STUDIES ON THE SALIVARY GLAND CHROMOSOMES OF SOME SPECIES OF BLACK FLIES (DIPTERA : SIMULIIDAE) FROM DARJEELING

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DEDICATED TO
MY
BELOVED PARENTS
AND
RESPECTED TEACHERS

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GENERAL INTRODUCTION

In the Simuliidae, analysis of salivary gland polytene chromosomes of larvae has contributed significantly to evolutionary and cytotaxonomical research. Additionally, such studies also have practical value in efforts to control diseases transmitted by Simuliid vectors. Our knowledge on the chromosomes of black flies has been accumulated considerably since the pioneering study of the salivary gland chromosomes of Simulium virgatum by Painter and Griffen (1937). Further studies (Kunze, 1953;Rothfels and Dunbar,1953) revealed that the gross features of salivary gland chromosomes could be useful in segregating the species cytologically. The substantial contribution made by earlier workers in the formative years of black fly cytology have been reviewed extensively by Rothfels (1979). Further impetus for the cytological investigation of black flies stems from the availability of sibling species which is rule rather than exception in Simuliidae (Rothfels, 1979). Recently, efforts have been made to study the cytotaxonomy (Procunier 1982; Gordon 1984; Brockhouse 1985; Hunter and Connolly, 1986; Post,1986; Elsen and Post 1989; Bedo,1989; Conn et al,1989)and ecology (Adler and Kim 1984; Adler, 1987) of sibling species since a good cytotaxonomic understanding and complementary ecological knowledge is important in deriving a vector control strategy which minimises the risk of spreading insecticide resistance (Post, 1986; Adler and Kim ,1984). However, the bulk of these cytological investigations have dealt with species

from North America and Europe. It is therefore of interest to extend such studies to black flies from other areas. The cytotaxonomic studies on black flies have been reviewed hereunder.

The chromosome complement of black flies

The basic chromosome complement of the Simuliidae consists of n = 3 chromosomes with median or submedian centromeres in all the genera so far studied. Accordingly, the polytene salivary gland nuclei contain three chromosomes with two homologues more or less intimately paired. The centromere regions in most species form a characteristic expanded regions dividing the polytene chromosomes in the same proportion as the mitotic metaphase chromosomes (ganglion or gonial). In some species, identification of centromere sites is confirmed by frequent ectopic pairing, while other species have a tight chromocenter. Each species typically has one major puff, Balbiani Ring (BR) and a nucleolar organiser region (NO). Additional landmarks include characteristic band grouping and parabalbiani (PB). Moreover, idiogram could be constructed from the measurements of each arms and are helpful to diagnose the species cytologically (Rothfels, 1979). Other important cytological features include supernumerary or B-chromosomes and aspects of male meiosis i.e.,chiasmate or achiasmate (Procunier,1975).

Cytological studies of different groups of Simuliidae

While compiling the cytological data on different groups of Simuliidae, classification suggested by Rothfels (1979) has been followed for practical convenience and consistency. Chromosome information now available for three subfamilies namely , Gymnopaiidinae, Prosimuliinae and Simuliinae. Of these , the subfamily Simuliinae has been extensively studied.

Gymnopaiidinae

In this subfamily chromosome data of seven species belonging to two genera are available at present (Rothfels and Freeman,1966; Chubareva,1977; Rothfels,1979). Species of Gymnopais differ from each other by inversion in each of the three chromosomes and in width of bands (Rothfels ,1979). Moreover,there is homology between the salivary gland chromosome map of Gymnopais and Twinnia (Chubareva,1977). However there are no cytologically determined sex chromosomes in the species of Twinnia (Rothfels & Freeman, 1966).

Prosimuliinae

This subfamily is better known cytologically than the previous one. Chromosome information is now available for five genera namely, Prosimulium , Heldon , Urosimulium , Gigantodax and Crozetia . On the basis of specific chromosomal characteristics, the species belonging to Prosimulium are placed in five major groups (Rothfels 1979) namely , hiritipes group , mixtum group , esselbaughi group

, magnum group and ursinum/ macropyga group. The member of hiritipes group (Basrur,1959) are characterised by the presence of vastly elaborated centromere region in chromosome I (transformed centromere, C_t). On the other hand, mixtum, esselbaughi and magnum groups of species are characterised by IIIL-1 (Rothfels, 1956), IIIL-2 (Basrur,1962 and Rothfels,1979) and IIIS-1 (Ottonen, 1966) inversions respectively. However, the fifth group does not have a cytological marker of its own and is represented by the standard Prosimulium sequence (Rothfels, 1979).

Chromosomally, the genus Helodon standard differs from that of Prosimulium in always having IIIS-2 inversion, frequently IIIS-3 inversions and never IL-1 (Rothfels and Freeman, 1966). In addition, all known members of the group share a basic inversion in IIIL (Rothfels, 1979). On the basis of apparent chromosomal deficiencies, Chubareva and Petrova (1969) have suggested that Helodon is phylogenetically younger than Prosimulium. However, according to Rothfels (1979), this conclusion may be correct cytologically but directionality can be read either way.

In the genus Urosimulium, our cytological knowledge is limited to a single species, Urosimulium stefanii (Frizzi et al., 1970), which is closely related to Prosimulium hiritipes group of species (Rothfels, 1979).

In the genus Gigantodax chromosome map has been prepared for Gigantodax bonarissorum and for two unnamed species (Rothfels, 1979). Certain land marks of these

species can be homologised with those of species in other genera. However, no serious attempt has yet been made to extend the knowledge on this genus.

The genus Crozetia is represented cytologically by a lone species , Eusimulium aureum (Rothfels and Mason, 1975), which is characterised by the presence of drumlike centromeres in all three chromosomes. The centromeres are expanded and deeply stained. The nucleolar organiser is in the base of IS, the balbiani ring (BR) is in the base of IIS and parabalbiani (PB) is distally located in IIL. However, heterozygous inversions were not encountered in any of the larvae. Meiosis in the male is found to be achiasmate. This genus has aroused considerable interest because of its isolated geographic position and abnormal head structure of its larvae (Rothfels, 1979).

Simuliinae

Extensive cytological work has been carried out on this subfamily. Chromosome information is now available for 13 genera belonging to five tribes namely , Austrosimuliini, Cnephiini, Eusimuliini, Wilhelmiini and Simuliini (Rothfels,1979).

Tribe Austrosimuliini

In the genus Austrosimulium detailed chromosomal studies has been carried out in three species namely, A. multicorne, A. laticorne and A. ungulatum, of which the

first named species is considered as standard (Rothfels, 1979). All the three species have three chromosomes identifiable by the standard Simuliid landmarks namely, the NO in the base of IS; the IS end is identical to that of other genera; IIS with the BR (Landau, 1962), and trapezoid (Rothfels et al., 1978); IIL with PB ; and IIIS with the frazzled end and blister. Chromosome III is identical in all three species. Sex chromosomes remain unknown in A. multicorne while A. laticorne and A. ungulatum have sex differential segments in 1L and 11L respectively.

It has also been suggested that A. tillyardi and other species of Austrosimulium differ from all other Simuliinae in a pericentric inversion in third chromosome (Chubareva and Petrova, 1975). However, very little information is available on Australian species of this genus. Bedo (1976) describe the polytene chromosomes from malpighian tubules of three Australian species of Austrosimulium namely, A. bancrofti, A. victoriae and A. torrentium. The chromosomes of these species are found to be thinner than those of Simulium species. However, of these three Austrosimulium species, satisfactory malpighian chromosome preparation could not be made using A. torrentium. However, the chromosome map of A. montanum are good enough to allow comparison with other species (Rothfels, 1979). The centromeres are well banded in A. bancrofti and they tend to form a chromocenters in A. torrentium. Nucleolar organiser of A. bancrofti is in IL and that of A. victoriae is in

first arm. Moreover, the chromosome of Austrosimulium appear to be smaller than those of Prosimulium, Simulium and Wilhelmia. This cytological feature may be phylogenetically significant (Rothfels,1979).

Tribe Cnephiini

Genus Cnephia

In this genus, five species are known cytologically (Basrur,1957; Petrova, 1972; Procunier,1975 a,b; 1982 a,b) namely, Cnephia dacotensis,C. pecuarum,C. eremites,C. ornithophilia and C. lapponica. Four of the five species have a chromosome number n = 3, with C. lapponica being reduced to n = 2 as a result of a fusion of chromosomes II and III. All species exhibit tight pairing of homologues. For n = 3, species the centromere regions of chromosomes I and II are expanded while the centromere region of chromosome III shows minimal expansion. NO is present in 1S while RB and PB are found in IIS and IIL respectively. The standard chromosome map of Cnephia has been described by Procunier (1982). Moreover, all members are male achiasmate. Sex chromosome differentiation varies from nonobservable in C. ornithophilia and C. eremites through C. pecuarum in which the standard and IS-5 sequence are distributed differentially over X and Y chromosomes, to the polytypic system of C. lapponica in which the X-chromosome is fixed for expression of the nucleolar organiser (NO) and the Y chromosome nonexpression. Further, all the five species

differ from each other by interspecific inversions and species-specific floating inversions. Besides autosomes, C. dacotensis and C. ornithophilia have B-chromosomes (Procunier, 1975a). However, the B-chromosomes of C. ornithophilia pair back on themselves, indicating that the ends are homologous for a considerable distance (Procunier, 1982 b).

On the other hand, the Australian species assigned to Cnephia are poorly studied cytologically. However, all the species studied so far revealed undifferentiated sex chromosomes, suggesting inclusion in a separate genus Paracnephia (Rothfels, 1979).

Genus Stegopterna

This genus is known cytologically from the study of three species namely, S. richteri, S. mutata (both diploid and triploid) and S. emergens (Madahar, 1969). In S. mutata, the only arrangement is found on the centromeric region of chromosome I and this region is associated with sex determination. The triploid females are fairly heterozygous and have four inversions in IL and two in IIL . The sexual diplnids are restricted to Southern Ontario and parthenogenetic chromosomal triploids are more widely distributed (Madahar, 1969).

Genus Metacnephia

This group is better known cytologically in comparison to the other genera of Cnephiini. Chromosome data are now

available for 15 species namely, Metacnephia korsakovi, M. pallipes, M. terterjani, M. subalpina, M. persica, M. kirjovavae, M. petrovae, M. pamiriensis, M. freytagi, M. crete, M. tredecimata, M. sommermanae, M. borealis, M. saskatchewana and M. amphora (Petrova, 1973a, 1973b, 1974, 1977; Procunier, 1982). All the species have a diploid chromosome number of n = 3 and they exhibit tight pairing of homologues with the centromere regions of chromosome I and II being expanded, while the centromere region of chromosome III shows minimum expansion. Chromosome I and II are metacentric, while chromosome III is submetacentric. The nucleolar organiser (NO) is present in the base of IS, ring of Balbiani (RB) in IIS, the parabalbiani (PB) in II L, and the blister (B) in IIIS. All members are male chiasmate and differ from related Cnephia by a whole arm interchange between chromosomes I and II. It has been suggested that the interchange is as characteristic cytological marker for Metacnephia (Procunier, 1982). Sex chromosome differentiation varies from X_0 Y_1 male of M. amphora to a complex system in M. borealis. The closest members of Metacnephia differ only in their sex chromosomes and share floating inversions. Among the members of Metacnephia, M. borealis is unique in having a large submetacentric B-chromosome (Procunier, 1982).

