I. INTRODUCTION

Meiosis in eukaryotes, the process of ensuring the numerical continuity of the chromosome complement of a species and genetic variation throughout the ensuing generations, has attracted and fascinated scientists for nearly a century. Throughout this period geneticists and cytologists have, in a concerted interplay, though not always in absolute harmony, contributed to the interpretation of the meiotic process, eventually resulting in the establishment of a basic framework which has essentially remained unaltered till date. Although the fundamental events of meiosis are universal, highly conserved and of high evolutionary stability, numerous modifications of meiosis have been described in different species and different taxonomic groups of plants and animals.

Like meiosis synaptonemal complex (SC) in eukaryotes, the coplaner set of proteinaceous three parallel strands which are coaxial to meiotic chromosomes, is also a structure of great evolutionary stability. Variants encountered in the details of its fine structure, its development and behaviour, and abnormalities are characteristic for relatively large taxonomic group.

Chromosome reduction and crossing over, the two special functions that characterize meiosis, depend on pairing and recombination respectively. These latter events are achieved by the formation of SC between the homologous chromosomes as evidenced by (i) the universality of its occurrence in eukaryotic organisms displaying four strand crossing over, (ii) the evolutionary stability of its structural organisation, and (iii) its role in the formation of chiasmata, the microscopically observable counterpart of crossing over (review: von Wettstein et al, 1984).

First demonstrated by Moses (1956) in pachytene spermatocytes of Crayfish, Procambarus clarkii, the SC has been found to occur in all eukaryotes, from protist to man (reviews: Moses, 1968; Westegaard and von Wettstein, 1972; Gillies, 1975a; von Wettstein et al, 1984) except few species viz., male Drosophila (Meyer, 1960; Rasmussen, 1973), male Glossina austeni (Craig-Cameron et al, 1973), and mitotic
race of Meloidogyne hapla (Goldstein and Triantaphyllou, 1978b). SC is assembled, rearranged, and disassembled during the first meiotic division. The lateral elements (LEs) (synonym: axial element, axial core, lateral component) provide simple, linear and faithful representations of chromosome behaviour in meiosis, the SC that they form being a representation of the bivalent (Moses, 1979). The discovery of SC has provided an incitement to continue the classical cytological approach towards an understanding and characterization of the ultrastructure of meiosis. SC analysis has become a powerful tool for the study of meiotic prophase and has been applied to various species (reviews: Westergaard and von Wettstein, 1972; von Wettstein et al, 1984). Chromosomal aberrations are also expressed by the structure and behaviour of SCs and meiotic abnormalities of various types have been studied by the analysis of SCs (Moses, 1977a,b; 1979).

Entire SCs are studied by two different preparative techniques: (a) 3-dimensional reconstructions from serial sections of whole nuclei (Sjostrand, 1967) and (b) whole mount microspreading (synonym: surface spreading) of nuclei in an air-water interface (Counce and Meyer, 1973). Three-dimensional reconstruction method has provided valuable information on SCs in intact nuclei (review: von Wettstein et al, 1984). Besides information on the exact positions of chromosomes and bivalents and the temporal changes hereof, which are important in elucidating the details of the pairing process, can only be obtained by 3-dimensional reconstruction method. However, this method is very slow, technically demanding, and often exhibits limitations, such as the difficulty of identifying or often not visible of kinetochores (Moses and Counce, 1974). On the other hand, microspreading provides flattened nuclei in which full SC complements, selectively stained are displayed. Terminal plagues for attachment to the nuclear envelope, and differentiations of the SC that represent kinetochores, are also stained, together, in some cells, with nucleoli, annuli of the nuclear envelope, and centrioles. The morphological details in such preparations correspond to those seen in thin sections with respect to axial and SC structure, attachment to the nuclear envelope etc. In addition, some details, such as the kinetochore, are often not visible in thin sections, but are distinct in the microspread preparations. While precise spatial
interrelationships are disrupted during the spreading process, such rela-
tionships as end-to-end associations, association with nucleoli, and
peripheral position of the sex body are maintained. This method is
quick, relatively simple, and technically undemanding. Results can
be obtained within one hour of collecting the tissue sample and a
large number of nuclei can be analyzed within a relatively short time.
Each of these two methods has its own advantages and disadvantages
and can give information that the other cannot. Both qualitative and
quantitative analyses of microspread preparations provide a powerful,
practical approach to the routine study of chromosomes at meiotic
prophase stages that in many organisms have so far been intractable
to detailed examination with the light microscope. SC analysis, in
surface spread preparations, can provide biological information about
chromosome behaviour in synapsis that has long lain beyond the limits
of light microscopic resolution, hidden in the confused appearance
of the early prophase meiotic chromosomes. The method promises
broad applicability to a range of hitherto unapproachable questions
of chromosome structure and behaviour in meiosis, such as the manner
and extent of XY pairing, chiasma formation, and the identification
and fate of chromosomal rearrangements (Moses, 1977b).

