). 21 species of Cyprinids have been reported by NAYYAR (1962, 63, 64, 66). Two species of the family Cyprinidae have been cytologically reported by SHARMA, PRASHAD and NAYYAR (1960). POST ALFRED (1966) has also reported the chromosome number of 10 species of Cyprinida. D. KAUR (1962) has given the cytological studies of four species of family Cyprinidae.

The chromosomes complement of Cyprinids were divided into 16 different Karyotypes by NAYYAR (1964) in different species of family Cyprinidae.

Large morphological variation exists among the complement having same chromosome number. These morphological variations are due to the presence of variable members of submetacentric and metacentric chromosomes. This phenomenon becomes clear with the following examples. 14 species of this family possess the diploid complement of 50 chromosomes. Only 8 species of these namely Sarcocheilichthys variegatus, Gnathopogon elongatus, Gnathopogon elongatus caeruleocentrus, Hemibarbus longirostris, Esomus danricus, Danio rerio, Oxygaster baicala and P. ticto ticto, happens to have the identical Karyotypes of 50 rod shaped
acrocentric chromosomes. This feature shows that these fishes have a close taxonomically affinity among them as far the number and morphology of chromosomes is concerned. The haploid sets of these species have been found to be formed of 25 chromosomes.

Four other cyprinds possess the same diploid chromosomes number (50). But the karyotypes differ from the above mentioned one in possessing 4 metacentric elements also with 46 acrocentrics. The fishes are Acheilognathus lanceolatus, Pseudorasbora perwa (MAKINO 1939) Zacco temminkei and Pungtungia herzi (NOGUSA 60) and thus they exhibit a close inter-relationship amongst themselves.

A strikingly peculiar type of chromosome complement has been reported by MAKINO (1939) Tribolodon hakuenise and NOGUSA (1955) for Abbottina rivularis. Both the Cyprinids are characterized by 2n complements of 6V and 44J shaped elements. Both have reported that the V shaped chromosomes have median or submedian constriction which are constant for each chromosome. No J shaped chromosomes have been met with in any of the Cyprinids studied in present investigations.

Another karyotype which differs in chromosome number from those of the above described species of Cyprinid has been found in Ctenopharyngodon idellus, Zacco platypus, Hemici grammophyris, Rasboralla and Puntius stigma. Here each cyprinid has got 48 rod shaped chromosomes with terminal and spindle fibre attachment and thus seem to have a kinship with the same chromosome number.

Contrary to this chromosome number (2n) including in P. stigma the chromosome number observed, the present work of
of \( Pauntius \) conchonius is \( (2n) 56 \).

\( \text{NOGUSA (1960) reported a different spermatogonial karyotype of Ishikaniia steenackeri which consisting of } 2V + 46 \)

\( \text{rod shaped chromosomes. According to him if both the } V's \text{ of Ishikaniia be considered equivalent to 4 rods on the basis of } \)

\( \text{ROBERTSONIAN LAW (1916). Total number of chromosomes becomes } 50 \)

\( \text{which has been obtained in majority of Cyprinids. Moreover } \)

\( \text{ROBERTSONIAN'S law for centric fusion seems applicable here, as } \)

\( \text{the evidence for phenomenon of centric fusion is available in fishes. } \)

\( \text{Acheilognathus rhombea (NOGUSA 1956) is characterised by centric fusion and variation in the morphology and number of } \)

\( \text{chromosomes and occurs in the same individual. NOGUSA has reported the occurrence of 3 types of } 2n \text{ complements i.e. (i) } 4V \text{ type, } \)

\( 4 \text{V's + 44R's, } 2n \text{ (44); (ii) } 2V \text{ type, } 2V's + 44R's, 2n \text{ (46); (iii) No } V \text{ type, } 48R's, 2n \text{ (48), in the above mentioned fishes.} \)

\( \text{Two Cyprinidae Morco steinderchneri and } \text{M. cernurus investigated by NOGUSA (1960) show that their mutual relationship by possessing the haploid number } 27, \text{ and same number has been found for } \text{Labeo gonius (D. KAUR 62), Labeo dero and L. gonius (NAYYAR 1964) and Labeo dero by present author. D. KAUR has reported the presence of two } J \text{ shaped and } 52 \text{ R's in diploid karyotype of } \text{Labeo gonius. Although the present author has found same number (n)27 but there are no } J \text{ shaped chromosomes but only acrocentric chromosomes have been observed.} \)

\( \text{A different haploid karyotype containing 26 chromosomes has been reported in each } \text{Psuedogobia esocinus and Barbus and Semimaculatus (NOGUSA) 1960. The diploid complex in the former} \)
species display 52 rods which in the later S V's and 46 R's shaped elements have been found. It is interesting to note that *Barbus conchonius* (Cyprinidae) worked out by the present author does not resemble either in its chromosome number or in morphology to its congeneric forms. The author has found the diploid complement of this fish as \((2n)56\) all the chromosomes are spherical in shape. *Puntius stigma*, *P. ticto ticto*, which possess 48, 52 rod shaped chromosomes as their diploid number respectively. Although *P. ticto ticto* and *P. stigma* differ from each other and both differ from *P. conchonius* (reported in the present investigation) in number. Thus it appears while describing this case it becomes clear that even allied species of fishes show variation in chromosome number and morphology.

