PART - II
CYTOGENETIC STUDIES
INTRODUCTION AND HISTORICAL REVIEW
Cytogenetics was developed from two originally separate sciences - cytology and genetics, which deals with the study of heredity through cytology and genetics. The science is concerned with the structure, number, function and movement of chromosomes.

Most of the early cytogenetic work in nematodes involves animal parasitic and free living forms and was conducted in the late part of the nineteenth century and the early part of this century. Some of the work is still considered as classic because it elucidated certain basic cytological and biological phenomena.

One of the most fundamental concepts of the fields of cytology and heredity is the discovery of the process of meiosis or reduction division, which was introduced by Van Beneden in 1883. In his report of studies on the eggs and spermatozoons of Parascaris equorum (Ascaris megaloccephala), he demonstrated that during the formation of the polar bodies, the chromosome number of the egg is reduced to one-half (meiosis) and that it is doubled again in the cleavage nucleus which is formed by the fusion of the egg and
sperm pronuclei (fertilization).

The realization that *Parascaris* germ cells were large, easily obtained and very simple in their nuclear organization, led to their use as study material in the rapid advances of cytology during the last decade of the nineteenth century.

The concept of individuality and physical continuity of the chromosomes was first introduced in cytology by Van Beneden (1883) and Boveri (1888) by demonstrating that *Parascaris* chromosomes persist during Interphase. The process of chromosome fragmentation and chromatin diminution was first discovered in nematodes. Boveri (1887) observed that the early cleavage divisions in *Parascaris* eggs were unusual in several respects, the process of chromosome fragmentation and chromatin diminution is repeated in one of the progeny of the blastomeres or propagation (P) cell line and eventually all the cells of the embryo are diminished, except the last two 'P' cells that become enclosed in the genital primordium of the larva and give rise to all the gonial cells of that individual.

As animal parasitic nematodes were recognized early as favourable material for cytological work, they were the second animal group after the insects to be
studied extensively with regard to chromosomal mechanism of sex determination. The X - 0 and multiple X chromosome situations were discovered in various nematodes as early as 1910 by Boveri, Gulick, Edwards and others. Although, these classic studies indicated that nematodes are favourable for cytological research, but relatively little work followed.

The first study on the gametogenesis in the order Oxyurida was done by Meves (1920) on Passalurus ambiguus (Family : Oxyuridae). Walton (1924, 1940, 1959) worked on the karyotypy of Syphacia obvelata and published work on the parasites and their chromosomes including the members of the order Oxyurida. Later, several studies on gametogenesis in various orders of Nematoda followed. Taylor (1960), Terry et al (1961), Poor (1967), Lejambre (1968) and Lejambre and Georgi (1970) reported studies on gametogenesis in animal nematodes.

Very little work has been done on oxyurid nematodes of vertebrates and invertebrates. Goswami (1976a,b; 1977) and Wharton (1979a) contributed to the cytogenetic studies on oxyurid nematodes.

The discovery of haplodiploid reproduction in the oxyurids of both vertebrate and invertebrate by Adamson (1984a, b) has given a new dimension to the cytogenetic research in the order Oxyurida. In haplodiploid
reproduction, males are haploid and develop from unfertilized eggs, whereas, females are diploid and develop from fertilized eggs.

The Oxyurida are the most recently recognized haplodiploid group and the only endoparasitic group in which this form of reproduction has been reported. Earlier reports of male diploid reproduction in Aspicularis kazakstanica (Goswami 1976a,b; 1977) and the interpretation of karyotypes in Syphacia obvelata and Passalurus ambigua, made by Walton (1924, 1959) were probably an error according to Adamson (1989), since Aspicularis tetraptera and Syphacia obvelata (Adamson 1984b) were found to be haplodiploid.

Haplodiploidy in the oxyurids of insect belonging to the superfamily Thelastomatoidea was first reported in two members of the family Thelastomatidae viz. Thelastoma spp. and Hammerschmidtella andersoni by Adamson (1984a). Haplodiploidy in H. diesingi was discovered by Adamson and Nasher (1987). Zervos (1988b) provided experimental evidence of haplodiploidy while studying developmental biology of Protrellus dixoni, whereas Pham Van Luc and Spiridonov (1990) reported experimental evidence of haplodiploidy in Blatticola blattae. However, only oogenetic studies were carried out on Leidynema appendiculatum and H. diesingi by
Cytological data for haplodiploidy are available for 18 species of Oxyurida belonging to genera parasitizing cockroaches, diplopods, lizards, tortoises, lagomorphs and rodents. Chromosome number in the group vary from $N = 3$ to $N = 6$. Available evidences suggest that haplodiploidy is characteristic of the entire order. Representatives of all major groups of Oxyurida (Thelastomatoidea, Pharyngodonidae, Oxyuridae and Heteroxynematidae) have been shown to be haplodiploids (Adamson, 1989).

With few exceptions, all efforts to gain information about the relationships among parasitic nematodes have been centred around comparative morphology, anatomy and physiology, leaving aside the cytological and cytogenetical aspects. Hence, in the present study, an attempt was made to investigate cytogenetical aspects on the members of the superfamily Thelastomatoidea. Studies on cytogenetical aspects have been done on nematodes in connection with gametogenesis and therefore, information is limited primarily to chromosomes of gonial cells, oogonia, spermatogonia, oocytes and spermatocytes.