The question of the relation between the SC, crossing over,
chiasmata and disjunction are relevant to the investigations on achias-
matic meiosis (Rasmussen, 1976). In Bombyx mori, crossing over and
chiasmata are limited to the male sex (Sturtevant, 1915; Maeda, 1939,
cited by Rasmussen, 1976). Since both sexes possess SCs Bombyx seems
to be a favourable organism for studying the relation between crossing
over, chiasmata, and the SC. Moreover, the achiasmatic female Bombyx
offers the advantage that chromosome pairing can be followed without
the complication that crossing over may introduce. The intriguing
problems of chromosome pairing with the aid of the SC which consist
of the preliminary recognition of homologous chromosomes, variations
in the precise temporal sequence of the organization of the SC, crossing
over, and regular disjunction of homologous chromosomes at metaphase
anaphase I have been extensively studied in Bombyx mori by 3-dimen-
sional reconstruction technique (Rasmussen, 1976; 1977a,b,c; Rasmussen
and Holm, 1979;1982; Holm and Rasmussen, 1980). The results of the same specimen obtained by 3-dimensional reconstruction and surface spreading methods show discrepancy (Counce and Meyer, 1973; Solari, 1980) and each can give information that the other cannot (Moses and Counce, 1974). Once the mechanics of meiotic prophase has been established by reconstruction procedures, the spreading technique will be helpful in analyzing a large spectrum of special cytogenetic situations (Rasmussen and Holm, 1980). Whole mount microspreading technique was, therefore, applied to female meiosis of *Bombyx mori* in order to compare the precision of information obtainable from microspreading analysis with that known from 3-dimensional reconstruction.

The insecta have been a fertile field of investigation on meiosis with numerous studies of the SC (review: von Wettstein et al, 1984) which have to a large extent contributed to the understanding of the basic scheme of meiosis. Furthermore, a number of the most intriguing modifications of the meiotic process have been reported from this taxonomic group. But SC and meiotic studies on Lepidoptera to which the silkworms belong have been limited to *Bombyx mori* (Rasmussen, 1976;1977a,b,c; Rasmussen and Holm, 1979;1982; Holm and Rasmussen, 1980), and *Ephesia kuehniella* (Traut, 1977). The general concept that Lepidopteran species have nonlocalized centromeres is still a disputed question. Gassner and Klemetson (1974) reported the presence of localized centromeres at metaphase I in an electron microscopic analysis of two species of Lepidoptera. Bigger (1975;1976) reported the existence of definite centromere-like constriction (primary centromere) at certain stages of the mitotic cycle of 5 Lepidopteran species. Danilova (1973) reported that metaphase II bivalents of *Bombyx* spermatoocytes also appear to contain localized centromeres. Differences in the nature of centromere have been observed at mitosis and meiosis in *Bombyx* by Murakami and Imai (1974) and Maeki (1980). Holm and Rasmussen (1980) reported the occurrence of 4 localized centromeres in each bivalent at metaphase I of *Bombyx* spermatoocytes. Surface spreading technique is well known for its potential to reveal centromere in SCs. Further studies on meiosis and SCs in other Lepidopteran species are needed.
Cytological studies of the class Reptilia are mainly on mitotic chromosomes. Meiotic chromosome studies on this taxonomic group reported so far include analysis of pachytene diplotene configurations (Wu, 1933; Asana and Mahabale, 1941; Bhatnagar, 1958; Becak et al, 1964; Gorman et al, 1967; Kral, 1969; Cole, 1971; Singh, 1974), chromosome replication (Bianchi et al, 1964), sex chromosome behaviour (Becak and Becak, 1972a;1981) and chromosomal aberration and chromatid exchange (Hall and Selander, 1973; Cole, 1977) at the light microscope level by squashing or air drying method. It is obvious that comprehensive information on the meiotic process of Reptiles cannot be obtained from these works. As far as we are aware no report on the SCs of any Reptilian species has been published till date.