However examples reverse to the above mentioned cases have been reported by MAKINO (1941) identical diploid sets for *Carassius carassius* and *Carassius auratus* each, species possessing rod shaped elements. On the other hand *C. c. specularis* and *C. c. communis* both possess \((n)52\) rod shaped elements as reported in the present investigations by the author. This number for these allied subspecies has been given by earlier workers also.

From the foregoing account it can be concluded that the members of the family Cyprinidae worked out cytologically so far exhibit a large number of chromosomes. The haploid number ranges from 22 to 52. However the number 25 occurs very frequently in this family, so it can be claimed as the model number for Cyprinidae. This confirms the findings of KAUR AND NAYYAR.
If the results of the present work on Cyprinidae are studied from the point of view of chromosome number the striking feature is the large range. The lowest number is \((n)24\) in \textit{Crossogobius}, \(27\) \((n)\) in \textit{Labeo dero}, \((n)28\) in \textit{P. conchoiinius}, \((n)38\) in \textit{Oreinus-plagioi stomus}, \((n)41\) \((n)\) in \textit{Schizothorax eocinus} and \((n)52\) in \textit{C. c. communis} and \textit{C. c. specularis}. If \((n)24\) is model number for Teleosts, the highest number \((n)52\) shows that a considerable period of time has elapsed till the formation of \((n)52\) in \textit{Cyprinus}. The terms of polyploidy the number \(24\) could rise to \(48\) by diploidy resulting in \((2n)96\) chromosomes, the addition of \(8\) chromosomes to make the \((2n)104\) in \textit{Cyprinus} may be explained by doubling of \((n)4\) chromosomes in evolution.

**Family Salmonidae:**

There has been no karyological work on Salmonids in India. The Salmonids are the only North American fishes for which detailed karyotype information has been available. The cytological investigations of the Salmonidae have been progressively carried out in many fishes. Reference to the new list of \textit{MAKINO} (1956) show that the chromosomes of 49 species and 4 hybrids have been reported in this family.

Earlier accounts published by \textit{BOHM} (1891), \textit{BLANG} (1894) \textit{BEHRS} (1898), \textit{OPPERMAN} (1913) and \textit{MRID} (1923) gave no evidences satisfactory at present day standards. Lately a series of cytological investigations has been published in this family by \textit{PROKOFIEVA} (1934), \textit{MAKINO} (1937), \textit{POMINI} (1939), \textit{SVERDSON} (1941, 45) \textit{EPEA} (1948) \textit{BUNGENBERG} (1955) with satisfactory results. \textit{NOGUSA} (1960) gave the chromosome number of Oncorhynchus nerka as
(n)54, O. rhodurus (n)50, O. masou (n)50, O. keta (n)50, Salmo iredeed
(n)52, Salvelinus fontenalis (n)50  

RAYMOND C. SIMON (1960) has reported the chromosome number of O. kisuech (n)60, O. gorbuechi (n)52; REES (1964) has reported the chromosome number of Salmo salar (n)52 and SVARDSON (1945) has reported (n)40 in Salmo trutta fario.

The author has found the chromosome number of Salmo trutta fario as (n)40 and Salvelinus fontenalis as (n)50 which confirms the findings of NOGUSA (1960) and REES (1964).

The variation in chromosome counts reported reflects either inaccuracy consequent upon inadequate cytological techniques for studying these small chromosomes, or possibly true chromosome polymorphism. It is worth noting that both SVARDSON and BOOTHROYD in view of these errors one must of course face the possibility that the variation in chromosome number reported between different Salmon stocks is more apparent than real.

Therefore while a real polymorphism in respect of structure seems well established by SVARDSON'S work. There must be some doubt as to whether the evidence on chromosome numbers to date establishes a firm and unequivocal case of polymorphism within the species.

Family Cottidae

The chromosome survey of this family has been done by MAKINO (1941) in the following two species; Misgurunus anguillicepsudatus and Barbatula oress, he reports (2n)52 and (2n)48 respectively. Barbatula oress includes two V shaped stelomitic elements. Thus the numerical relation of chromosomes between the related forms, Misgurunus and Barbatula may be
explicable as a result of the formation of multiple chromosome by means of the union of two rods at their inner ends.