In the present study, cytogenetical aspects during oogenesis and spermatogenesis were investigated. This
part of the thesis comprises of three chapters, based on the members of three families of Thelastomatoidea. The first chapter deals with the oogenesis on the members of four genera of the family Thelastomatidae namely Cameronia, Gryllophila Hammerschmidtiiella, Thelastoma and gametogenesis in the genus Leidynema. The second chapter is associated with the oogenesis in the members of three genera of the family Chitwoodiellidae namely Binema, Isobinema and Chitwoodiella and gametogenesis in the genus Mirzaiella. The third chapter is concerned with the oogenesis in the genus Protrellatus of the family Protrelloidiidae.

To the best of our knowledge, the present study reports the chromosome numbers of seven genera of the Thelastomatoidea for the first time viz., Cameronia Gryllophila, Binema, Isobinema, Chitwoodiella, Mirzaiella and Protrellatus whereas, the chromosome numbers of Thelastoma, Leidynema and Hammerschmidtiiella have been reported for the first time in India.
MATERIALS AND METHODS
MATERIALS AND METHODS

Collection of the host and isolation of the parasite is described in the first part. Cytogenetical method described by Triantaphyllou (1981) is adopted in the present study.

1. **Selection of nematode material and preparation of slides for temporary mounts**

   The best material for studying oogenesis of insect nematode consists of live, relatively young, egg producing females and relatively young males for spermatogenesis.

   Slides for temporary mount were prepared by placing nematodes in a drop of normal saline on glass slides. Worms were cut beneath the esophagus to obtain the reproductive tract. Rest of the body parts of the worms were discarded. The slides with reproductive tracts adhering to them were air-dried for few seconds and marked with diamond pencil and then proceeded for hydrolysis.

2. **Hydrolysis:**

   Slight hydrolysis carried out at room temperature before fixation, improves the quality of the preparation considerably. Hydrolysis was carried out by immersing the slides with adhered material in 1N
HCl solution, prepared by mixing about 10 ml of reagent HCl in 100 ml distilled water. The slides were kept in HCl for 5 to 10 minutes and then removed and wiped dry with tissue paper leaving only the material wet.

3. **Fixation:**

   Fixative was prepared by mixing three parts of absolute ethyl alcohol and one part of glacial acetic acid in a couplin dish. The slides were immersed in couplin dish containing fresh fixative for 20 to 30 minutes and then removed from the fixative and wiped dry, leaving only the material wet.

4. **Staining:**

   2% propionic orcein stain was prepared as follows: 2.2 gm of orcein stain was added to 100 ml of glacial propionic acid and boiled gently for at least 20 minutes. The solution was cooled and then diluted by the addition of 100 ml of distilled water and passed through a fine filter for removal of any undissolved stain particles and stored in air-tight glass dropping bottles to prevent evaporation of the acid and minimize precipitation of the stain particles.

   Fixed material was then proceeded for staining by placing one or two drops of stain on the slides and covered with petridish to prevent evaporation of the acid and precipitation of the stain particles.
materials were kept in stain for about 20 to 40 minutes. The slides were then removed and held horizontally on a piece of tissue paper to drain the excess stain.

5. Mounting and Sealing:

To make the stained material stable for a longer period, prior to mounting, the coverslips were dipped for a few seconds in a solution of 50 ml of 45% propionic acid containing few drops of 2% propionic orcein stain and these coverslips were placed on the material. Excess of acid solution was absorbed with tissue paper and the slides were sealed with nail polish. This type of mounting and sealing keeps the stain stable for 2 to 3 days.

6. Examination of the material and Photomicrography:

The sealed slides were examined under 100 x magnification of microscopes of high resolution. The slides with good preparation were photomicrographed using microscope equipped with camera. Nikon Optiphot - 2 microscope was used in this study.
GAMETOGENESIS IN THELASTOMATOIDEA
GAMETOGENESIS IN THE SUPERFAMILY THELASTOMATOIDEA

(i) Oogenesis:

In oogenesis (the development of ova in the female gonad) a number of maturation processes occur, beginning with the small oogonia in the germinative (apical) portion of the ovary and finishing with the large egg in the uterus.

Female reproductive system:

The female reproductive system of nematode consists of paired ovaries, oviducts, spermatheca (either one or two) and uteri which are connected to a common vagina. The ovary is divisible into a short germinative zone and a long growth zone which extends upto the oviduct. Oogenesis can be studied under the following headings.

Multiplication of Oogonia:

Multiplication of oogonia occurs in the germinative zone of ovary. Oogonial cells are straight line descendants of the propagation 'P' cells of the embryo and appear to be set aside from the remaining somatic cells during the early cleavage divisions. Oogonial divisions usually start in third stage larvae and continue upto early adult stage. They are normal mitotic divisions and result in the production of a
large number of oogonia, all the which have the somatic (2n) chromosome number. Very few oogonial divisions have been observed in adult females. The less advanced cells of the germinal region located toward the apex of the ovary are usually at Prophase, those in the middle are at Metaphase and the most advanced cells, located toward the growth zone are usually at Anaphase or Telophase stage. The chromosomes are not discrete at any stage of the mitotic cycle. Cytological analysis of oogonial division is difficult due to their small size.

**Maturation of Oocytes:**

The oocytes undergo normal meiosis. The beginning of the meiosis is marked by the disappearance of nucleolus. In a short region of the ovary located next to the germinative zone, the chromatin of the oocytes stains heavily and forms a dense network of fine strands. This peculiar behaviour of chromatin indicates the occurrence of synapsis (pairing of homologous chromosomes) in the cells of that short region (zone of synapsis).