Genus Sulcicnephia

Only three species namely, Sulcicnephia ovtshinnikovi, S. lobashovi and S. petrovae of this genus

have been worked out cytologically (Chubareva and Petrova, 1975). They have suggested homology between the first named species and Austrosimulium tillyardianum. However, the cytological knowledge of this group is still inadequate.

Genus Ectemnia

Only two species of this genus have been cytologically worked out so far (Madahar, 1967). The comparison between Ectemnia invenusta and Ectemnia taeniatifrons revealed that the chromosomes of E. invenusta are tightly paired. The nucleolar organiser is at the base of IS, while the Balbiani Ring is towards the centromere in IIS. Inversion polymorphism occurs atleast in IIS and IIIL. On the otherhand, the chromosomes of E. taeniatifrons are loosely pairod. The nucleolar organiser is in the center of 1S while BR is in the middle of 11S. No polymorphism were found in this species.

Tribe : Eusimuliini

Genus Eusimulium

Our cytological knowledge of Eusimulium is still fragmentary. Dunbar (1962) divided this taxon into several species groups, of which aureum and vernum groups, have been studied in detail. Eusimulium aureum group has n=2 chromosomes. The very long dicentric first chromosome is due to the fusion of chromosomes II and III i.e. I of aureum = II and III of other simuliids; II of aureum = I of other

simuliids (Dunbar,1958). Chubareva(1974) also reported n=2 for Eusimulium brachyantherum. Therefore, this group would be a promising one in which to search for reduction of the chromosome complement to the theoretical level of one (Rothfels,1979). On the otherhand, Eusimulium vernum group has a chromosome number n=3,in common with most blackflies. In general the pairing of the homologues is loose, although the degree of pairing varies among 12 cytotypes within Eusimulium vernum group. The NO is in the base of IS arm throughout the vernum complex. Two of the cytotypes have a chromocenter while four carry supernumerary chromosomes. Moreover, five of the total of six chromosome arms are involved in sex determination in the various member of this complex (Brockhouse, 1984, 1985). Study of the cytotaxonomy of seven species in the E. vernum group (Hunter and Connolley, 1986) further revealed that there exist two cytological lineages within E. vernum group. E. aestivum, E. impar, E. pugetense, and E. quebecense belong to one lineage, while E. gouldingi, E. croxtoni and Simulium sp. to the other. The former lineage is characterised by the fixed inversion IIIS-1 and by IIIL - 1? (fixed and floating); the latter lineage by fixed inversions IL-1,2,3&4; IIL-4,and IIIL-4,5 and by IIIL - 6 (fixed and floating).

Genus Inseliellum

Only two species of this genus namely, Inseliellum tahitiense and I. oviceps have been studied cytologically

(Rothfels, 1979). Chromosomally these two species are extremely close. Four of the six arms are identical. No more than four inversion differences exist. In both the species, females are hetrogametic and are more closely related to each other than to any other Eusimulium so far studied. However, I. tahitiense is male chiasmate whereas I. oviceps is male achiasmate.

Tribe Wilhelmiini

Genus Wilhelmia

Our knowledge on the chromosome of this genus is limited to few species only. W. equina is one of the first black fly species studied chromosomally (Montalenti 1947). Extensive inversion polymorphism exists while no sex chromosome has been recorded in this species so far studied (Grinchuk, 1968,1969; Grinchuk & Chubareva, 1972,1975).

Genus Edwardsellum

Species of this genus received considerable cytotaxonomic attention since this genus includes the vectors of human onchocerciasis in Africa (Dunbar,1960, Vajime & Dunbar,1975). Twenty-five sibling species of Edwardsellum damnosum have been described so far (Quillevere et al.,1976).

Tribe Simuliini

This tribe include three cytologically studied group namely, Psilozia, Shewellomyia and Simulium. Except Simulium, cytological studies are piecemeal in other groups.

In the following discussion Psilozia and Shewellomyia have been given subgeneric rank (Cup & Gordon, 1983) for our practical convenience.

Subgenus : Psilozia

Simulium (Psilozia) vittatum was the first blackfly species to be mapped (Rothfels and Dunbar, 1953). The congeneric species S. (P.) argus also showed the same gross feature as S. (P.) vittatum, with chromosome arms IL, IIS and IIIS identical. However, IIL differs by two fixed inversions and IS is homologous upto the centromeric region (Pasternak, 1964).

Subgenus :Shewellomyia

The species in Simulium (Shewellomyia) have been examined by conventional staining and quinacrine fluorescence staining methods (Bedo ,1975). Simulium (Sh.) pictipes Hagen consists of three siblings, pictipes A, pictipes B, and S. (Sh.) longistylatum. In all three siblings, the haploid chromosome number is three. Specific differences include a simple and a complex inversions, a shift of basal bands between the short arm of the second and third chromosomes, details of the sex chromosome and the amount of DNA in certain individual bands and expanded centromeric regions. However, the unique situation is that Y chromosome markers are located in a different element of the complement in each of the three species. Thus S. pictipes A

has heteroband in IIL, and S. pictipes B and S. (Sh.) longistylatum have Y chromosome inversions in IIIL and IS respectively (Bedo, 1975).

Genus Simulium

This genus is better known cytologically than the other groups of Simuliidae. S. tuberosum contains four to five siblings (Landau,1962; Rothfels,1981). All major species - specific inversions were found on IIS and all are sex related. The banding sequence of other arms were identical in all the siblings except for 83 floating inversions. The A,B sibling has been found in Europe and North America and is thought to be true S. tuberosum (Rothfels,1981). The geographical distribution of sibling of S. tuberosum was further studied by Mason (1982). He observed four new siblings which like originals have fixed differences in chromosome arm IIS. One of these, FGI, distinguished by a high degree of polymorphism and the presence of fixed differences from the tuberosum standard in arms IS, IL and IIIL, occurred both in Alaska and Norway. A comparison of the standard tuberosum, the S. venustum and the FGI sibling chromosome pattern revealed that the FGI sibling to be much closer to the venustum standard, than any other tuberosum sibling (Mason,1982).Sex chromosome polymorphism in S. tuberosum complex has also been studied by Mason (1984). He observed that the closely related siblings species could be distinguished by the banding pattern on their sex chromosome. Simulium tuberosum differs from the venustum

standard by a total of 16 inversions and from all other Simulium species by having the NO in the base of IIIL in other species (Rothfels, 1979). Moreover, Simulium vittatum was found to be composed of 3 sibling species, two of which are defined as IIIL-1 and one as IS-7 cytospecies (Rothfels and Featherston, 1981). There are several polymorphisms, many of which are shared by both siblings and which vary in their frequencies within each sibling. Further studies (Adler and Kim, 1984) revealed ecological difference between IIIL-1 and IS-7 siblings. The species S. decorum which is a member of Simulium argyreatum / decorum complex also have sibling species. The decorum sibling is distinguished from the other two by the presence of a heavy band at the base of IL and by chromosome III being the sex chromosome (Rothfels, 1981). Furthermore, one of the largest complexes in North America is that of the Simulium venustum /S. verecundum which includes the principal noxious biter of man. Study of polytene chromosomes show that both S. venustum and S. verecundum include a minimum of seven sibling species designated by their IIS sequence (Rothfels et al., 1978). The basic chromosome complement of S. venustum/verecundum (n=3), in arm association and arm ratios, is same as in S. tuberosum, except that the NO is in the base of IIIL rather than IIIS. This change in position of NO is common to all other members of Simulium so far studied (Rothfels et al., 1978). Moreover, S. verecundum lineage differs from its venustum counterparts by 10 fixed

inversions (Rothfels, 1981). Limnological features are also found to be associated with the distribution of the cytotypes of this complex (Gordon and Cupp, 1980).

However, no sibling species were found in jenningsi group (Gordon, 1984). Three species S. jenningsi, S. fibrinflatum and S. luggeri differ by 6 fixed inversions and by 19 floating inversions, 4 of which are related to sex determination in S. jenningsi. Sibling species was also revealed by the analysis of polytene chromosomes of five S. neornatipes populations (Bedo, 1984). These sibling species share a common standard polytene chromosome banding sequence which differ from Australian S. ornatipes complex standard by five fixed inversions. The neornatipes species are distinguished from each other by additional fixed inversions and differentiated sex chromosomes. It has been suggested from the study that the rearrangements themselves have no direct role in the speciation process of the group.

The cytotaxonomy of S. sanctipauli and S. soubrense has been described by Post (1986). It has been noted that chromosomal inversion 2L-7 is not only responsible to separate the above named species. However, two newly recognised inversions 1L-A and 2L-A can be used in combination to identify S. sanctipauli, S. soubrense and a new species S. soubrense B. Adler (1987) described the polytene chromosome of S. loerchae Adler, a new species in the S. vernum group. The chromosome number is found to be n=3. It has a fixed inversion at 1L -2 and possesses a

primitive X_O Y_O sex chromosome system and lacks autosomal rearrangement. It has been suggested that this species has been derived from the vernum standard by three inversions. Furthermore, a comparison of the polytene chromosomes of S. furculatum and the S. vernum standard revealed that the former does not belong to the S. vernum species group (Hunter, 1989).

The study of the polytene chromosome banding patterns of 11 members of the S. metallicum complex revealed that, as in other members of Simulium, the chromosome complement consists of n=3 (Conn et al., 1989). For chromosome I, the puffed region followed by three heavy bands in section 12 and 13 in the IS which serves to distinguish IS from IL. Arms IS and IL may also be separated by the banding patterns in their ends. Chromosomes II and III are characterised by standard land marks ; Balbiani ring and the double bubble in IIS, parabalbiani and grey band in IIL, the blister and the capsule in IIIS and the basal marker in IIIL. Centromere regions in chromosome II and III of all members of the S. metallicum complex examined so far are rather bulbous and uniform in expression. Bands are discernible in the centromeres but they do not stain darkly. Construction of cytophylogeny separates the 11 members of S. metallicum complex into three lineages. Elsen and Post (1989) found a new subspecies within the Simulium damnosum complex on the basis of analysis of polytene chromosome and the larval morphology. It is named S. (Edwardsellum) squamosum

kitetense ssp.n. Chromosomally, it is most similar to S. squamosum from which it differs by 3 new fixed inversions.

Bedo (1989) studied the polytene banding patterns of S. ruficorne populations from two islands and a continental African locality. A standard map was prepared and compared with that of S. ornatipes-neornatipes species complex in Australia and New Caledonia shows striking similarities, 90% banding homology between the two standards and three shared inversions between the lineages further emphasised their similarity. These result corroborate a close taxonomic relationship between S.ruficorne and S.ornatipes.

CHROMOSOMAL POLYMORPHISM IN SIMULIIDAE

Many species of animals appear to be cytologically monomorphic, while others show various kinds of chromosomal polymorhism. Dipteran polytene chromosome provide a high resolution system for the accurate and detailed study of chromosome polymorphism. Most investigators exploiting these chromosomes have examined rearrangements of polytene chromosome banding pattern resulting from inversions or less commonly translocation. However, with the availability of the staining procedure, it is now possible to study band and nucleolar polymorphisms.