NOGUSA (1960) has reported the karyotypes of two cobitids viz Lefua echigonia (2n)50 and Cobitis biwae (2n)64. The former species has 50 chromosomes which are telomitic elements, while the latter possess 54 chromosomes composed of 4 prominent V shaped and 50 telomitic ones. There is no similarity between above mentioned species in their chromosomes. POST ALFERED (1966) has reported (n)24 in Misgurnus fossilis which is the same number as above mentioned cobitids.

The two cobitids studied in the present investigations are Nemachilus kashmiriensis and Botia birdi. The spermatogonial metaphase plate of Nemachilus kashmiriensis exhibits (2n)52 spherical elements where the diploid set of Botia birdi is composed of (2n)64 chromosomes. The two fishes not only differ in the number of chromosomes but they also differ in the morphology. In both the fishes chromosomes are small in size.

From the above account it seems none of the cobitids worked out up till now have got similar chromosome number. The chromosome number in the family cobitidae ranges from 25 to 32 in hiploid number. Thus the evolution of the chromosomes number in the family cobitidae appears to show a gradual increase viz. 25 to 32 (The authors, Nemachilus kashmiriensis (n)26, Botia birdi (n)32 and NOGUSA'S Echigonius (n)25).

MAKINO and NOGUSA (1960) are of the opinion that Cyprinidae show close affinity to the family cobitidae and NOGUSA thinks that this statement is also in agreement with MATSUBARA (1955) who
regarded Cobitidae as a subgroup of Cyprinidae. MAKINO made the statement that chromosomes of *Achelognathus lanceolatus* and *Pseudorsbora parva*, in respect of number and morphology seem to be closely related to the Cobitids. *Misgurnus anguillicaudatus* (2n) 52 r's and *Barbatula oreas* (2n) 48,4V's + 44 R's, the same view has been hold by NOGUSA.

But after studying Karyotypes of different families already worked out the present author does not find this view reasonable on the grounds that not only the Cobitidae, but a number of other fishes belonging to primitive as well as specialized families show a resemblance to their Karyotype to Cyprinidae.

In respect to chromosome number and morphology for example the Karyotype of 48 r's which has been found for many Cyprinidae also occur in the some members of family Serranidae (*Coreoperca-kawambari*), Selarginidae (*Sillago sihama*), Hexagrammidae (*Hexagrammon otakii*), Cottidae (*Cottus pollux*), Cyprinodontidae (*Xiphophorus* and *Platypoecilus*) and Pleuronectidae (*Limanda yokohamni* and *Kareias bicoloratus*).

Moreover so far Karyotypes of only seven Cobitids are known (including the present two) and these resemble those of Cyprinids. There is a possibility that future investigations on family Cobitidae may reveal different Karyotypes. The author feels that at present it is not proper to establish inter-relationship between these two families on the basis of such scanty cytological investigations. More so since Cyprinidae is an extremely huge family and could naturally exhibit large numerical variations in chromosome number; and Cobitidae being a small family may show similarity in chromosome number with some of the
members of the Cyprinidae, without having any phylogenetic importance.

**Family Poeciliidae:**

The diploid chromosome complement of Gambusia affinis holbrooki investigated by the present author consists of \((2n)\) 48 small rod shaped elements. The haploid number is 24. The karyological study of this fish has been made by earlier workers also. GEISER reports the presence of only \((2n)\) 36 chromosomes in the same species, SHARMA, PRASHAD and NAYYAR (1960) have reported only \((2n)\) 46 chromosomes in Gambusia affinis. FRANKLIN, ROBERTS, L. (1965) has reported \((2n)\) 48 in Gambusia so the present investigations agree with ROBERTS results. The other members of the family Poeciliidae have been worked out by POST ALFRED (1966). The fishes are Belonesox helianus \((n)\) 24; Heterandria formosa \((n)\) 24; Lebistes reticulatus \((n)\) 23; Limia melonogaster \((n)\) 24; Mollinesia latipenna \((n)\) 24; Mollinesia sphenops \((n)\) 24; M. velifera (REGAN 1914) \((n)\) 26.

It is thus apparent that the family Poeciliidae have a fairly constant and comparable number of chromosomes, \((2n)\) 48 being the most commonest number followed by \((2n)\) 46 in Lebistes reticulatus. The family Poeciliidae is thus highly evolved uniform family.
2. Sex determining mechanism in class Teleostomi:

In all the species worked out by the present author nothing has been brought to light which even suggests the occurrence of morphologically or behaviourally recognizable sex chromosomes during mitotic or meiotic cycles. No heteromorphic pair has ever been observed as it is in higher vertebrates i.e. Mammals. No heteropycnosis of any chromosome which might be considered sex chromosomes, has ever been observed in meiotic prophase of the 12 fishes studied.

Apart from the existence of chromosomes which are considered to be found in the heterochromatic regions by GEHLER and RIS etc. All the chromosomes at anaphase divide synchronously and move to the opposite poles of the spindle nearly at the same time.