The process of synapsis including Leptotene, Zygotene and Pachytene stages described in detail in other animals, cannot be clearly differentiated in these nematodes. Following the zone of synapsis, the
young oocytes enter the 'growth zone' of the ovary and start increasing in size (growth period) with proportional increase in the size of their nuclei. When the oocytes are midway down the growth zone, the double chromatin threads in their nuclei become diffused, loose their stainability and eventually may disappear completely (diffuse state). As the oocytes migrate farther down the ovary, they continue to increase in size, particularly those approaching the oviducts. In oocytes within the oviduct or occasionally in those at the proximal end of the ovary, the chromosomes condensed and appeared as paired structures connected by chiasmata. Sperms were seen in some but not in all eggs at this time and then the process of shell formation started. The chiasmata terminalized resulting in bivalents each consisting of two homologous chromosomes attached end to end. The nuclear membrane and nucleoli disappeared and the bivalents moved to the peripheral cytoplasm.

Reduction division proceeded identically in fertilized and unfertilized eggs. Homologous chromosomes separated yielding first polar body which lay on the inside edge of the egg shell. No further division occurred in this polar body.

The second meiotic division was not followed by
cytokinesis and the second polar body came to lie on the inside of the egg cytoplasm. After completion of both meiotic divisions, eggs had either one or two pronuclei depending on whether or not they had been fertilized. In fertilized eggs the pronuclei fused re-establishing the diploid chromosome number (2n) prior to first cleavage. Unfertilized eggs entered cleavage with a haploid complement of chromosomes.

(ii) **Spermatogenesis:**

In haplodiploids, spermatogenesis is modified to avoid further reduction of the chromosome complement of gametes in the male and hence a single equational maturation division occurs in haplodiploid Oxyurida. In males there are no homologous chromosomes and a reduction division is not possible, everything occurs as if meiosis began at the second meiotic division. Chromosomes in the final division of oxyurid spermatocytes are meiotic rather than mitotic in forms, but they undergo an equational division.

**Male reproductive system:**

The males have one testis (monorchic) which leads into a common seminal vesicle and vas deferens before entering the cloacal chamber, a common opening for the reproductive and digestive systems and a single copulatory structure, a spicule. On the surface of the
cuticle surrounding the cloacal opening there are a series of genital papillae which have sensory function.

The testis is divisible into three zones
(i) A germinative zone in which mitosis was observed, comprising one-half to two thirds of the length of the organ.
(ii) A growth zone in which mitosis is absent, here the young spermatocytes start increasing in size with proportional increase in the size of their nuclei.
(iii) A transformation zone in which maturation division occurs just before the seminal vesicle. Interphase nuclei in the germinative zone were similar to those observed in the ovaries. Mitosis was observed in this region. Interphase nuclei in the growth zone contained fine grained homologous staining chromatin.

Chromosomes present at the beginning of the transformation zone were long, filamentous and darkly stained and gradually condensed to form short double chromosomes with fuzzy irregular outlines. A single maturation division was observed, during anaphase the two groups of chromosomes headed in opposite directions which condensed further to form 'tail' of the spermatid.
RESULTS
CHAPTER - I
RESULTS

CHAPTER - I

FAMILY : THELASTOMATIDAE

(i) **Genus : Cameronia**

**Species: aspiculata**

Oogenesis follows the pattern described in the superfamily Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 1 A) The oocytes undergo normal meiosis, which is marked by the disappearance of the nucleolus. Zygotene stage (Fig. 1 B), showing synopsis of homologous chromosomes was observed in the synaptic zone of ovary, located at the beginning of the growth zone. However, other meiotic Prophase stages described in detail in other animals could not be clearly differentiated in these nematodes. The oocytes undergo growth phase and start increasing in size progressively. Sperms were present in both the spermathecae (Fig. 1 C) The oocytes approaching the oviducts were in diffused stage and appeared almost unstained. At the beginning of the uterus, the oocytes were in Metaphase I stage (Fig. 1 D) and in early Anaphase I stage (Fig. 1 E) in the anterior region of the uterus. The oocytes reveal four bivalents at their
posterior end. The chromosomes show oval appearance and due to their small size could not be measured. The bivalents separated yielding first polar body. The second meiotic division was completed by the formation of second polar body. After the completion of both meiotic divisions, eggs had either one or two pronuclei depending on whether or not they had been fertilized. In fertilized eggs, the pronuclei fused re-establishing the diploid chromosome number (2n) prior to first cleavage. Unfertilized eggs entered cleavage with a haploid complement of chromosomes. The diploid chromosome number 2n = 8.

(ii) Genus : Gryillophila
Species : nihali n. sp.

The oogonial cells were in the Interphase stage (Fig. 2 A) The oocytes undergo normal meiosis. Zygotene stage (Fig 2 B), showing synapsis of homologous chromosomes was observed in the synaptic zone. However, other meiotic Prophase stages could not be differentiated. The oocytes undergo growth phase and increase in size with the proportional increase in the size of their nuclei (Fig. 2 C). The oocytes approaching the oviducts were in diffused stage and appeared less stained (Fig. 2 D). At the beginning of
the uterus, the oocytes were in Metaphase I stage (Fig. 2 E) and revealed five bivalents at its posterior end. The diploid chromosome number is re-established by the fusion of pronuclei. The diploid chromosome number 2n = 10.