The family Simuliidae offers excellent opportunity for studies of this kind. Polymorphism for inversions, interchanges and band width are established in populations

while their frequencies may fluctuate seasonally, altitudinally or geographically. They are considered to be essentially established or balanced (Rothfels, 1980).

Interchanges

Cases of natural interchange polymorphism are not known in blackflies (Rothfels, 1980). However, in some populations of Cnephia lapponica (Norway) metaphase I of males reveal whole arm interchange of the n=2 chromosome, giving an $X_1X_2Y_1Y_2-X_1X_1X_2X_2$ system of sex determination. Male meiosis is achiasmate and disjunction alternate (Rothfels, 1980).

Pericentric inversions

Large pericentric inversions are not known as polymorphism in blackflies. Though some smaller autosomal ones are well documented in Prosimulium mixtum (Rothfels & Freeman, 1977), small pericentric inversions are common as sex differential segments and many of them exist as sex chromosome polymorphism. Small pericentric inversions were also reported in S. ornatipes (Bedo, 1977). Such small pericentric inversions may be favoured because crossing over with resultant duplication- deficiency is minimal around the centromere which may be embedded in heterochromatin (Rothfels, 1980).

Paracentric inversions

Paracentric inversion form the basis from which most polymorphisms arise. It appears probable that paracentric

inversion polymorphism may be long lived and survive one or more speciation events. They may be fixed in one line of descent and lost from another (Rothfels,1980). A number of examples are on record regarding sharing of inversion polymorphism among sibling or related species;e.g.,in S. venustum/verecundum (Rothfels et al.,1978), S. tuberosum (Landau,1962), S. damnosum (Vajime and Dunbar,1975) and S. ornatipes (Bedo,1977). A recently-studied (Rothfels,1980) interesting case is that of S. vittatum distributed throughout North America, Greenland, Iceland and Faroes. In temperate North America , two sibling species are recognised, IIIL-1 and IS-7, differing in their sex chromosome. The IIIL-1 sibling has males heterozygous for a Y-chromosome inversion (IIIL S/1), and females homozygous for the standard form (IIIL S/S). The IS-7 males are heterozygous for a chromosome I inversion (I S - S/7), with females homozygous for the inversion (7/7). There are no fixed inversion differences between siblings, but very large numbers of autosomal inversion polymorphism, most of which are shared between siblings, at very different frequencies. In pure population of III L-1, the Y-chromosome typically has the inversion and the five particular autosomal inverted sequences are relatively infrequent, generally of the order of 20%. The 111L-1 sibling has been found in Southern Canada from Quebec to Saskatchewan and in the north-eastern states, west to Wisconsin and south to Louisiana. On the other hand, pure 1S-7 sibling populations are characterized

by the IS-7 X-chromosome. In eastern North America, this sex linkage may only be partial, while in the western provinces sex linkage appears to be complete and additional inversions may be superimposed both on 'X and Y'. In eastern North America, all five types of autosomal inversions polymorphism are characterised by high frequencies of the inverted sequences of the order of 60% or more.The IS-7 sibling is distributed through southern Ontario, Michigan, Pennsylvania and west to British Columbia and Alaska. The two siblings are widely sympatric in Ontario, New York state, Michigan and Wisconsin, and frequently occur in the same stream or more or less in synchrony and through several generations a year. Where they co-exist, each sibling maintains cytological integrity. Therefore, the inversion polymorphism may be fixed in some populations while in others they have been lost. It has been suggested (Rothfels,1980) that paracentric inversions are responsible for most of the chromosomal restructuring in Simuliidae. Furthermore, Dubovy and knoz (1982), concluded from the study of inversion polymorphism in Simulium argyreatum that the heterozygote inversions IS 1,IL 3 and IIIS 1 play an important role in the adaptation to the changing environment.

While studying the polytene chromosome polymorphism in the sibling species of S. ornatipes A, Bedo (1979b) observed that the populations of this species can be separated into two groups A1 and A2. The chromosomal system of A1 and A2

show distinct differences. A1 has a flexible system with high levels of polymorphism and geographic variation. By contrast A2 has a more rigid system with far less polymorphism and geographic variation. The results highlight different adaptive mechanism in two species.

Nucleolar polymorphism

Nucleolar organiser show heteromorphism for expression and rare secondary nucleoli are found on all chromosomes. Heterozygosity of nucleolar expression has often been reported in Simuliidae, although its frequency within a species is normally rare (Basrur, 1959; Dunbar, 1958, 1959, 1967; Procunier, 1975a; Ottonen, 1966; Rothfels and Freeman,, 1966). A dark nucleolar organiser band replaces the nucleolus in all cases. The secondary nucleoli of S. ornatipes A, with the exception of that in IIIS, also appear to originate from heavily stained bands (Bedo, 1977). Secondary nucleoli which are also found in several other black fly species should be considered with the problem of diversity of main nucleolar sites in the Simuliidae. Characteristically, perinucleolar inversions are not involved in intraspecific nucleolar shifts. It would appear, therefore, that a multiplicity of potential nucleolar sites may exist within the genome, one such site being dominant to the virtual exclusion of others in any given species. Secondary nucleoli are manifestations of incomplete suppression of some of these sites (Bedo, 1977)

or sometimes secondary nucleolus replaces the main one as found in Eusimulium aureum (Dunbar, 1959).Therefore,it has been suggested (Bedo, 1977) that nucleolar polymorphism in different black fly species is due to the multiplicity of sites for ribosomal genes and their selective amplification.

STUDY OF POLYTENE CHROMOSOME IN SIMULIUM BY BANDING TECHNIQUE

Different banding techniques namely., C - G - and Q - banding have been developed to study the linear differentiation of chromosomes. The development of C-band technique (Pardue and Gall, 1970; Sumner, 1972, Gallaghar et al., 1973) by utilising Giemsa staining and development of G - banding (Caspersson et al., 1969) by using fluorochromes are widely used for the identification of centromeric region. It has been believed the C-banding procedures reveal areas of constitutive heterochromatin (Arrighi et al., 1970). In Simulium polytene chromosome consistently show C-banding of centromere regions, telomeres, nucleolar organiser and numerous interstitial sites (Bedo, 1975b). The interstitial C-banding sites correspond to morphologically single polytene band. Interstitial C-bands in S. ornatipes are scattered throughout the complement, whereas in S. melatum they are clustered. Mitotic chromosome of both species show a single centric C-band with indication of two weak interstitial bands in S. ornatipes suggesting that many C-band regions detectable in polytene chromosome are not resolved by present technique in mitotic chromosome. Contrary to current opinion that C-banding is diagnostic for

constitutive heterochromatin, the interstitial C-band sites of polytene chromosome are regarded as euchromatin. However, the heterochromatic pericentric regions of S. ornatipes are not C-banded. It has been suggested that polytene chromosomes of Simulium are promising system for the elucidation of C-banding mechanism. Quinacrine fluorescence is also used to detect the centromeric regions of ganglion and polytene chromosomes of the species of pictipes group (Bedo, 1975a). Moreover, in males of S. pictipesA, X and Y chromosomes could be distinguished from each other from their fluorescence characteristics.

DNA REPLICATION IN THE POLYTENE CHROMOSOME OF SIMULIUM

Although considerable work has been carried out on the replication pattern of Dipteran polytene chromosome (Kalisch and Hägele,1973, 1976 ;Hägele and Kalisch, 1974 ; Hägele, 1973; Gall et al.,1971; Lakhotia and Roy, 1979 ; Lakhotia and Mukherjee, 1970), lone attempt has been made to study the replication pattern of polytene chromosome in Simulium ornatipes (Bedo, 1982) since the polytene chromosome system of this species is rich in inversion polymorphisms, presence of amplified and supernumerary polymorphic band as well as all three chromosome pairs are C- band positive (Bedo,1975b,1977,1979a,b). Study of the replicative behaviour of the heterochromatic and C-banding regions of polytene chromosomes of S. ornatipes, using H^3 and C^{14} thymidine shows that chromosome synthesis follows three

distinct phases namely, a short phase of initiation in puffs and interbands spreading to more condensed regions; a long continuous labeling phase and a discontinuously labelled end phase. Analysis of H^3 labeling patterns indicated that while heterochromatic bands replicate there is no clear correlation between heterochromatic or C-banding regions and band replication time. The major characteristic governing band replication time appears to be band size and density. However, in some bands this relationship is modified, by DNA organisation, influencing the efficiency of replicons. The existence of great variability in homologous band replication time, even within a chromosome pair, indicates that the control of band replication is highly autonomous. Therefore, it has been suggested (Bedo,1982) that the polymorphisms at the molecular level determine this variation. Moreover, replicative behaviour of nucleolar organiser is somewhat unusual in S. ornatipes. The long replication time of active nucleolar organiser in contrast to the short replication of condensed inactive organisers is either due to the differential polytenisation of ribosomal DNA or due to the amplification of ribosomal DNA, by active nucleolar organiser (Bedo,1982). However, further investigations are desired in this direction.

SUPERNUMERARY CHROMOSOMES IN SIMULIIDAE

In natural populations of certain species of animals and plants the supernumerary chromosomes (B-chromosome) are present in some individuals but not in others. In some cases

the majority of population may carry supernumeraries, while in other instances the frequency of individuals carrying them is very low. In animals, the B-chromosomes of classical type have been described in flat worms (Melander,1950), snails (Evans, 1960), Isopoda (Rocchi,1967), grasshoppers, scale insects, Heteropteran, Lepidopteran, beetles and in some Diptera (White,1973). In Simuliidae, however, our knowledge on the B-chromosome is still inadequate. Procunier (1975 b), reported the presence of B-chromosomes in Cnephia dacotensis and Cnephia ornithophilia. In Metacnephia borealis, (Procunier,1982 a), the B is large submetacentric chromosome and in polytenized state it is approximately one-third the length of the IIS arm. The same author (Procunier,1982 b) also observed the interdependence of B-chromosomes, nucleolar organiser expression and larval development in the black fly species Cnephia dacotensis and Cnephia ornithophilia. This system may account for the wider range and occupation of more diversified habitats of the members of these two species, possessing B-chromosomes. Mitotic B-chromosomes smaller than the normal autosomes were also encountered in Simulium (Eusimulium) gracilis and Simulium (Eusimulium) ghoomense (Dey and Wangdi, 1984).

MEDICAL AND ECONOMIC IMPORTANCE OF BLACK FLIES

Black flies are economically and medically important because of the blood sucking habits of the adult females of many species. Because of their blood-seeking behaviour,

injection of saliva during ingestion of blood, and their occasional large numbers the black flies can be detrimental to animal production namely, weight loss and reproductive dysfunction (Freeden, 1977), decrease in milk and egg production (Jamnback, 1973; Steelman, 1976 ; Watts, 1976), dermatitis and skin lesions (Gräfner, 1981); death due to toxemia and systemic shock (Watts, 1976 ; Steelman, 1976; Freeden, 1977); bovine onchocerciasis (Watts, 1976; Steelman, 1976) and avian leucocytozoonosis (Watts, 1976; Snoddy and Noblet, 1976; Fallis, 1980) to human health namely, dermatitis, systemic reaction to bite (Jamnback, 1973; Watts, 1976; Newson, 1977) and human onchocerciasis (Watts, 1976); and to recreational and agricultural land use; namely, nuisance and loss of tourist revenue (Newson, 1977; Merritt and Newson, 1978), decrease in work efficiency in field and forest (Jamnback, 1973; Watts, 1976).