Old workers like MOENKHAUS (1904), PINNEY (1918), TURNER (1919) and GEISER (1924) who do not refer to sex chromosomes cytologically. They hold two different views regarding the sex chromosome condition in this group of animals.

According to the reports of earlier workers FOLEY (1926), VAUPEL (1929), RALSTON (1934), BENNINGTON (1936) and BARIGOZZI, the males in Teleosts during the first division of meiosis, have been concluded to be sex chromosomes by above mentioned workers.

But other workers IRIKI (1932), MAKINO (1931), a, b, 1939, 1941), SVARDSON and WICKBOM (1939), NOGURA (1943, 50, 51 a, 1954, 1957 b, 1960), JAKOWSKA SOHIE (1950) et al held the view that male specimens examined by them had no detectable sex chromosomes.
None of these workers could observe any sign of heteropycnosis at mitotic prophase and they have concluded that males examined by them do not display sex chromosomes recognizable either by structure or behaviour.

The question naturally leads us to examine the observations on the basis of which the workers of first category that cytologically differentiated sex chromosomes are present. The authors who have reported the sex chromosomes in males during meiosis have observed a chromosome pair which are unlike the others in form and behaviour in first meiotic division.

Foley (1926) has observed two large L shaped chromosomes and considers the males of *Umbrla limi* as X-X type. Vaipul (1929) suggests the presence of X-Y pair in the males of *Lebistes reticulatus* as the members of this pair pass to the poles during first meiotic stage ahead of others, while the remaining are still in metaphase. The only such case in the present thesis is that of *Botia birdi*, in which one or two chromosomes are seen to pass to the spindle poles ahead of the others in primary spermatogonial anaphase.

Ralston (1934) in his study on chromosomes of *Xiphophorus platypoecilus* and their hybrids considers the sex chromosomes to be most conspicuous. He states that there is either 22 condition in the males or XY in which the X and Y are morphologically identical. Since in other animals the sex is seldom morphologically identical one is led to the assumption that chromosomes of Teleosts are 22 type.

Bennington (1936) reports that the large lagging chromosomes of *Betta splendens* in the primary spermatocyte
Division are sex chromosomes being a primitive group of animals. He considers that development of sex chromosomes in Teleosts is in an early stage, hence they are neither greatly differentiated from the autosomes nor do they show any disparity between them and thus are existing in 'nascent condition'.

The study of the fish cytology is universally beset with many difficulties. The extremely small size of the male germ cells as well as chromosomes, difficulty in preservation of chromosomes etc. are the factors which immediately lead to the idea that the chromosomal elements which have been indicated by the above mentioned authors as sex chromosomes are nothing but the ordinary bivalents which got protruded abnormally owing to the faulty fixation. Moreover the authors have described the presence of heteromarphic pair on the basis of cytological investigations, have not given any account of heterophycnosis of that particular pair at meiotic prophase. In spite of this the illustrations given by them do not prove the fact that structurally the so called sex chromosomes resemble those found in insects and mammals. The large frontispiece piece of the chromosomes of B. birdi as drawn from Microphotograph at the diskinetic stage of Oogonial meiosis, has been included in the thesis, as it was search for sex chromosomes. The plate show's V's, rods and dots, most of them being in the paired condition. No definite conclusion could be established for sex chromosomes even here, but the peculiar single ring and double ringed ones seen in the plate, which are different from the chromosomes give an indication that they may be the sex chromosomes.
Oogonial mitosis and meiosis has not been reported in other fishes since they were unclear in preparations to the Karyotypes of spermatogenesis.

The above argument again helps us to state the evidence in favour of occurrence of sex chromosomes in teleosts are not conclusive but the structure so described are aberrations caused by inadequate fixation.

 MAKINAG (1934, 39, 41, a, b) repeatedly claimed in his studies on fish karyotype that there are no identifiable sex chromosomes in this group.

Recently NOGUSA (1955 a, 57 a) contrary, to the above findings, for he succeeded first time in demonstrating a clear cut and definite cytological evidence in favour of the occurrence of XY mechanism in males of *Morguranda obscura* (Gobiidae) and *Cottus pollux* (Cottidae). He reported in both the species an X chromosome larger in size than Y, though he was unable to identify this XY pair in spermatogonial and second spermatocyte. He found following evidences for its existence in the primary spermatocytes:

(1) The XY pair is traceable as definite heteropycnotic body throughout the growth period.

(2) The XY bivalent is clearly distinguishable from the autosomal bivalents owing to its characteristic heteromorphic structure. The XY pair is extremely peculiar owing to its mode of conjugation.

(3) The X segregates from the Y in the first division and they migrate to the opposite poles of the spindle.
Thus apparently NOGUSA has established conclusive
cytological evidence of the male heterogamy. In Mygrunda obscura
and Cottus pollux SHARMA, PRASHAD and MULLYAR (1960) have also
described the occurrence of feulgen positive heterophagomenetic body
during Leptotene, Zygotene and Pachytene stages of spermatogenesis
in Esostus danricus (Cyprinidae) but they could not make out its
exact nature and morphological entity at and after diakinesis.