(iii) Genus : Hammerschmidtella
Species : diesingi

The oogonial cells were in the Interphase stage (Fig. 3 A). Zygotene stage (Fig. 3 B), showing pairing of homologous chromosomes, which was observed in the synaptic zone of ovary, but other meiotic Prophase stages could not be differentiated. The oocytes approaching the oviducts were in diffused stage (Fig. 3 C). At the beginning of the uterus, the oocytes were in early Metaphase I stage (Fig. 3 D) and were followed by late Metaphase I stage (Fig. 3 E). The oocytes at this stage revealed five bivalents at its posterior end. After the completion of both meiotic divisions, eggs had either one or two pronuclei depending on whether or not they had been fertilized. In fertilized eggs, the pronuclei fused re-establishing the diploid chromosome number. Unfertilized eggs entered cleavage with a haploid complement of chromosome. The diploid chromosome number 2n = 10.
(iv) **Genus**: *Lexdynema*

**Species**: *appendiculatum*

Oogenesis: Oogenesis follows the pattern described in the superfamily Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 4 A). Zygote stage (Fig. 4 B), showing synapsis of homologous chromosomes was observed in the synaptic zone of ovary. However, other meiotic Prophase stages could be differentiated. The oocytes undergo growth phase and increase in size with the proportional increase in the size of their nuclei. The oocytes approaching the oviducts were in diffused stage and appeared less stained. Sperms were present in both the spermathecae (Fig. 4 C). At the beginning of the uterus, the oocytes were in Metaphase I stage (Fig. 4 D) revealing five bivalents. The bivalents separated yielding first polar body in the middle of uterus (Fig. 4 E) revealing very small five univalents. After completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. Fertilized eggs, re-established the diploid chromosome number (2n) by the fusion of the pronuclei. Unfertilized eggs entered cleavage with a haploid complement of chromosomes (2n). The diploid chromosome number 2n = 10.
Spermatogenesis:

Spermatogenesis follows the pattern described in the superfamily Thelastomatoidea. The spermatogonial cells were in the Interphase stage (Fig. 5 A). Spermatocytes undergo single equational division and hence no synapsis was observed. The spermatocytes increased in size in the growth zone (Fig. 5 B). The Metaphase stage, revealing five small univalent chromosomes, were observed at the beginning of the transformation zone of testis (Fig. 5 C & D). Sperm formation was observed at the end of transformation zone. The males are haploid and the haploid chromosome number n = 5.

(v) Genus : Thelastoma

Species : basirí

The oogonial cells were in the Interphase stage (Fig. 6 A). The oocytes undergo normal meiosis. Zygotene stage (Fig. 6 B), showing synapsis of homologous chromosome, was observed in the synaptic zone of ovary, but other Prophase stages could not be differentiated. The oocytes approaching the oviducts were in diffused stage and appeared less stained (Fig. 6 C). Sperms were present in spermatheca (Fig. 6 D). At the beginning of uterus, the oocytes
were in Metaphase I stage (Fig. 6 E) and revealed four distinct bivalents. After the completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. The diploid chromosome number is re-established by the fusion of the pronuclei. The diploid chromosomes number $2n = 8$. 
CHAPTER - II
(i) **Genus**: Binema  
**Species**: mirzaia

Oogenesis follows the pattern described in the superfamily Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 7 A). The oocytes undergo normal meiosis. Zygote stage (Fig. 7 B), showing synopsis of homologous chromosomes was observed in the synaptic zone of ovary, located at the beginning of the growth zone. However, other meiotic Prophase stages could not be clearly differentiated. The oocytes undergo growth phase and start increasing in size. Sperms were present in both the spermathecae (Fig. 7 C). The oocytes approaching the oviducts were in diffused stage and appeared almost unstained. At the beginning of uterus, the oocytes were in Metaphase I stage (Fig. 7 D). The oocytes revealed three bivalents at their posterior end. The chromosomes appear oval and due to the small size could not be measured. The bivalents separated yielding first polar body. After completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. The diploid chromosome number is
re-established by the fusion of the two pronuclei. The diploid chromosome number \(2n = 6\).

(ii) Genus: *Chitwoodiella*

Species: *ovofilamenta*

Oogenesis follows the pattern described in the superfamily *Thelastomatoidea*. The oogonial cells were in the Interphase stage (Fig. 8 A). The oocytes undergo normal meiosis. Zygotene stage (Fig. 8 B), showing synapsis of homologous chromosomes was observed in the synaptic zone of ovary. However, other meiotic Prophase stages could not be clearly differentiated. The oocytes undergo growth phase in the growth zone of ovary. Sperms were present in both the spermathecae (Fig. 8 C). The oocytes approaching the oviducts appeared almost unstained and at the beginning of uterus they were in early (Fig. 8 D) and late Metaphase I stages (Fig. 8 E). The oocytes reveal three bivalents at its posterior end. The bivalents separated yielding first polar body. After completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. The diploid chromosome number in fertilized eggs is re-established by the fusion of pronuclei. The diploid chromosome number \(2n = 6\).
(iii) **Genus**: *Isobinema*

**Species**: *flagellocerca*

Oogenesis follows the pattern described in Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 9 A). The oocytes undergo normal meiosis. Zygotene stage (Fig. 9 B), was observed in the synaptic zone of ovary, showing synapsis of homologous chromosomes, but other meiotic Prophase stages could not be clearly differentiated. The oocytes undergo growth phase in the growth zone of ovary. The oocytes approaching the oviducts were in diffused stage and appeared almost unstained. Sperms were present in both the spermathecae (Fig. 9 C). At the beginning of uterus, the oocytes were in early (Fig. 9 D) and late Metaphase I (Fig. 9 E) stages. The oocytes revealed four bivalents, which separated yielding first polar body. After completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. The diploid chromosome number in fertilized egg is re-established by the fusion of pronuclei. The diploid chromosome number \( 2n = 8 \).