It has also been observed that the black fly species of Darjeeling and adjoining hill areas are serious nuisance to local population. Their biting habit results in ulceration and occasional fever (Black fly fever). Similar reports have also been received from Arunachal Pradesh and Assam regions of North East India. Of all the species involved, S. (S) himalayense is specially responsible for causing annoyance to human and cattle populations (Das et al, 1985).

It is clear from the foregoing review that the generic and specific identification of the members of the Simuliidae

is very difficult. This difficulty is further compounded by the discovery of sibling species which are diagnosed on the basis of chromosomal criteria (Rothfels, 1956). Several groups within different genera have been studied extensively by the cytological method (Rothfels,1979). Therefore, the analysis of salivary gland chromosomes of larvae has contributed significantly towards the evolutionary and cytotaxonomic research. Simuliidae is a small family comprising of about 1270 species of aquatic flies (Crosskey,1981). Of these, about sixteen species have been reported from Darjeeling and adjoining hill areas (Datta,1973, 1974a,b, 1975; Datta and Pal,1975). However, our knowledge on chromosome of Himalayan Simuliidae is still inadequate (Dey and Wangdi,1984 a,b).Therefore,the present work on the study of salivary gland chromosome of black flies from Darjeeling and adjoining hill area will contribute significantly towards the understanding of the cytotaxonomy and evolution of Simulium fauna of this region.

INTRODUCTION

The modern work on the giant chromosome of the Diptera dates from the work of Heitz and Bauer (1933), Painter (1933) and King and Beams, (1934), who clearly interpreted them as chromosomes, realised the significance of the bands and pointed out their importance for detailed cytogenetic investigations. Polytene chromosomes have been extremely important in cytogenetics for two main reasons. On the one hand, studies of their detailed structure and especially of DNA repliction cycle and the puffing phenomenon have led to new insights on fundamental problems such as the nature and mode of action of genes. On the other hand, comparison of banding sequences of different individuals, populations and species have been of great significance in the analysis of evolutionary cytogenetic processes as well as in cytotaxonomic work. Though considerable work has been carried out on the salivary gland chromosomes of different dipteran species (White, 1973), very little is known on the giant chromosomes of Simuliidae. As in other dipterans, the giant salivary gland chromosome of black flies also provide a wealth of descriptive morphological detail of the characteristic expanded centromeric regions, in the location of specific nucleolar site, in the degree of pairing of homologues and in the ultimate discernible banding pattern. Though detailed studies of polytene chromosomes of numerous Canadian and European species of Simuliidae have been carried out by Rothfels and Dunbar,(1953); Rothfels,

(1956,1979,1980,1981); Dunbar (1958,1959,1965 and 1967) ; Basrur(1959,1962) ; Rothfels and Basrur (1960); Landau (1962); Pasternak (1964); Carlsson (1966); Ottonen (1966); Rothfels and Nambiar (1981); Procunier (1987,1982 a,b); Bedo (1975 a,b, 1984 and 1989); Brockhouse (1985); Conn,et. al.,(1989), little is known on the chromosomes of Himalayan black flies (Dey and Wangdi,1984 a,b). Keeping these facts in view,the present work has been undertaken by the author to study the polytene chromosome and to prepare the standard map of each species which will facilitate not only the identification of sibling species,but also will be helpful for cytotaxonomic study. In the present investigation the following five species of Himalayan black flies have been studied :

1. Simulium (Simulium) dentatum Puri, 1932.
2. Simulium (Simulium) singtamense Datta and Pal, 1975.
3. Simulium (Simulium) himalayense Puri, 1932.
4. Simulium (Eusimulium) praelargum Datta, 1973.
5. Simulium (Eusimulium) ghoomense Datta,1975.

Of these species, standard maps of both the sexes of only S. (S.) dentatum were prepared. On the other hand , standard map of only female was prepared in S. (S.) singtamense , S. (S.) himalayense and S. (E.) ghoomense. In case of S. (E.) praelargum, standard map of male sex was prepared. However, the ganglion chromosome of only S. (S.) himalayense and S. (E.) praelargum were studied.

MATERIALS AND METHODS:

Materials

The hill areas of Darjeeling district a part of Himalayas, encompassing three hill sub - divisions namely, Darjeeling, Kurseong and Kalimpong. The total area of these three sub - divisions is about 833 sq. miles. Darjeeling district is the northern most district and the smallest district of the state of West Bengal, India. It lies between 26° 31' and 27° 13'North latitude and between 87° 59' and 88° 53' East longitude. Darjeeling is situated at an altitude of 2134 meters, experiencing average rain fall of about 320cm. The Darjeeling area is dotted with small natural springs forming the ideal sites for black fly breeding (Plate 1,Fig.-1). The temperature during the summer ranges from 15°- 25°C; while in winter, minimum 1.5°C with occasional snow fall. The average humidity is 85%. The penultimate larvae were mostly available during the month of June to November, when the temperature of water ranges from 15° to 20°C.

The penultimate larvae of five species of Simuliidae constitute the material for present investigation. They were mainly collected from different streams of Darjeeling and adjoining hill areas during the period of June, 1986 to October, 1990. Table - 1 gives the classified list of species, place of collection, date of collection, altitude, temperature of water, sex of the larvae and relative abundance.

Table : 1 : List of Black fly species used as material with some associated ecological data

Taxon	Place of Collection	Date of Collection	Temp. of Water at the time collection (°C)	Altitude of the collection site (in meter)	Sex of the specimen	Relative abundance
Family : Simuliidae Sub Family : Simuliinae Tribe : Simuliini.						
i. Simulium (Simulium) dentatum	Lebong (Stream)	24:10:1989	15	1650	male & female	Abundant (15 - 25 %)
ii. Simulium (Simulium) singtamense	Victoria Falls	17:10:1988	16	2132	female	Rare (below 5 %)
iii. Simulium (Simulium) himalayense	Lebong (Stream)	24:10:1989	15	1650	female	Dominant (over 25 %)
iv. Simulium (Eusimulium) praelarqum	Happy Valley (Stream)	01:08:1990	18	1500	male	Abundant (15 - 25 %)
v. Simulium (Eusimulium) ghoomense	Victoria Falls	17:10:1988	16	2132	female	Rare (below 5 %)

All the species were identified by the author himself by following the species identification key by Datta, 1973 , 1974a,b, 1975 , Datta and Pal , 1975 .

Methods :

Collection and fixation of larvae

The penultimate instar larvae (Plate 1,Fig.-2) were collected from different small streams of Darjeeling and adjoining areas, using curved forceps and then kept immersed in water. They were then transferred to a plastic petridish. The white background helped to sort out the larvae with black respiratory histoblast present on either side of the larvae antero-laterally. Only the larvae with such developed histoblast which represents the advanced stage of development of larvae were collected. The immature larvae without such structure were thrown back to their habitat. Many methods were used for the purpose of fixation of larvae as per need and all the procedures produced satisfactory results. They are as follows :

Firstly, the collected larvae were placed on moist filter paper in petridishes. The covered petridishes were buried in crushed ice, allowing storage of larvae without deterioration of salivary gland chromosomes. The collections were readily transported to the laboratory for fixation and further processing. Intact larvae were fixed by plunging them into a freshly prepared acetic acid - alcohol mixture (one part acetic acid to three parts ethanol). The fixative was replaced after two to three hours to compensate for dilution of the original mixture by the larval body fluids. The vials were properly labeled with necessary informative data such as place of collection, date of collection, number of specimens, the temperature of spring water. The vial

containing fixed specimens were stored in the refrigerator until required for slide preparation. This procedure is most suitable when the collections are made from a distant locations.

Secondly, the larvae of desired instars may be collected along with water in short specimen jars with wide mouth. The live specimens were taken to the laboratory and were taken out of the container with the help of small painting brush. The larvae were then soaked in a blotting paper and fixed in freshly prepared aceto-alcohol in a glass vial. They were then labelled and stored in a refrigerator. This procedure is followed only when the collections could be transported within half an hour to the laboratory.

Thirdly, the larvae could also be fixed in the field directly in 70% ethanol.

Moreover, the live specimens could be brought to the laboratory with minimum swing and disturbance and kept alive in water for few hours by supplying oxygen from aerator.

Identification of the collected <u>Simulium</u> larvae:

The fixed larvae were screened and identified on the basis of diagnostic characters described by Datta (1973, 1974a, b, 1975), Datta and Pal (1975); like size and colour of the larvae, head spots on the cephalic apotome, cephalic fan, antenna, postgenal cleft, hypostomium, mandible, respiratory histoblast, rectal gills and anal sclerites.

Dissection of larvae

The fixed final or penultimate instar larvae, recognised from the presence of well developed respiratory filaments of histoblasts, were dissected in 95% ethanol under a dissecting binocular microscope. The dissection was made with a pair of fine needles and a cataract blade. The larvae were cut open ventrally to pick up the salivary glands.

Temporary chromosome preparation

Polytene chromosome

The pair of larval salivary gland,was dissected out from fixed larvae, and was placed on a grooved slide containing 50% acetic acid for softening. After 1 to 2 minutes, the glands were stained in 1 % orcein in equal parts of 25 % lactic and propionic acids, on a slide for about 10 minutes. The glands should further be cleaned off the jelly-like content in 50 % acetic acid for better spreading of the chromosomes. The epithelial layer of the gland was restained for about 10 minutes. The stained epithelium was transferred to a very clean slide on a drop of mixture of 50% lactic and propionic acids and squashed under the cover glass with thumb pressure and then sealed with nail polish or DPX mounting medium. The slide was labelled and observed under compound microscope (Olympus). The glands, squashed in a mixture of 50% lacto-propionic acids, looked fresh even after three months. On the other hand, the permanent preparations lacked clarity and always

tend to be destained, and the chromosomal morphology also tends to be distorted. Therefore, the chromosome studies were always made from the temporary preparations only.

Mitotic chromosome

The penultimate instar larvae were treated with 0.25% of colchicine for 2-3 hours and then fixed in freshly prepared aceto-alcohol (1:3) for 20 minutes. The neural ganglia were dissected out and then softened with 50% acetic acid (depending on the condition of the gland). The glands were stained in 1% lacto-propionic- orcein for 30 minutes. The stained glands were squashed in a mixture of lactic acid and propionic acid (1:1) under a cover glass with a gentle and uniform thumb pressure. The slides were then sealed and observed under microscope.

Detection of NOR:

In all the species under present investigation, NOR is present in the interstitial region which could clearly be detected since in case of maximal expression a marked discontinuity is produced in the chromosome which flares outward into the nucleolus disrupting the surrounding banding pattern (Bedo, 1979a; Procunier, 1982a,b; Rothfels et al., 1978).

Sexing of larvae

The larvae, after dissecting out the salivary glands, were immediately sexed. The 95% alcohol in which the larvae

PLATE - 1

Fig. 1 : Showing one of the collection sites of Simuliid larvae.

Fig. 2 : Dorsal view of the Simuliid larva.