The mechanism of sex determination has also been worked
out in many fishes and discussed from genetical standpoint by
several genetists. A number of sex linked genes are known in
Lebistes reticulatus (WINGE 1923, 1932), WINGE and DITTERESON
(1947) and in three species of Xiphophorus (Platy-pecilus
maculatus, Xiphadium, Variatus). (BELLAMY 1922, 1936), BELLAMY
and OWAL (1960), KOSSWIG (1939), GORDON (1951, 37, 46, 47), CHAM and
GORDON (1951), WINGE (1922, 33, 32), carried out genetical
experiments on Lebistes reticulatus and arrived at the conclusion
that male is heterogametic. Both X and Y carry the sex linked
genes. In which he concluded that autosomes also carry sex linked
genes. In which the male is XX type and female XY type.

AIDA (1936) obtained similar results in Aplocheilus latipes.
The sex reversed female of male genotype XY was obtained by him.
After crossing the abnormal female with normal male, the male
and female offsprings were produced in the ratio of 1:3.

$\frac{1}{3}$ of its male offsprings were males of YY type and these
produced only male offsprings. On the basis of these results
AIDA proposed a new hypothesis of sex differentiation. The male
and female determining genes remain distributed on autosomes and are set into activity by certain amount of stimulating genes. The sex differentiation is caused by the difference in the quantity of the stimulating genes. When the difference is great the female genes are only activated and action of male genes gets suppressed. If it is small just the reverse happens.

KOSSWIG (1931-41) carried out researches on Platypoecilus and Xiphophorus, he has also concluded that sex is determined by interplay of autosomal genes, so called T genes which act as polymeric factors. The total sum of T genes determines the sex, for instance, all combinations between \( T^0 \) - \( T^1 \) can be defined as weak or female or those between \( T^1 \) - \( T^2 \) as strong and male. Apart from the T genes the genetic constitution is labile with regard to external influences which orient the fundamentally bipotential organism towards one or the other sex.

The genetic investigations of sex determination regarding Betta splendens (EBERHARDT 1943) offer good evidence of the sex in fishes. Betta has a normal sex ratio i.e. 1 : 1 in normal environmental condition. Unfavourable environment viz., unsuitable food and space, water conditions favour a statistical pre-dominance of males.

BELLAMY showed through his genetical work that males of Poecilid are homogametic and the similar conditions have been observed by GORDON (1937) and BREIDER (1942) in the domesticated stocks of this fish. Later on GORDON (1946,47,51) has analysed that one wild stock Xiphophorus maculatus shows male heterogamety, while the another has female heterogamety.
He finds that pair of chromosomes responsible for sex determination in *X. maculatus* is the same one in all the stocks. Four different types of the chromosome exist which may be called *W*, *X*, *Y* and *Z*. *W* is strongly female determining and *Y* is strongly male determining. He described the genetical constitution of three natural populations (i.e. wild):

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<td>third</td>
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After crossing the *WY* female with *XY* male, Gordon (1952) obtained 4 classes of offsprings in equal numbers *WY* and *WX* (both females) and *XY* and *YY* (both males). Upon back crossing the *XY* female with parent *XY* male a 3:1 sex ratio was obtained, *WX*, *WY* and *XX* (all females) and *XY* (Male).

The general picture that emerges out of these works is that the knowledge of mechanism of sex determination in Teleosts is in its infancy; the sex chromosome vary little different from autosomes and hence, not distinguishable morphologically. The primitive mechanism of sex determination seems to be unstable in many different ways i.e. in *Laevis* selection of minor sex determining genes in the autosomes may lead to a new sex determining mechanism has been found, which depends on the mutation of the "main" sex genes; this labileness seems to have actually occured in the course of evolution. Again it has been observed that intersexuality or sex reversal is not uncommon feature in fishes and sex has been controlled environmentally. In all likelihood, all the Teleosts go through a Protogynous hermaphroditic stage (Svardson and Wickbom 1942) but permanent intersexuality owing to genetic lack of balance is not found.
3. Diffuse stage:

The present work on the spermatogenesis of twelve fishes of Kashmir, the author came across diffuse stage in all the fishes except Salmonidae. It was found that meiotic pachytene prophase is followed by diffuse stage. During this stage the nucleus shows few small irregular chromatin wisps which do not stain well with nuclear dyes. The structure of the spermatocyte at this stage appears similar to that of the resting stage, but the nuclear material lies scattered as chromatin dots in the nucleoplasm in resting nucleus whereas nucleolus being completely absent and can not be seen in this stage.