(iv) **Genus**: *Mirzaiella*

**Species**: *asiatica*
Oogenesis: Oogenesis follows the pattern described in the Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 10 A). The oocytes undergo normal meiosis. Zygote stage (Fig. 10 B), showing synapsis of homologous chromosomes was observed in the synaptic zone of ovary. The oocytes undergo growth phase in the growth zone of ovary. The oocytes approaching the oviduct were in diffused stage. Sperms were present in both spermathecae (Fig. 10 C). At the beginning of uterus, the oocytes were in Metaphase I stage. (Fig. 10 D), revealing six bivalents which separated yielding first polar body. After completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. The diploid chromosome number is re-established in the fertilized eggs by the fusion of pronuclei. The diploid chromosome number $2n = 12$.

Spermatogenesis:

Spermatogenesis follows the pattern described in the superfamily Thelastomatoidea. The reflexed testis showing spermatogenesis is photomicrographed (Fig. 11 A). The spermatogonial cells were in the Interphase stage (Fig. 11 B). Spermatocytes undergo single equational division and hence no synapsis was observed. The spermatocytes undergo growth phase in the growth
zone of testis. At the beginning of the transformation zone of testis, the spermatocytes were in Metaphase I stage (Fig. 11 C) revealing six small univalent chromosomes. Sperm formation was observed at the end of transformation zone (Fig. 11 D). The males are haploid as they derive from unfertilized eggs. The haploid chromosome number $n = 6$. 
CHAPTER - III
(i) Genus : *Protrellatus*

*species* : *siddharthi* n. *sp.*

Oogenesis follows the pattern described in the superfamily Thelastomatoidea. The oogonial cells were in the Interphase stage (Fig. 12 A). The oocytes undergo normal meiosis. Zygote stage (Fig. 12 B), showing synapsis of homologous chromosomes was observed in the synaptic zone of ovary. However, other meiotic Prophase stages could not be clearly differentiated. The oocytes undergo growth phase in the growth zone of ovary. The oocytes approaching the oviducts were in diffused stage and appeared less stained (Fig. 12 C). Sperms were present in spermatheca (Fig. 12 D). At the beginning of uterus, the oocytes were in Metaphase I stage (Fig. 12 E), revealing six bivalents at its posterior end. The bivalents separated yielding first polar body. After the completion of both meiotic divisions, eggs had either one or two pronuclei, depending on whether or not they had been fertilized. In fertilized eggs, the pronuclei fused re-establishing the diploid chromosome number. The diploid chromosome number \(2n = 12\).
DISCUSSION
DISCUSSION

Available evidences suggest that cytogenetical aspects have been done on nematodes only in connection with gametogenesis and therefore information is limited. The work done is primarily on gonial cells, oogonia, spermatogonia, oocytes and spermatocytes.

Cytogenetical studies in the members of the superfamily Thelastomatoidea (Oxyurida) revealed that this group of nematodes are haplodiploids. Haplodiploidy otherwise referred to as male haploidy, arrhenotoky or male parthenogenesis, is a system of reproduction in which males develop from unfertilized eggs and are haploid whereas females develop from fertilized eggs and are diploid. Cytological data are available for 18 species of Oxyurida belonging to 10 genera parasitizing cockroaches, diplopods, lizards tortoises, lagomorphs and rodents, all are haplodiploid. Chromosome numbers in the group vary from \( N = 3 \) to \( N = 6 \) (Adamson, 1989).

A Haplodiploidy in the Animal Kingdom:

Haplodiploidy in the animal kingdom can be discussed under the following headings.

1. Taxonomic distribution:

Haplodiploidy operates within more or less large
groups of closely related species. Besides the Oxyurida, haplodiploidy is characteristic of monogonant rotifers, the Thysanoptera and Hymenoptera, some Homoptera and some insects of Scolytidae, Coleoptera (Scott, 1936; Huges-Schrader, 1948; Entwhistle, 1964; Birky and Gilbert, 1971; Oliver, 1971; White, 1973).

2. **Ecological similarities among haplodiploid taxa:**

Restriction of haplodiploidy to a few higher taxa, suggests that approach to this form of reproduction depends on ecological and genetic (rather than morphological and physiological) factors (Hartl and Brown, 1970). Although species exhibiting this form of reproduction are a phylogenetically diverse, they do have strong ecological similarities. Most are colonizing species with relatively low vagility and viscous population structure i.e populations are divided into small semi-isolated sub-populations of closely related individuals.

B. **Obstacles in the Development of Haplodiploidy:**

The relative success in terms of numbers of species of some haplodiploid groups (e.g. Hymenoptera, Thysanoptera, Monogonant rotifers, non-metastigmate Acarina and Oxyurida) suggests that haplodiploidy has not been a barrier to transpecific evolution (Whiting, 1945; Hartl and Brown, 1970; White, 1973).
(i). Population structure:

Male haplodiploids are hemizygous and deleterious recessives are not sheltered from selection in the male. Haplodiploidy is therefore unlikely to arise in populations which depend to a greater extent upon heterozygosity (Brown, 1964; Borgia, 1980). However, deleterious recessives are expected to be more rapidly screened from and should therefore be less frequent in haplodiploid populations.