Fig. 3 : One of the dissected out ovaries of female Simuliid larvae. (X3000 approx.)

Fig. 4 : Pair of dissected out testes of male Simuliid larva. (X3000 approx).

PLATE - I

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2

3

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were dissected was dripped off. They were then treated with a mixture of saturated aqueous solution of Picric acid 50 cc, Acetic acid (50%) 20 cc,Formalin (5%) 20 cc and 10 cc of Ethanol for two minutes. The slides containing larva was then placed on black background of the dissecting Binocular and the gonads were located. This process of sexing of larvae was carried out along with the conventional method of staining with Feulgen stain. Although the gonads are embedded deep in the fat body in the abdominal region, the chemical mixture used above automatically clears the fat and the white gonads could be observed against the black background under Binocular without much effort. The ovaries were elongated (Plate 1,Fig.-3) and the testes looked round/oval (Plate 1,Fig.- 4).

Photomicrography

The good plates were photographed with the help of Olympus PM6 Camera. Both slow speed 25 ASA ORWO and high speed 125 ASA ORWO black and white films were used. The negatives were developed by A902 fine grain developer. Printing was made in glossy bromide paper (Agfa), using Agfa A901 paper developer.

Preparation and Nomenclature of Polytene Chromosome Map

Conventions for mapping polytene chromosomes of Simuliidae have been developed in series of papers from the laboratory of Professor K.H. Rothfels (Rothfels and Dunbar, 1953; Rothfels, 1956; Basrur,1959; Dunbar,1959). A

comprehensive description of mapping and nomenclature is presented by Basrur (1959) and this has largely been adhered to by subsequent workers. The relevant conventions, together with some new terminology has been presented by Bedo (1977) which is being followed here.

Composite photographic maps were made,using photos from different chromosomes, showing each sections at its best. Only the best pieces of chromosomes were used so the composite picture displayed the whole chromosome at its best. This may require slight adjustment to magnification of its print used to achieve a good match with the next one in the composite. To find the total complement length (TCL) each individual chromosome was measured by following the method of Rothfels and Dunbar (1953). Flattened and evenly stretched chromosomes were outlined with a camera lucida and traced with malleable copper wire which was then straightened and measured on a stage micrometer scale reproduced under the same optical system. The length of all the chromosomes was added to get TCL and then fraction of this total for each individual chromosome was calculated to get its % TCL. The chromosomes were then numbered in descending order of length, using Roman numericals (I, II, III). Long and short arms are indicated by the capital letter 'L' or 'S' written after the chromosome number (IS, IIIL etc.). The entire complement is divided into 100 major sections and each chromosome is being assigned approximately the same number of sections as its percentage of total

complement length (% TCL). The major sections are numbered in Arabic numericals beginning at the tip of IS and running through the centromere of chromosome I continuing through chromosomes II and III to the tip of IIIL. Each major section is further subdivided into two or three sections labelled A, B or C in the same direction as major section numbering. Individual band can be specified by writing the section number , subsection letter and the position of the band within the subsection. The chromosomes have been displayed with the short arm to the left and long arm to the right. Along with photographic maps, hand drawing maps were also prepared to show the detailed banding pattern.

In our account of polytene elements we have not described every band or indeed every major division, for in many cases they are adequately demarcated in the maps and figures. Our aim has been to present an overall picture of each arm and to concentrate attention at those features which make useful markers.

OBSERVATIONS

As in most of the investigated species of Simuliidae, all the five species studied here also revealed n=3 chromosomes. Measurements of the polytene complements are summarised in Table 2.

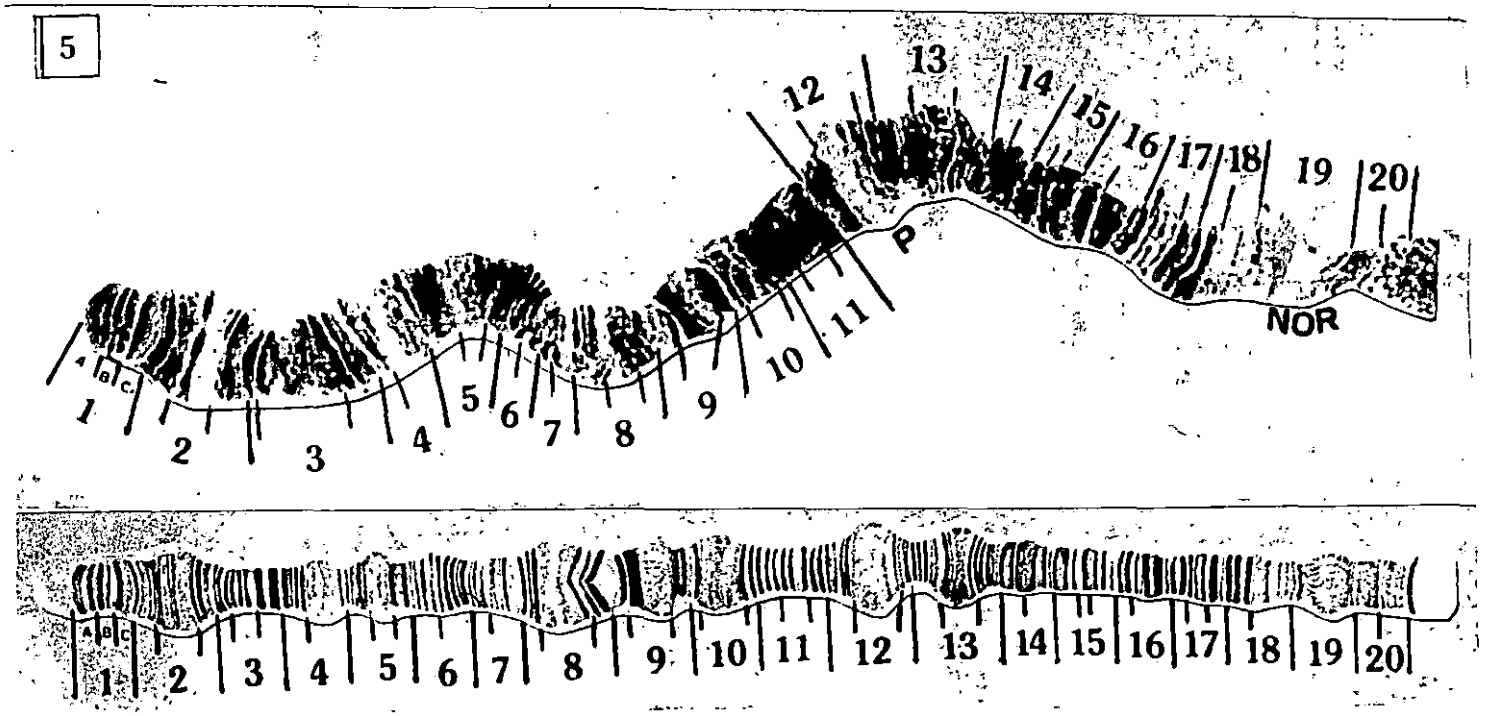
Table : 2 : Measurement of polytene chromosomes of five species of Simuliidae .

Name of the species		IS	IL	IIS	IIL	IIIS	IIIL
S. (S.) dentatum (Female)	% TCL of arms	19:22	21.33	14.28	15.34	13.23	16.60
	% TCL of chromosome	40.55		29.62		29.83	
	Secs. Assigned per arm	20	20	14	16	13	17
	Arm ratio	1		1.14		1.3	
S. (S.) singlamense (Female)	% TCL of arms	21.70	22.50	13.23	18.22	8.19	16.15
	% TCL of chromosome	44.20		31.45		24.34	
	Secs. Assigned per arm	22	23	13	18	8	16
	Arm ratio	1.04		1.38		2.0	
S. (S.) himalayense (Female)	% TCL of arms	20.12	21.16	11.81	18.80	10.09	18.02
	% TCL of chromosome	41.28		30.61		28.11	
	Secs. Assigned per arm	20	21	12	19	10	18
	Arm ratio	1.05		1.58		1.8	
S. (E.) praelargum (Male)	% TCL of arms	19.93	19.97	13.37	19.41	11.70	15.62
	% TCL of chromosome	39.90		32.78		27.32	
	Secs. Assigned per arm	20	20	13	19	12	16
	Arm ratio	1		1.46		1.33	
S. (E.) ghoomense (Female)	% TCL of arms	16.83	19.17	12.81	23.09	9.97	18.03
	% TCL of chromosome	40.00		32.00		28.00	
	Secs. Assigned per arm	17	23	13	19	10	18
	Arm ratio	1.35		1.38		1.8	

PLATE - 2

Figs. 5 - 6 : Standard photocomposite map and free hand pencil drawing of IS (sections 1 - 20) and IL (sections 21 - 40) of S. (S.) dentatum female. Abbreviations used - C, Centromere; NOR, Nucleolar Organiser Region; P, Puff. (X3000 approx.).

PLATE - 2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B
C
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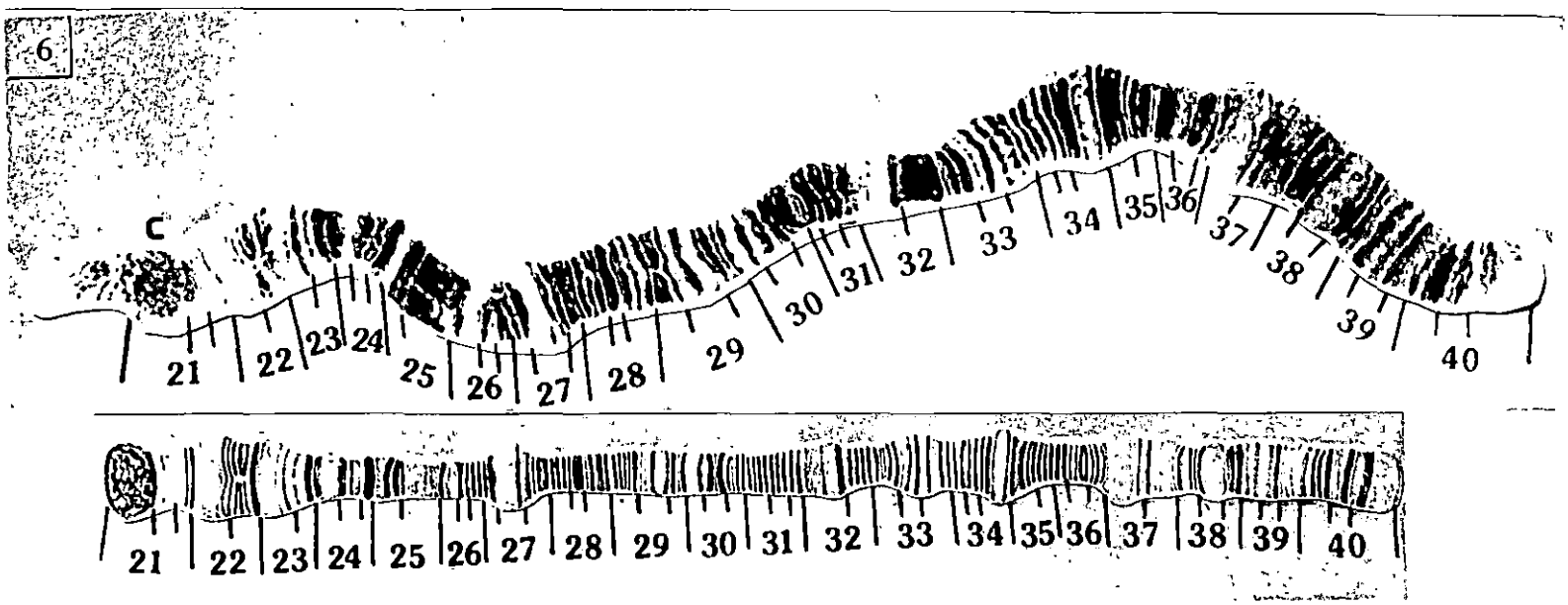
P
NOR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Family – Simuliidae

Subfamily – Simuliinae

Tribe – Simuliini

Simulium (Simulium) dentatum(female) Puri,1932.