The occurrence of this stage has been reported by various workers in Teleosts i.e. VAEPEL (1929) in LEBES Lebistes reticulatus, RALSTON (1935) in Platypoecilus, BENNINGTON (1936) in Betta splendens, NOGUSA (1955) in Zacco temmincki, SHARMA PRASHAD and NAYYAR (1960) in G. affinis, Esoximus cannicola, Danio rerio, D. KAUR (1962) in Notopterus notopterus, O. bacalla Puntius stigma, P. ticto ticto and Xenentodon. All these above mentioned workers have come across diffuse stage which is preceded by a Pachytene stage and is succeeded by a typical Diakinesis, while the bivalents reappear in the final form of tetrads.

The presence of a diffuse stage has been observed in the spermatocytes of some insects particularly in Hemiptera, FRANG SCHRAEDER (1940, 41) has reported diffuse stage in two hemipterans i.e. Rhytodorinia senilis and Edeusa irrorata, this phase follows some times after synopsis, he expected that in
the ordinary course of events homologous chromosomes must lie in paired condition and this natural assumption of duality has been strikingly shown by the diffuse nuclei of *R. senilis*. WILSON (1925) is also of the opinion that diffuse period should regarding as highly modified diplotene in which the duality in the latter however, obscure it may be, persists throughout in some manner, SCHRADER (1941) claims that at this stage the appearance of chromatin approaches, that of the resting stage, and all the chromosomal structures, both Euchromatic and Heterochromatin become diffuse.

The presence of diffuse stage has never been reported in the spermatocytes of higher Vertebrates. WILSON (1925) suggests that chromosomes at this stage are characterized by their shape and have got rougher countours, and often branched, in extreme cases may be lost from view in nuclear space. Thus he correlates this determination of the bivalent threads, with the growth of Auxocytes, the greater the growth of the Auxocytes the greater the nuclear diffusion. SCHRADER (1940,41) correlates this stage with the growth of nucleus.

But contrary to the above view we have never observed the increase in nuclear size during diffuse stage nor has been reported by previous workers. This is either due to the fact that the size of spermatocyte nucleus in fish is very small and hence increase in size at different stages is not marked or there is every possibility that nucleus may not grow in size at this stage. During the present investigations the author has not found any
marked increase in size of nucleus from resting stage to Diakinesis. So it is a unique phenomenon found only in fishes that there occurs a diffuse stage after the pachytene.
4. Evolution of chromosome number and form in class Teleostomi.

The Taxonomy, variation and distribution of animals have been dealt with by a number of taxonomists and ecologists. On the other hand, chromosome cytology has made considerable contributions to animal taxonomy. Cytological data serves as useful and basic criteria for diagnosis of species and for understanding the mechanism of evolution of organisms, since changes in chromosomes play an significant role in the formation of species (WHITE 1954). In view of the above consideration, the cytological feature of the placenta is of great interest in connection with the evolution of vertebrates. At the present time the number of the described species is believed to be somewhat 30,000 of this total, about 800 have been studied by chromosome cytologists till today up to more or less degree as referred to in the monograph published by MAXIMO (1956).

A glance at the histogram based on the haploid chromosome number (Pl. XXXII Fig. 200) makes it clear that the class Teleostomi show considerable variation in the chromosome numbers that is from 24(n) to 62(n). The histogram also discloses that the number 24 (n) is met in two species out of 12 species studied by the author. Number 24 has also been found in certain members belonging to several families of the Teleostomi by other workers.

POST ALFRED (1965) has found number 24 in 79 species of the class Teleostomi. He states if the number(n)24 has phylogenetic significance for fishes. The haploid chromosome
number dominates within Teleosts that have been studied up till now, very strongly; not only as compared to every single number deviating from it, but also compares with all of them together. It is impossible that this preferred number appears accidently so often in most different families from all the five continents. It must be assumed that accident can not be responsible for the increased appearance of \((n)24\) that means either \((n)24\) must have occurred mostly independently as a trend, or this number is characteristic inherited from a common ancestor. The assumption of a trend can be excluded as no case is known where the distribution of inherited properties in a definite number of chromosomes brings an advantage or a disadvantage to a species.

According to POST ALFRED (1965) the question of the ancestral significance of the number 24 within narrowly bounded systematic groups, for example subspecies of a species or also species of a genus is relatively easier to answer. In the ideal case i.e. excluding errors of the one who systematises, it concerns the ancestral units with monophyletic origin.

If these forms do not deviate from each other in their chromosome numbers, then it can be assumed with certainty that the common ancestor was already in possession of this number.