(ii). Initiation of development in the absence of fertilization:

In many organisms, the stimulus of sperm entry is essential for further development of the ovum. This is not true in haplodiploids, where males develop from unfertilized eggs and this obstacle must be overcome by all parthenogens. The occurrence of thelytoky (parthenogenetic production of female) in most animal groups is frequent and this suggests that this obstacle is likely to be not highly restrictive. Though Thelytoky is rare in the Nematoda, it is the sole form of reproduction in number of species of Tylenchida, parasitic on cultivated plants (Triantaphyllou, 1971).

(iii). Form of sex determination of progenitor population:

Certain modes of sex determination (e.g genic balance or dominant Y mechanism) would probably prevent
the origin of Haplodiploidy, since haploids would be female. Huges-Schrader (1948) claimed that all haplodiploid coccids arose from progenitor populations with an XX/XO system for sex determination. Little is known about sex determination in nematodes, but most appear to have an XX/XO system (Anya, 1976).

(iv). **Inviability of haploid tissue:**

Haploid tissue is generally of low viability. This is because there is no protection for deleterious recessives and the second problem associated with haploidy is that of gene dosage. Endomitotic divisions could offset this problem, thus only the germinal tissue of the male need be haploid.

(v). **Modification of spermatogenesis:**

In haplodiploids, spermatogenesis must be modified to avoid further reduction of the chromosome complement of gametes in the male. A single equational division occurs in haplodiploid rotifers (Whitney, 1929); Coccoids (Huges-Schrader, 1948) and Oxyurida (Adamson, 1981; 1984a, b; Adamson and Petter 1983a, b).

(vi). **Necessity of fertilizing only part of the egg output:**

This constraint arises from the fact that the proportion of males in haplodiploids is proportional to the number of unfertilized eggs.
In certain Oxyurida of diplopods, females are didelphic but a seminal receptacle and consequently sperms are present in one horn of the reproductive system. Adamson (1984 C, 1985) interprets the unpaired seminal receptacle as an adaptation to haplodiploidy. Inseminated females are thereby able to ensure both sexes in their progeny: fertilized eggs, destined to develop as females are produced in one uterine horn, while unfertilized eggs, destined to develop as males are produced in the other.

However, this asymmetry in the reproductive system has only been described in a few genera (Thelastoma, Desmicola, Cornostoma) but the problem presumably affects all haplodiploids. Other mechanisms must exist that allow females to produce unfertilized eggs after insemination. Adamson (1983) suggested a mechanism whereby secretions in the upper oviduct of Gyrinicola batrachiensis could mediated egg fertilization by stimulating shell development in ova or by interfering with sperm activity.

The results of the cytogenetic studies on the members of the family Thelastomatidae indicates the occurrence of haplodiploid reproduction in this group of nematodes. The diploid chromosome number $2n = 8$ in Thelastoma basiri is in agreement with Thelastoma spp.
The diploid chromosome number \(2n = 10\) in *Hammerschmidtella diesingi* is in agreement with Adamson (1984a) and Cutillas et al. (1985). The studies on gametogenesis in *L. appendiculatum* shows that females are diploid with \(2n = 10\), males are haploid with \(n = 5\) and it is in conformity with the studies on *H. diesingi* by Adamson (1984a). The diploid chromosome number \(2n = 8\) in *Cameronia biovata* and \(2n = 10\) in *Gryllophila basiri* are in conformity with the studies on the members of this family (Adamson, 1984a, Cutillas et al., 1985) viz. *Thelastoma* spp. (\(2n = 8\)) and *H. diesingi* (\(2n = 10\)). The cytogenetic studies on the members of the family Thelastomatidae indicates that the chromosome number in the group varies form \(N = 4\) to \(N = 5\).

The results of the cytogenetic studies on the members of the family Chitwoodiellidae shows the occurrence of haplodiploid reproduction in this group. The diploid chromosome number \(2n = 6\) in *Binema mirzaia*, *Chitwoodiella ovofilamenta*, \(2n = 12\) in *Mirzaella asiatica*, \(2n = 8\) in *Isobinema flagellocerca* are in agreement with Adamson (1989). The studies on gametogenesis in *M. asiatica* shows that females are diploid (\(2n = 6\)) and males are haploid (\(n = 3\)). The cytogenetic studies on the members of the family
Chitwoodiellidae has been done for the first time. The chromosome number in the group varies from $N = 3$ to $N = 6$.

The results of the cytogenetic studies on the member of the family Protrelloididae viz., Protrellatus siddharthi n. sp. shows that the females are diploid with $2n = 12$ and is in agreement with the studies in the order Oxyurida (Adamson 1984 b). The diploid chromosome of the genus Protrellatus is reported for the first time.

The asymmetry in the reproductive system was observed in Thelastoma basiri, H. diesingi and Protrellatus siddharthi n. sp. and conforms well with the studies of Adamson (1989). On the contrary, paired seminal recaptacle (spermatheca) containing sperms were observed in C. biovata, G. basiri, L. appendiculatum, B. mirzaia, C. ovofilamenta, I. flagellocerca and M. asiatica, hence some other mechanism must exists in these nematodes that allow females to produce unfertilized eggs even after insemination and this will require further studies on the secretions of oviducts and uteri in these nematodes as suggested by Adamson (1989).
CONCLUSIONS

From the cytogenetic studies on the members of the superfamily Thelastomatoidea, following conclusions were drawn:

1 - The members of the family Thelastomatidae and Chitwoodiellidae were found to be haplodiploid i.e. female are diploid and develop from fertilized eggs and males are haploid developing from unfertilized eggs.