Among the Reptiles common garden lizard, *Calotes versicolor* will be a suitable material for cytological studies because of its low chromosome number (2n=34, Makino and Asana, 1948) and its common occurrence. The simple and rapid surface microspreading technique for SC analysis has been successfully applied to several plant and animal species of different taxonomic groups (review: von Wettstein et al, 1984). If the method works for Reptilian meiotic chromosomes also, information on the Reptilian meiosis and SC can be easily and quickly obtained as in the case of other animals. The present study of the SC analysis of male *Calotes versicolor* using surface spreading technique was, therefore, undertaken in order to fill up the lacuna of the class Reptilia in the explosive growth of research in meiosis and SCs.

Karyological information on birds in general is very scanty. Domestic fowl, *Gallus domesticus* is the only Avian species which has been extensively studied at the genetic and cytological levels. Female is the heterogametic sex of this species (Suzuki, 1930, cited by Ohno, 1961). The diploid chromosome number of this bird is 78 (Pollock and Fechheimer, 1976) consisting of 18 large chromosomes and 60 small chromosomes. The large chromosomes are generally called macrochromosomes, while the small ones are referred to as microchromosomes. No essential difference was observed in the behaviour and structure between the macrochromosomes and microchromosomes.
(Ohno, 1961; Ford and Woollam, 1964; Owen, 1965). The sequential changes of the chromosomal axes during presynaptic and synaptic stages in chicken oocytes has been described at electron microscope level (Solari, 1977). But Solari's study focussed on the behaviour of ZW chromosomes. Kaebling and Fechheimer (1983) have reported the SC karyotyping in spermatocytes to this species on the basis of relative lengths and centromeric positions and the comparison of the lengths of macrosynaptonemal complexes (macroSCs) to those of the respective mitotic and meiotic chromosomes. The present study deals with the development and behaviour of autosomal SCs in Gallus spermatocytes.

In this investigation we have also extended the earlier studies of Gallus SCs in oocytes to spermatocytes. Furthermore, the aim of this study is to obtain a comparative account of meiotic prophase in animals of different taxonomic groups through SC analysis at the light microscope level. The present study of light microscopic analysis of the development and behaviour of the SCs of male domestic fowl was undertaken to represent meiotic prophase of the class Aves. The reasons for using domestic fowl as a sample of birds are: (a) it is the most readily available species of bird and (b) it is the only species of bird which has been extensively studied at the genetic and cytological level.

Mammalian meiosis has been extensively studied in several species and variations are encountered in the nature of chromosome pairing and disjunction, number of nucleolus organizer regions (NORs), occurrence of nuclear dense bodies (NDBs) etc., (reviews: Westergaard and von Wettstein, 1972; Gillies, 1975a; Moses, 1977a; Rasmussen and Holm, 1980; von Wettstein et al, 1984).

The domestic pig, Sus scrofa domestica has been an object of attentive cytogenetic study because of its low chromosome number (2n=38, Krallinger, 1931). So far as our knowledge is concerned, the meiosis and the development and behaviour of SCs in this species have not been described except the pachytene SC complement (Schwarzacher et al, 1984).

Investigations on pig NORs in somatic cell (Vejalainen and Rimaila-Parnainen, 1978; Grafodatskii, 1981) and germ cell (Mayr and
Hager, 1980; Schwarzacher et al., 1984) revealed interstrain and intercellular intraindividual polymorphism to the expression of the NORs. Further observations on the NORs of pig germ cells are needed in order to correlate NOR expression in somatic and germ cells.

Variable number of chromocenters has been observed in air-dried preparations of pig spermatocytes (Schwarzacher et al., 1984). Further study is needed to illustrate the development and behaviour of chromocenters at different meiotic prophase stages and the nature of chromosomal association to form the chromocenter. The meiotic prophase in male porcine through SC analysis was, therefore, studied.