All the Hypherschryson species have 24 chromosomes in the haploid set and to the number 24 for them could be of ancestral significance. Because of the same reason as with in a genus can, however, the number 24 be present so often with in larger systematic units, because this implies only a temporal shift of the same statement.
If \((n)24\) should be common to many Teleosts as old
inheritance, so it should be assumed that during the evolution
of this class -rich- in forms mainly changes in the amount of
genesis of the individual elements of the set of the species took
place, without the possibility of a statement regarding their
nature (point - or structural - mutations) often there are quite
remarkable difference between species with 24 chromosomes;

Regarding the origin of the species with chromosome numbers
deviating from \((n)24\). Besides the dominating number 24, there are
a series of other values, each of which however occur only
seldom and so lead to the guess, that it resulted from the
ancestor number through secondary processes. If one considers,
that for the evolution of the modern Teleosts, about \(150 \times 10^6\)
years were available, so one should expect, that depending upon
the time at which these changes of the chromosome number occurred
bigger or smaller systematic unite will show these secondary
deviations. Changes in chromosomes depends on :-

(1) Loss of the individual chromosome. This would cause
disturbance in the genetic balance, and it is improbable
that they were retained in the evolution. Of course it may be
that we are dealing with elements poor in genes, that are
unknown till now in the case of fishes.

(2) Fusion or interchromosomal translocation between two or more
non-homologous chromosomes of a haploid set.

(3) Separation of double armed chromosomes. In this case, each
part should have retained a Kinetochore, so as not to remain
behind in the equitorial plane during anaphase.
(4) Hyperploidie, it being assumed that this disturbance is not lethal for the genetic balance.

(5) Polyploidy without balance difficulties.

The model number 24, can not be said to be ancestral for the whole group as it has been found in most of the families primitive as well as specialized for example family Esoxidae and Engraulidae which are considered to be primitive families include members (Esox lucius and Engraulis japonicus) with 24 chromosomes. While the members of the family Cottidae and Pleuronectidae e.g. Cottus pollux, Limanda, Yokohema and Koreius bicoloratus, which have been regarded as specialized animals in the course of evolution also possess the same number.

There is another peculiar feature in the chromosome number of fishes, members of the same order and even same family are found with considerable divergence in chromosome numbers. In family Cyprinidae the chromosome number varies from 22(n) to 52(n).

On the other hand in family Salmonidae (excluding the previous erroneous data of BOHM, 1891, BLANC 1894, BEHRENS 1898 and OPPERMANN 1915) occurrence of high chromosome number have been reported nearly in all species investigated with the only exception of Coregonus wartmanni caeruleus (n)18 KUPKA (1948). Except a light difference in the chromosome number, the chromosome pattern of S. t. faio and Salmo fontinalis by PROKOFFIEVE 1934 and SVARDSON (1945) are quite in agreement with each other and also with the present findings of Salmo trutta faio (n)40 and Salvelinus fontenalis (n)50. The 2n number in these fishes varies
The variation of chromosome number within three groups is significant.

(2) Difference of the chromosome number between the three species lies within approximately 10 chromosomes.

(3) The chromosome number in each group is almost a multiple of 10 i.e. 30-40-50 and

(4) With the increase of chromosome number, the number of the V-shaped chromosomes increases contrary to ROBERTSON's LAW.

Thus SVARDSON (1945) suggested the existence of Polyploidy in Salmonid fishes. The salmonid present an old polyploid series, with a basic doubling and not by centric fusion or misdivision. SVARDSON's hypothesis has drawn much attention and repeatedly been discussed by WHITE (1946), KU-KA (1948), MATTHEY (1949), FISHERBERG and BEATTY (1953), BUNGENBERG (1951) and BOOTHROYD (1957) regarding the occurrence of polyploid relationship in animals.

BUNGENBERG (1955) studied the chromosome of Salmo irideus and observed 60 chromosomes in cleavage mitosis of which at least 40 chromosomes have median or submedian centromeres. Based on the data from Salmo irideus BUNGENBERG suggested that the chromosomes would change in number according to 'ROBERTSON'S LAW.' BOOTHROYD (1957) reported 80 diploid chromosomes in Salmo salar.
from Canadian population. This is somewhat lower than the number (2n)60 reported by both PROKOFIEVA and SVARDSON for European salmon populations. BOOTHROYD stated that Canadian population did not fit directly to SVARDSON'S Hypothesis (1945).