2 - The chromosome number in the members of the family Thelastomatidae viz. Cameronia, Gryllophila, Hammerschmidtia, Leidynema and Thelastoma varies from N = 4 to N = 5. Whereas the chromosome number in the members of the family Chitwoodiellidae viz. Binema, Chitwoodiella, Isobinema and Mirzaiella varies from N = 3 to N = 6. The chromosome number in the member of the family Protrellooididae viz. Protrellatus is N = 6.

3 - Oogenesis and chromosome numbers are reported for the first time in seven members of the superfamily Thelastomatoidea, viz., Cameronia biovata, Gryllophila basiri, Binema mirzaia, Chitwoodiella ovofilamenta, Isobinema flagellocerca, Mirzaiella asiatica and Protrellatus siddharthi n.sp.
4 - Oogenesis and chromosome numbers are reported for the first time from India in three members of the family Thelastomatidae viz. *Hammerschmidtiiella diesingi*, *Leidynema appendiculatum* and *Thelastoma basiri*.

5 - Spermatogenesis is studied for the first time in two members of the superfamily Thelastomoidea, viz., *L. appendiculatum* and *M. asiatica*.

6 - Lastly, it is concluded that the chromosome number in the members of the superfamily Thelastomoidea varies from $N = 3$ to $N = 6$ and this study is in agreement with the earlier studies.
PHOTOMICROGRAPHS
Fig. 1. Photomicrographs of Oogenesis in *Cameronia aspiculata*

A. Oogonial cells in Interphase stage (x 4600).
B. Zygotene stage showing synapsis (x 4800).
C. Sperms in spermatheca (x 3500).
D. Oocytes in Metaphase I stage (x 6800).
E. Oocytes in early Anaphase I stage (x 6800).
Fig. 1.
Fig. 2. Photomicrographs of Oogenesis in *Gryllophila nihali* n. sp.

A. Oogonial cells in Interphase stage (x 2800).
B. Zygotene stage showing synapsis (x 3800).
C. Oocytes in the growth zone of ovary (x 4000).
D. Oocytes approaching the oviduct (x 3800).
E. Oocytes in Metaphase I stage (x 6500).
Fig. 3. Photomicrographs of Oogenensis in Hammerschmidtia diesingi.

A. Oogonial cells in Interphase stage (x 4000).
B. Zygotene stage showing synapsis (x 5000).
C. Oocytes approaching the oviduct (x 3800).
D. Oocytes in early Metaphase I stage (x 6250).
E. Oocytes in late Metaphase I stage (x 6300).
Fig. 4. Photomicrographs of Oogenesis in *Leidynema appendiculatum*.

A. Oogonial cells in Interphase stage (x 4200).
B. Zygote stage showing synapsis (x 3800).
C. Sperms in spermatheca (x 4400).
D. Oocytes in Metaphase I stage (x 5700).
E. Oocytes showing first polar body (x 6200).
Fig. 5. Photomicrographs of Spermatogenesis in *Leidynema appendiculatum*.

A. Spermatogonial cells in Interphase stage (x 6700).

B. Spermatocytes in the growth zone of testis (x 4200).

C. Spermatocytes in early Metaphase stage (x 4200).

D. Spermatocytes in late Metaphase stage (x 4200).
Fig. 6. Photomicrographs of Oogenesis in Thelastoma basiri

A. Oogonial cells in Interphase stage (x 3800).
B. Zygotene stage showing synapsis (x 3800).
C. Oocytes approaching the oviduct (x 3800).
D. Sperms in spermatheca (x 800).
E. Oocytes in Metaphase I stage (x 3000).
Fig. 7. Photomicrographs of Oogenesis in *Binema mirzaia*.

A. Oogonial cells in Interphase stage (x 4700).
B. Zygote stage showing synapsis (x 4200).
C. Sperms in spermatheca (x 1100).
D. Oocytes in Metaphase I stage (x 6000).
Fig. 8. Photomicrographs of Oogenesis in Chitwoodiella ovofilamenta.

A. Oogonial cells in Interphase stage (x 2800).
B. Zygotene stage showing synapsis (x 3300).
C. Sperms in spermatheca (x 1300).
D. Oocytes in early Metaphase I stage (x 4700).
E. Oocytes in late Metaphase I stage (x 4700).
Photomicrographs of Oogenesis in Isobinema flagellocerca.

A. Oogonial cells in Interphase stage (x 3800).
B. Zygotene stage showing synapsis (x 4500).
C. Sperms in spermatheca (x 1500).
D. Oocytes in early Metaphase I stage. (x 4500).
E. Oocytes in late Metaphase I stage (x 4500).
Fig. 10. Photomicrographs of Oogenesis in *Mirzaella asiatica*,

A. Oogonial cells in Interphase stage (x 3200).
B. Zygotene stage showing synapsis (x 3200).
C. Sperms in spermatheca (x 1300).
D. Oocytes in Metaphase I stage (x 4700).
Photomicrographs of Spermatogenesis in *Mirzaella asiatica*.

A. Whole gonad showing spermatogenesis (x 3200).
B. Spermatogonial cells in Interphase stage (x 3200).
C. Spermatocytes in Metaphase stage (3200).
D. Transformation zone of testis showing sperm formation (x 3200).
Fig. 12. Photomicrographs of Oogenesis in *Protrellatus siddharthi* n. sp.