In the studies on the development and behaviour of SCs in species with asynchronous meiocyte development there is some question as to the exact meiotic stage described. There is also the question of duration of the meiotic stage to which SCs are referred. The problems of determining the duration of meiotic prophase stages and referring the stages of SCs to them have been solved in various ways. The appearance of different meiotic cell stages coincide with easily recognizable spermatid stages (Oakberg, 1956a). It is thus possible to determine the structure of the SC in each stage of the meiotic prophase in a thin section (Solari, 1970a). Meiotic stages are easily identified by light microscopy in thick section, and in thin section cut adjacent to it, the SC of the same identified cells can be analysed by electron microscopy (Solari, 1964). However, the above methods require 3-dimensional reconstructions of serial sections because it is not possible to study the entire SCs in one preparation (Solari, 1970a; Holm and Rasmussen, 1977).

In fact, it seems that much of the controversy and uncertainty concerning chromosome structure is due to the difficulty of sequentially relating light and electron microscopic images to a particular stage of meiosis, especially in those systems that have several chromosomes (Roth, 1966). Various phenomena like synopsis of the XY axes, appearance of single axial elements, developmental changes of the centrioles and the behaviour of nucleolar structure can be used to identify prophase stages in whole mount preparation of SC (Tres, 1977; 1979; Moses, 1977a; Solari and Counce, 1977; Dresser and Moses, 1980). But the
synapsis and desynapsis of XY pair are generally out of phase with the autosomes, and because there is variation in this asynchrony from cell to cell, use of XY pair to define various stages of meiotic prophase must be made with caution (Moses, 1977a) and there is possibility of misinterpretation of results (Dietrich and Mulder, 1981). The development of a method of sequential analysis of meiosis in male mice using hydroxyurea and/or triaziquone (HU/T) (Oud et al, 1979; Oud and Reutlinger, 1981; Dietrich and Mulder, 1981) has solved this problem. Treatment of the chemical(s) gives rise to two gaps in spermatocyte development with a small fraction of spermatocytes in between, which are at about the same meiotic stage. By studying the testicular material at successive periods after the treatment it is possible to determine the sequence of the different stages. The restricted spermatocyte population created by HU/T treatment spans a difference in developmental age of about 1.5 days and consists of 2-3 stages of the epithelial cycle (Oakberg, 1956a,b). This implicates that it is rather difficult to give an accurate timing of short lasting stages (Oud and Reutlinger, 1981). It is thus possible that the correlation between the characters and age of the meiotic cells, which is the basic aim of sequential analysis, is less specific. Thus this method suffers from difference in the ages of intra- and intergap spermatocyte population and the interference of the restricted spermatocyte population by germ cells of advanced stages. This problem will be solved if the meiotic cycle under study begins with a population of spermatogonia of the same age which are the oldest germ cells of the testes as yet. In male rats, gossypol, a reversible male antifertility agent, kills the meiotic and post meiotic cells except the undifferentiated spermatogonia and seminiferous tubules are depopulated leaving a layer of cells consisting of undifferentiated spermatogonia and sertoli cells only (Xue et al, 1980; Bhagirath and Kundu, 1983). It should be possible to determine the sequence of SC development at different meiotic prophase stages by examining the meiotic cells on successive days after termination of gossypol treatment without the inherent problem associated with HU/T treatment method. Besides the above mentioned problems of HU/T treatment method the possibility that some cells may have been damaged by HU/T treatment cannot be discarded (Die-
trich and de Boer, 1983). This drawback is absent from gossypol treatment method because this chemical is nontoxic (Xue et al, 1980), does not induce abnormalities to meiotic chromosomes (Bhagirath and Kundu, 1985) and mitotic chromosomes (Tsui et al, 1983), and nonmutagenic (see Bhagirath and Kundu, 1985 for review in section 4.9 of this text).

Using whole mount microspreading preparations it is possible to study all the SCs in their entirety in one spermatocyte (Counce and Meyer, 1973). But the interrelations within the seminiferous tubules are eliminated by the preparation technique and thus it is no longer possible to recognize the different meiotic stages by comparing them with spermatid stages in the same preparations (Dietrich and Mulder, 1981). But it will be possible to identify the meiotic stages of the synchronously repopulating spermatocytes in spread preparations after gossypol treatment as they are the oldest germ cells in the testes. Dietrich and Mulder (1981) further argue that chromatin and SCs cannot be visualized in one whole mount microspread preparation and it excludes a direct sequential analysis of the development of the SCs through the prophase of spermatocytes. Contrary to it the authors state that "Using the whole mount spreading technique for light microscopy it is easy to distinguish between the different prophase stages of male meiosis and to recognize diplotene by the presence of axial elements and the shape of the SCs". Indeed analysis of SC development and behaviour through meiotic prophase stages of the synchronously developing first generation meiocytes by a variety of methods including surface spreading is possible (Speed, 1982; Dietrich and Mulder, 1983; Roth, 1966; Fiil and Moens, 1973). Therefore the testes of gossypol treated male rats can be easily and reproducibly staged for examination by whole mount microspreading method.