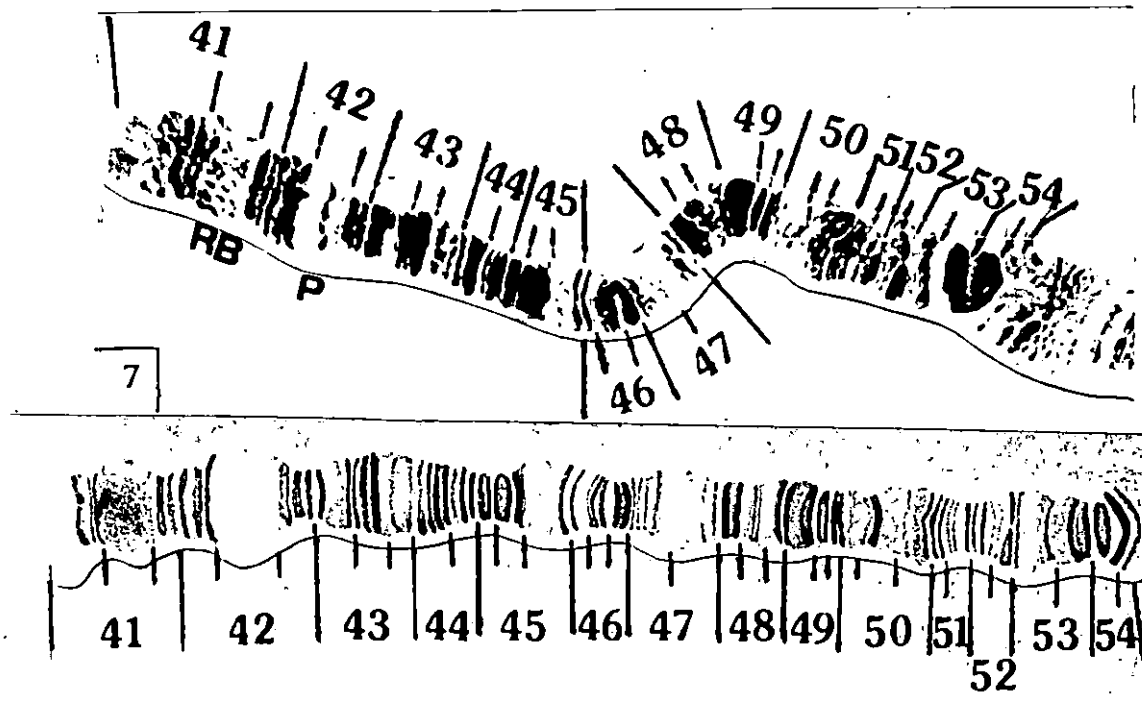
This species revealed three single polytene chromosomes n=3, each one is tightly synapsed and having prominent centromeres. The percentage of total complementary length of each chromosome arm is given in Table 2. The length of the largest chromosomes was 40.55% of TCL; while II and III were 29.62% and 29.83% respectively. The difference between II and III chromosomes was very less. The photocomposite map of each chromosome was prepared on the basis of sections assigned per arm.

Chromosome I. This chromosome (Plate 2, Fig.5 & 6) is distinguished from other chromosome by its greater length, metacentric nature and the presence of prominent nucleolar organiser region in the section 19 at the base of IS (Fig. 5). In IS (Fig. 5) several characteristic landmarks provide useful aid for chromosomal analysis. The expression of the nucleolus is more or less same in all IS chromosome. Three heavy group of bands are present in section 17C/18A near the nucleolus region. There is a puff in section 12. A prominent constriction in section 3 also provides a convenient marker for identification of IS. section 7 tends to be somewhat

PLATE - 3

Figs. 7 - 8 : Standard photocomposite map and free hand pencil drawing of IIS (sections 41 - 54) and IIL (sections 55 - 70) of S. (S.) dentatum female. Abbreviations used - C, Centromere; P, Puff; PBR, Parabalbiani Ring and RB Ring of Balbiani. (X3000 approx.).

PLATE 3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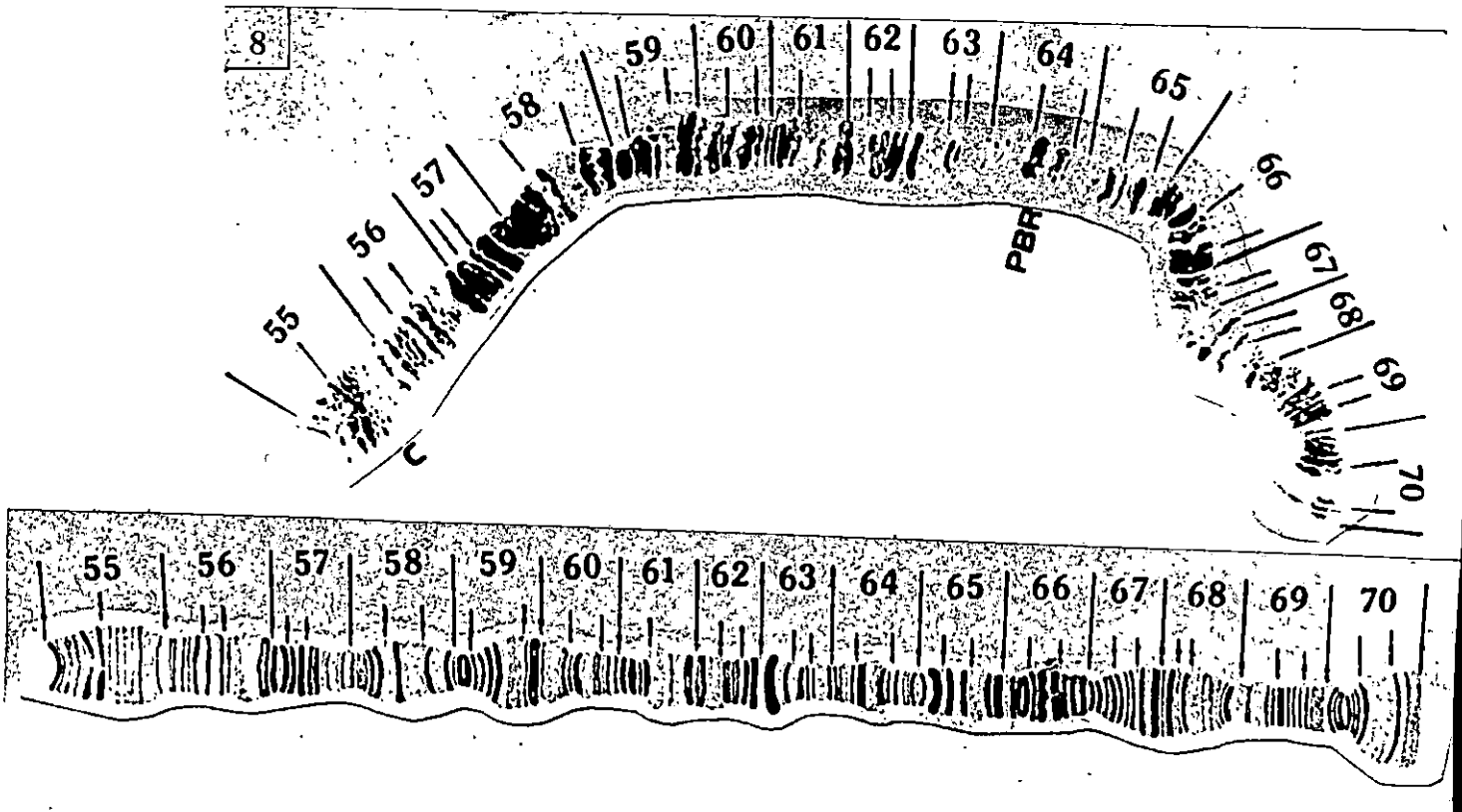
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smaller in diameter. A shield-like pattern in section 9 is also a useful marker.

The long arm (IL) (Plate 2, Fig.6) has preponderance of dark bands which vary considerably in thickness, texture and grouping. A prominent homogeneously stained centromere is present in section 21A. Of particular importance are a series of bands in section 30/31, a prominent deeply stained band in the section 24C following unstained gap. There lies a glazed band in the section 38B.

Chromosome II. This chromosome (Plate 3, Figs,7 & 8) is somewhat shorter than chromosome I and is metacentric in nature. The centromere is prominent with a deep band at its center.

Chromosome IIS is rich in morphological characteristics. The most striking being the Balbiani Ring near its tip in section 41B. A large pale puff is found in section 42 next to a series of prominent bands in the section 42A.

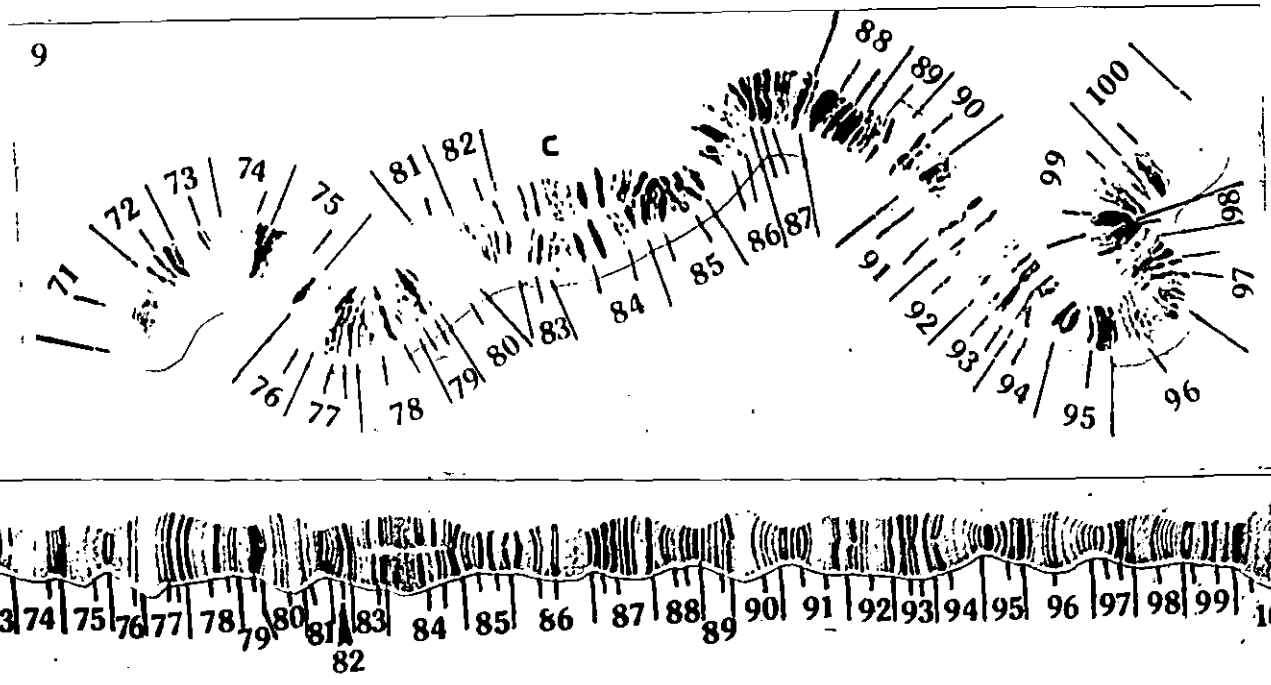
In IIL the centromere (section 55) is characterised by two deeply stained and some fine lightly stained bands (Plate 3, Fig. 8). There is a glazed band in section 58C, followed by a constricted neck region in section 59A. Other marker band includes a dark band at 63A followed by a group of three prominent bands in section 65, while in section 64B lies Parabalbiani. The tip of IIL is flared with a prominent band in the center.