A. Oogonial cells in Interphase stage (x 4500).
B. Zygotene stage showing synapsis (x 4500).
C. Oocytes approaching the oviduct (x 4500).
D. Sperms in spermatheca (x 3000).
E. Oocytes in Metaphase I stage (x 4500).
SUMMARY

The present investigation, on the nematode parasites of various insects found in Aligarh and SiddharthNagar (Uttar Pradesh), North India, embodies two parts. The first part deals with the Taxonomic studies and the second part is associated with Cytogenetic studies.

I - TAXONOMIC STUDIES

Investigations of various insect hosts found in Aligarh and SiddharthNagar, North India, for the parasitism by the members of the superfamily Thelastomatoidea revealed 16 species of nematodes. These species belong to three families, 10 known genera, 5 new species and 11 known species, out of which 4 species are reported for the first time from North India. In addition, the diagnosis of the superfamily, 3 families and 6 genera has been emended. Identification keys to the species of nine genera and comparative measurement charts of the new species have also been provided.

Phylum : Nematoda
Class : Secernentea
Order : Oxyurida
Superfamily: Thelastomatoidea

I. The families are:
1. Thelastomatidae
2. Chitwoodiellidae
3. Protrellooididae

II. The genera are:
1. Blatticola
2. Cameronia
3. Gryllophila
4. Hammerschmidtiella
5. Leidynema
6. Binema
7. Chitwoodiella
8. Isobinema
9. Mirzaiaella
10. Protrellatus

III. The new species are:
1. Cameronia basiri n. sp.
2. Gryllophila nihali n. sp.
3. Binema adamsii n. sp.
4. Chitwoodiella tridentata n.sp
5. Protrellatus siddharthi n.sp.

IV. The known species are:
1. Blatticola blattae
2. Cameronia aspiculata
3. Hammerschmidtiella diesingi
4. Leidynema appendiculatum
5 L. periplaneti
6 Binema mirzaia
7 Chitwoodiella ovofilamenta
8 Isobinema flagellocerca
9 Mirzaella asiatica
10 M. alii
11 M. haroldi

V. The first records of known species from North India:
1 L. periplaneti
2 Leidynema appendiculatum
3 Cameronia aspiculata
4 Mirzaella haroldi

VI. Diagnosis emended:

Superfamily : Thelastomatoidea
Families : 1 Thelastomatidae
           2 Chitwoodiellidae
           3 Protrelloididae

Genera : 1 Cameronia
         2 Gryllophilus
         3 Binema
         4 Chitwoodiella
         5 Isobinema
         6 Protrellatus
VII. **Scanning electron microscopy performed for the first time:**

- **Genus**: *Leidynema*
- **Species**: *appendiculatum*

VIII. **Scanning electron microscopy performed for the first time in India:**

- **Genus**: *Hammerschmidtiella*
- **Species**: *diesingi*

Several species of insects found in Aligarh and SiddharthNagar were examined, but only five species of insects were found to harbour this group of nematodes. The highest rate of infection was found in mole cricket, *Gryllotalpa africana*. It harboured 11 species of nematodes representing 2 families and 6 genera. American cockroach, *Periplaneta americana* harboured 2 species representing single family and 2 genera. The rate of infection was low in *Blatta orientalis* (1 species), *Blatella germanica* (1 species) and *Gryllus domesticus* (1 new species). The worm burden in these insects was found to be regulated by temperature. At extreme temperatures, very few nematodes were recovered with very low activity.

Finally, it may be concluded that the incidence of nematode parasitism is low in the insects of Aligarh and SiddharthNagar (Uttar Pradesh), North India.
II. CYTOGENETIC STUDIES:

Cytogenetic studies in the members of the superfamily Thelastomatoidea have been done for the first time in India. Oogenesis has been studied in 10 genera, whereas spermatogenesis has been studied in only two genera due to the scarce availability of males in this group of nematodes.

I. Oogenesis studied in the following genera:

1. Cameronia
2. Gryllophila
3. Hammerschmidtia
4. Leidynema
5. Thelastoma
6. Binema
7. Chitwoodiella
8. Isobinema
9. Mirzaella
10. Protrellatus

II. Spermatogenesis studied in the genera for the first time:

1. Leidynema
2. Mirzaella

III. Chromosome numbers reported for the first time:

1. Cameronia \( (2n = 8) \)
<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Ploidy (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Gryllophila</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Binema</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Chitwoodiella</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Isobinema</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Mirzaiella</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Protrellatus</td>
<td>12</td>
</tr>
</tbody>
</table>

**IV. Chromosome numbers reported for the first time from India:**

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Ploidy (2n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hammerschmidtiiella</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Leidynema</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Thelastoma</td>
<td>8</td>
</tr>
</tbody>
</table>

The cytogenetic studies on the members of the superfamily Thelastomatoidea indicate that this group of nematodes are haplodiploids i.e. males are haploid and develop from unfertilized eggs, whereas, females are diploid and develop from fertilized eggs. The diploid chromosome number in the family Thelastomatidae varies from $2n = 8$ to $2n = 10$; whereas, in the family Chitwoodiellidae it varies from $2n = 6$ to $2n = 12$ and in the family Protrellooididae it is $2n = 12$. In general the chromosome number in this group of nematodes (superfamily : Thelastomatoidea) varies from $N = 3$ to $N = 6$ and it is in agreement with the earlier studies.
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