KUPKA (1948) reported 70 or 72 as diploid number for Coregonus asperi maraenoides, C. esquius albellus and C. shinzi duplex and 36 for C. wartmanni operulus. He showed that polyploidy occurs in Salmonid fishes. SVARDSON'S and KUPKA'S arguments in favour of polyploidy in coregonids are not in agreement with one another, since SVARDSON claimed that the species with (2n)80 (Coregonus lavaretus and C. albula) are octoploids with a basic haploid number of 10, while KUPKA regarded the species with (2n)70 or 72 as exhibiting tetraploidy by comparison with C. asperi maraenoides (n)18 having a basic haploid number of 18. MATTHEY (1949) tends to agree with SVARDSON'S concept; he stated that even in the case where evidence of the chromosome number appears to be in favour of the hypothesis of polyploidy, the number of major chromosomes arms is in disagreement with it. From 1935 to 1963 following authors determined the chromosome number for Salmonids: MAKINO (1935), POMINI (1939), SVARDSON (1945), KUPKA (1948), EMBIIBL (1953), LEIDER (1956), SNOMALAINEN (1958) and BARGETZI (1960) and KARBE (1963): thereby an irregular series was obtained with (2n)36, (2n)58, (2n)60, (2n)70, (2n)72, (2n)74, (2n)80, (2n)84, (2n)96, (2n)100, (2n)102. All the authors obtained the number exclusively by studying the mitosis. SVARDSON (1945) points out that with in the nuclei of the embryo, the chromosomes have tendency to stick to their centromeres, so that the danger is
there, that often two low values are found. BERGETZI (1960) had also pointed this later. KARBE (1963) has now found for the Bodensee, exclusively (n)48 and (2n)96 chromosomes, where by the haploid value was obtained by investigating the meiosis for the diploid value, he then investigated the metaphase of the division of the spermatogonia and embryonal mitosis there by comes to the following conclusions:

1. The chromosome number is constant in the case of Bodensee and few further forms in the generative tissue, whereas they show considerably scatter in the somatic tissue of the cellular plate, so that this tissue should be used to determine the chromosome number only with reservation.

2. The chromosome number is, in the case of all the forms named, with greatest probability haploid 48 and diploid 96.

It has been shown by present work that the chromosome numbers should be established by the study of only germ cells as for example in Salmo trutta fario (n)40 which is the same number as reported by early workers. Which shows that there is no change in the chromosome number of germ cells.

Except KARBE, NOGUSA, D. KAUR and NAYYAR used gonads to determine the chromosome number of the Salmonidae and it is remarkable that NOGUSA obtained values, that deviate from (n)48 only now essentially i.e. (n)50 in the case of the three species of the genus Onchorhynchus and for Salvelinus fontinalis, (n)52 for Salmo iridens and (n)54 for O. nerka.

According to (MANDEL et al. 1955; ALFEBRY et al. 1955, OHNO et al. 1964 a, 1964 b, BECAK (1964) radiation from an immediate
ancestor in to a multitude of species appears to have occurred with little or no change with total genetic content. Mutations of individual genes in different directions as well as the continuous re-arrangement of linkage groups have played a major role in Vertebrate evolution. One conspicuous way of reducing the number of linkage groups is known as ROBERTSONIAN change. At first one new metacentric is formed by centric fusion of two telocentrics in certain individuals causing chromosomal polymorphism within the species. Latter individuals which have become homozygous for this change evolve into a new species with 2n chromosomes no. two less than before. The ROBERTSONIAN change had been generally assumed to be unidirectional.

S. OKMO, O. STENIUS, K. FAISET and M. T. ZAITES (1965) have found in Salmo iredeus that ROBERTSONIAN process can be reversed. Not can only one metacentric form at the expense of two telocentrics, but two functional telocentrics can result from the splitting of metacentric.

The results of the two Salmonidae Salmo trutta fario and Salvelinus fontinalis studied in the present investigation are in accordance with those reported by earlier workers.

It is well known that like most of the primitive groups Teleosts also frequently possess rod shaped acrocentric chromosomes, metacentric or sub-metacentric elements occur rarely. If MATTHEY'S concept (1945,49) of "Nombre fundamental" is applied holding each metacentric chromosome as equal to two major chromosomes arms and each acrocentric as one, as is
convienient for many groups such as *Drosophila*, *Grasshopper* and *Vertebrates*. It has been found that it does not work completely for fishes.

From the foregoing account conclusion can be drawn that evolution of species in Teleosts has not involved simply by fragmentation or fusion or structural change involving both breakage and fusion of chromosomes on the other hand considering that the fishes are primitive animals, one can put forth the view that the deviation in chromosome number can be possibly according to NAVASHIN'S (1932) 'Dislocation hypothesis of evolution of chromosomes'. This hypothesis explains that each chromosome is monocentric and an evolutionary change in chromosome number must involve duplication of an centromere together with a region around it. While a decrease in number must mean a permanent loss from Karyotype of region containing centeromere. Hence it seems plausible that variation in chromosome numbers in the members of the family or even in allied species may be due to the complete loss or duplication of the centeromere at random.

NOGUSA (1960) on the basis of his observed facts states that the lancelet (*Amphioxus*) shows a considerable Karyological kinship to some of the cyclostomes. In general appearance the chromosomes of Lancelet also resemble the chromosomes of various Teleosts. These facts led him to say that there certainly does exist a Phylogenetic relationship between Lancelet and lower vertebrates. However on the grounds of considerable differences between the chromosomes of *Klaasombranchii* and teleostomi he considers that possibly teleostomi may be a separated divergent group from *Klaasombranchii*. 