Chromosome III The higher arm ratio serves to

PLATE – 4

Fig. 9 : Standard photocomposite map and free hand pencil drawing of IIIS (sections 71 – 83),IIIL (sections 84 – 100) of S. (S.) dentatum female. Abbreviations used – C, Centromere.(X3000 approx.).

PLATE - 4


9
C
71
72
73
74
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94
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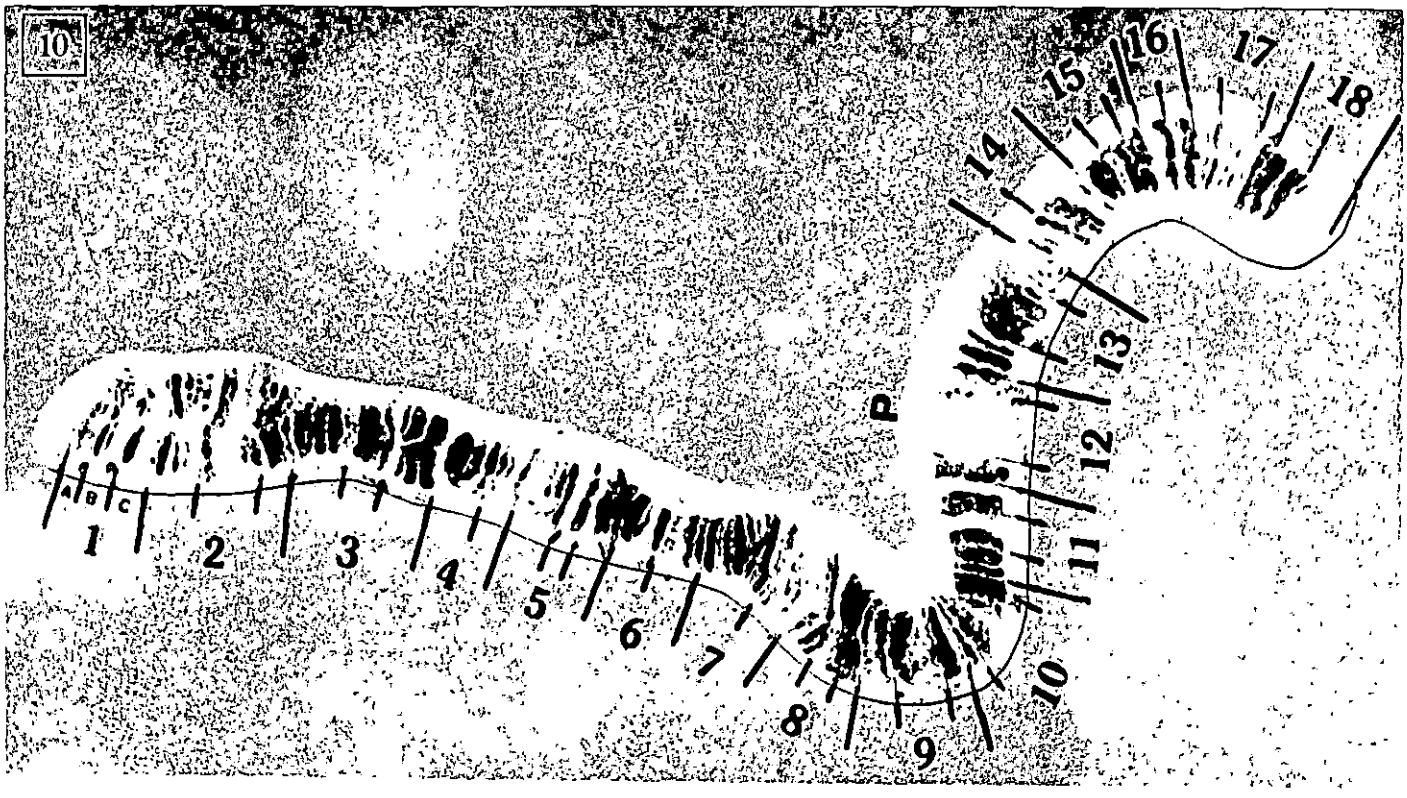
98
99
100

PLATE - 5

Figs. 10 - 11 : Standard photocomposite map of IS (sections 1 - 20) and IL (sections 21 - 40) of S. (S.) dentatum male. Abbreviations used - P, Puff; NOR, Nucleolar Organiser Region; C, Centromere.(X3000 approx.).

PLATE - 5


10
A
B
C
1
2
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7
8
9
P
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11
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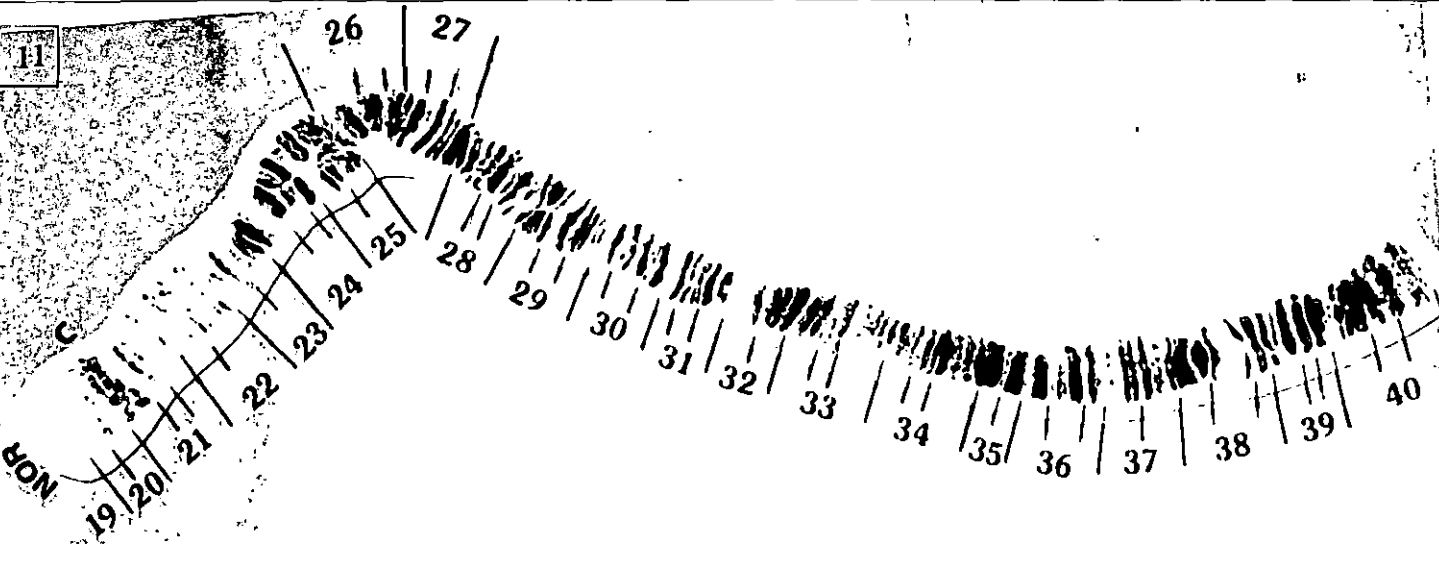
17
18


11
26
27
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23
22
21
20
19
C
NOR
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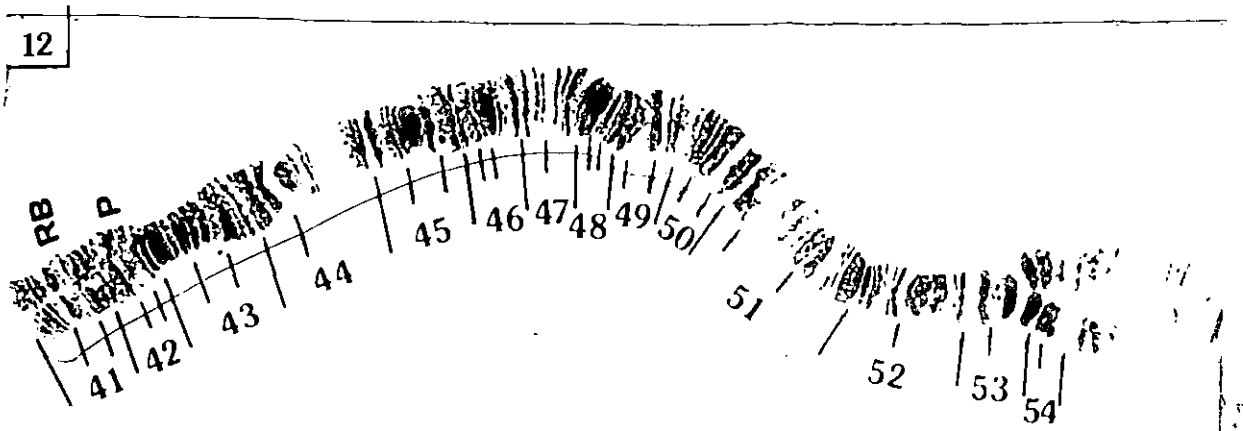
PLATE - 6

Figs. 12 - 13 : Standard photocomposite map of IIS (sections 41 - 54) and IIL (sections 55 - 70) of S. (S.) dentatum male. Abbreviations used - RB, Ring of Balbiani; P, Puff; C, Centromere and PBR, Parabalbiani Ring. (X3000 approx.).