Male meiosis in the albino rat, Rattus norvegicus has been described by Ohno et al, (1958) at the light microscope level using air drying method. The ultrastructure and the composition of the SC during pachytene of albino rats have been studied (Solari, 1972; Dumontier and Sheridan, 1977). These papers dealt mainly with the effects of fixatives, salts, dehydrating agents and DNase on the structure and morphology of SC. Development and behaviour of SC and the timing
of the meiotic prophase of male albino rats have not yet been described. Sequential analysis of the SC through meiotic prophase stages of the male albino rat using gossypol treatment and whole mount surface spreading technique was, therefore, undertaken to obtain further information on the development and behaviour of SCs at different meiotic stages. (This work consists of a paper entitled "Sequential analysis of synaptonemal complexes in the repopulating spermatocytes of Rattus norvegicus after restricting the germ cell population to spermatogonia by gossypol treatment" in collaboration with S.C. Kundu which will appear in European Journal of Cell Biology Vol.40, No.2, 1986).

The present study includes also the application of SC analysis to cytogenetic studies and part of this chapter consists of a published paper (presented in section 4.9 of this text) entitled "Effects of the male contraceptive agent gossypol on meiotic chromosomes of the male rat" in collaboration with S.C. Kundu, Cytogenetic. Cell Genet. 39:228-230 (1985).

SCs of species hybrids may be normal (Moses et al, 1979) or abnormal (Menzel and Price, 1966; La Cour and Wells, 1973; Abirached-Darmency et al, 1984) depending on whether the two species are genetically balanced or not (La Cour and Wells, 1973). The question as to the nature of chromosome pairing and SC formation is more intriguing in hybrids of two species having wide disparity of chromosome number and morphology.

Jolly et al, (1969) succeeded to produce fully fertile hybrids of two silkworm species, Chinese Antheraea pernyi (n=49) and Indian A. roylei (n=30) and the hybrid has been named as A. proylei. They observed 30, and 30-49 chromosomal units in the metaphase I of the F1 and F2 respectively of both the cross and reciprocals (Jolly et al, 1979). Unfortunately pairing behaviour of the parental chromosomes in the primary and early subsequent hybrid generations and backcross progenies was not studied.

Careful examination of Effective Rearing Ratio indicates a sign of low viability in F2 (Tazima, 1974). Rearing records at Regional Tasar Research Station at Imphal and several other Tasar Farms of Manipur in 1975-1981 showed a sign of adverse effects on viability in filial generation twenties and thirties. For the assurance of the
crop the stabilization of chromosome constitution and/or regular disjunction at meiosis are urgently needed as far as the use of filial generations of the hybrid is contemplated. SC analysis in the subsequent filial generations of this hybrid to understand the nature of chromosome pairing and segregation is of utmost importance not only from scientific but also from industrial points of view. The study will also provide information as to the behaviour of chromosomes in the preceeding generations. Moreover, the structure of the SC will provide information on the chromosome homology and genetic balance between the two species. The present study was, therefore, undertaken in order to understand (a) the chromosome homology and genetic balance between the two parental species, (b) the chromosome constitution, pairing behaviour and disjunction in the current and the preceeding generations of the hybrid and to predict (c) the future chromosome constitution and fate of the hybrid.

The aim of the present study in general are: (a) application of surface spreading technique in preparing SCs from spermatocytes and oocytes of animals of different taxonomic groups, (b) to compare the precision of information obtainable from microspreading analysis with that known from 3-dimensional reconstruction, (c) comparative study of meiotic prophase in animals of different taxonomic groups with the analysis of SCs, (d) application of SC analysis to sequential analysis of meiotic prophase in male animals using gossypol, a male contraceptive agent, and (e) application of SC analysis to cytogenetic study.

The present text describes the main results of the work primarily obtained at the light microscope level by surface spreading and silver staining of meiotic nuclei of animals of different taxonomic groups, of treated animals and hybrids. The interpretation of the qualitative and quantitative data are incorporated into the general scheme of meiotic prophase.