PLATE - 6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49
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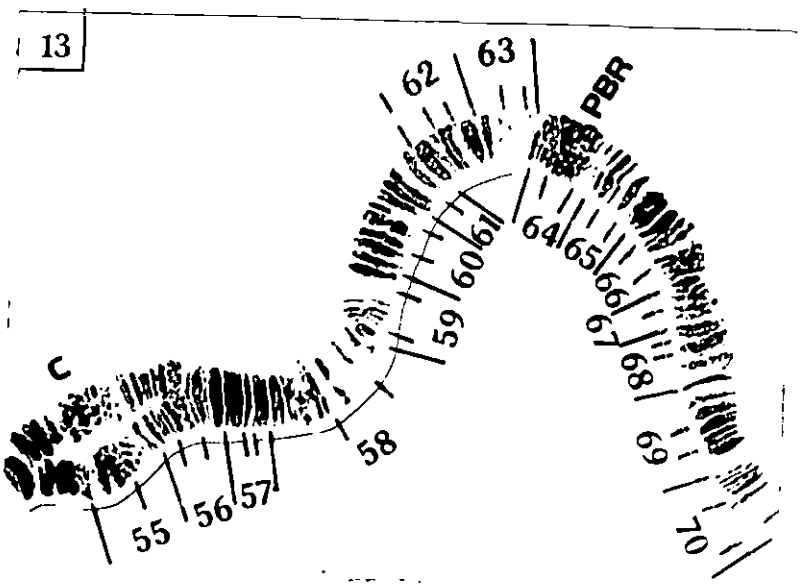
52
53
54


13
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PBR
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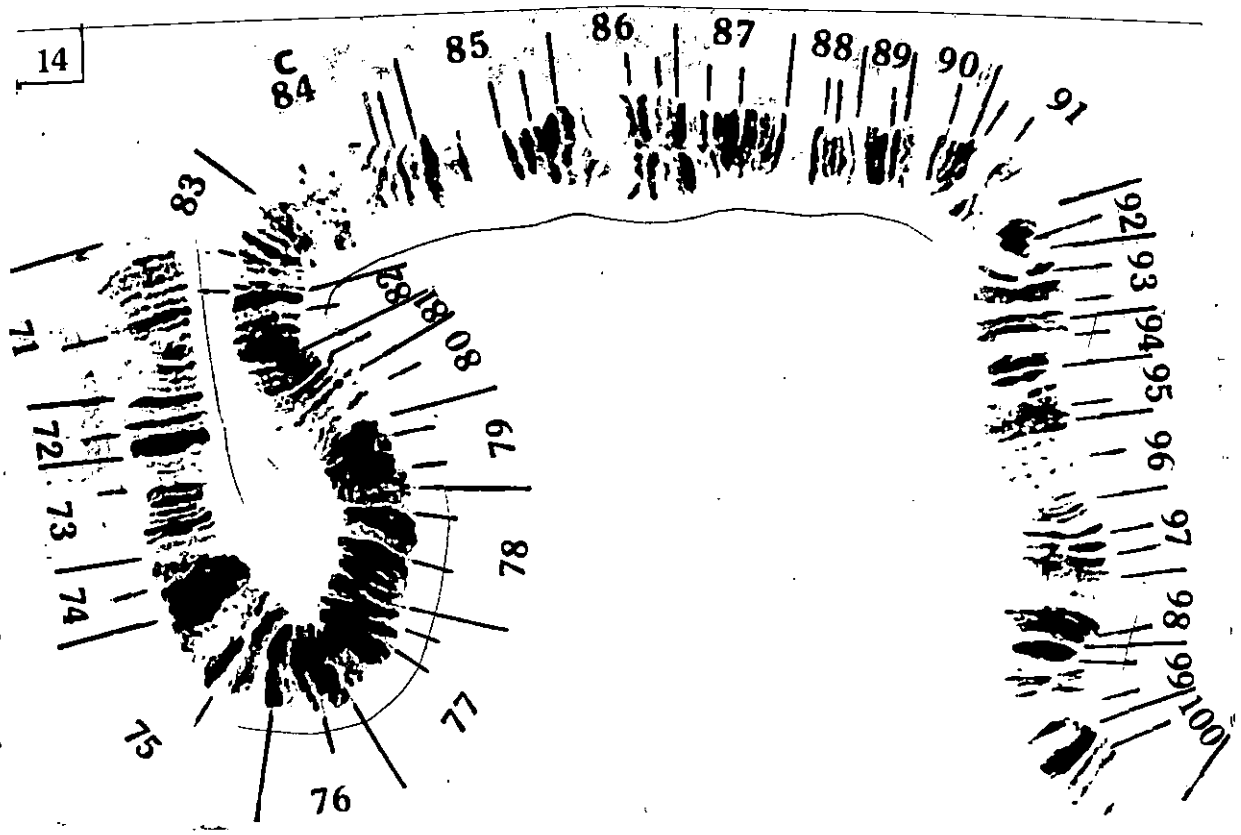
PLATE – 7

Fig. 14 : Standard photocomposite map of IIIS (sections 71 – 83), IIIL (sections 84 – 100) of S. (S.) dentatum male. Abbreviations used – C, Centromere. (X3000 approx.).

PLATE - 7


14
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100
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73
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71

distinguish chromosome III from chromosome II with which it shares a comparable length. Prominent groups of bands interspersed along both IIIS and III L (Plate 4,Fig.9). This chromosome is metacentric but the centromere is not as prominent as observed in other two chromosomes. Tip of IIIS (section 71) is provided with fine and very light thread - like bands. There is a group of deeply stained bands in the sections - 77, 78 and 79 followed by a bulge in the section 80. The centromere is provided with light and deeply stained bands in section 84. Centromeric region is also asynapsed while rest of the chromosome is tightly synapsed.

The long arm, IIIL is also characterised by some prominent banding group. A group of dark bands are present in the section 87 followed by a series of paired bands in sections 92 and 93. There is a prominent constriction in the section 95A. The tip of IIIL (section 100) is bulbous and very lightly stained.

Simulium (Simulium.) dentatum (Male) Puri, 1932

In male S. (S.) dentatum (Plates, 5-7 Figs.10-14), the chromosome number was found to be n=3, as observed in female dentatum. The general morphology, characteristic landmarks and banding patterns are similar in both male and female. However, sex specific bands or inversions were not encountered in any of the male individuals so far studied.

Comment The haploid count, the general banding pattern and characteristic landmarks of all the three polytene

chromosomes of S. (S.) dentatum agree with those of other Simulium species, as reported by other workers (Bedo, 1975; Rothfels et al.,1978; Rothfels and Dunbar, 1953 and Gordon, 1984). A comparison of banding patterns of male and female individuals of S. (S.) dentatum did not reveal any remarkable differences, such as difference in banding pattern, presence or absence of inversions. Furthermore, the present study did not reveal any sex specific locus or sex chromosome either in male or female individuals, though several workers (Bedo, 1975 , 1976, 1989; Rothfels et al., 1978; Gordon, 1984; Post, 1985; Elsen and Post, 1989) reported the presence of sex specific inversions or bands in different species of Simulium.

Family - Simuliidae

Subfamily - Simuliinae

Tribe - Simuliini

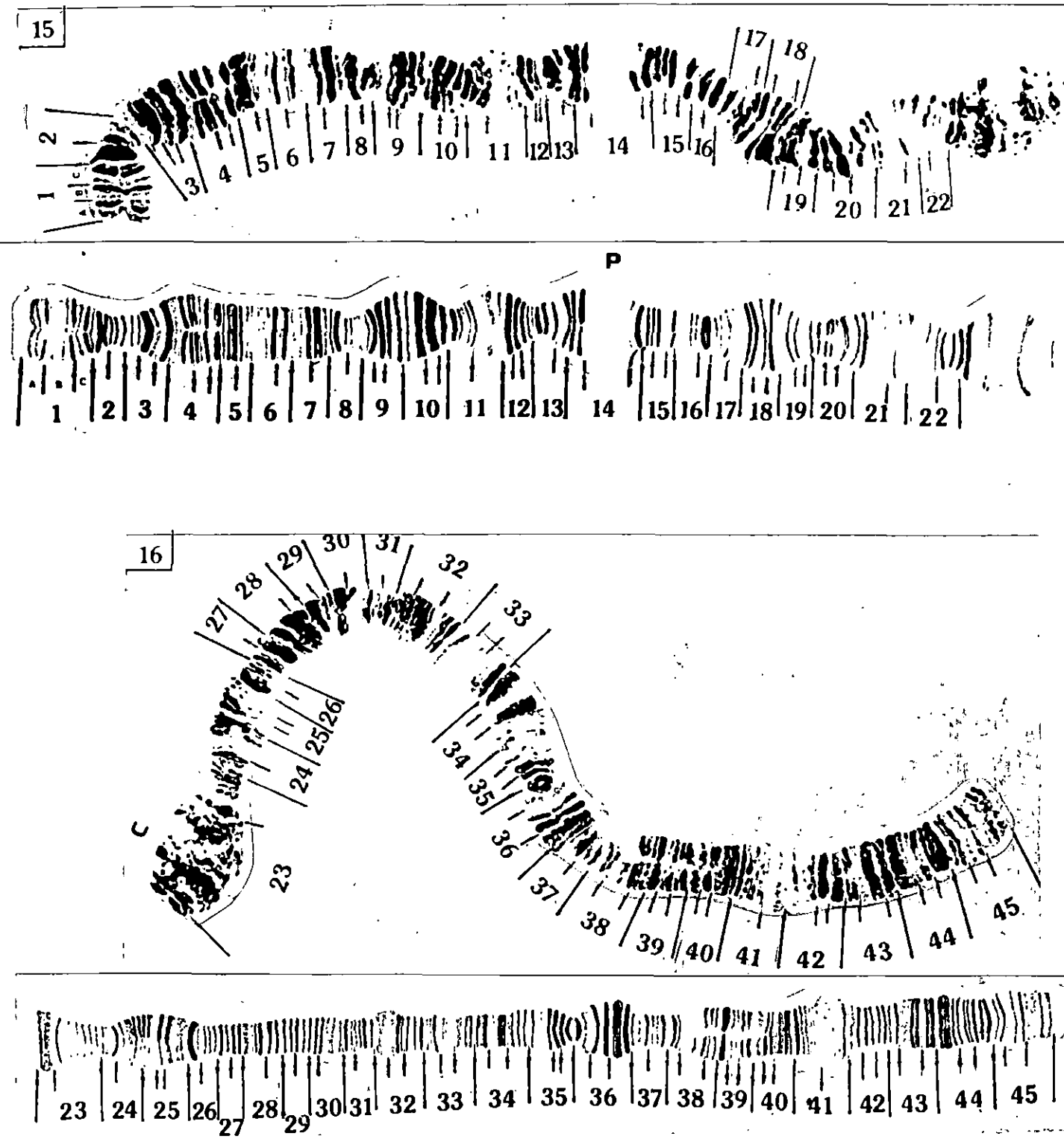
Simulium (Simulium.) singtamense(Female),Datta and Pal,1975

As in S. (S.) dentatum, this species also revealed three polytene chromosomes each one is tightly synapsed and very prominent centromeres are present in all the three chromosomes. The percentage (%) of total complementary length of each chromosome arm is given in Table-2. The length of the longest chromosome was 44.20 % of TCL, while II and III chromosomes were 31.45 % and 24.34% respectively. Therefore, there is remarkable difference between the length

PLATE - 8

Figs. 15 - 16 : Standard photcomposite map and free hand pencil drawing of IS (sections 1 - 22) and IL (sections 23 - 45) of S. (S.) singtamense female. Abbreviations used - P, Puff; C, Centromere. (X3000 approx.).

PLATE - 8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20
21
22
P
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of all three chromosomes. Photocomposite and hand drawing maps of each chromosome were prepared on the basis of sections assigned per arm.

Chromosome I: This chromosome (Plate 8, Figs.15 and 16) is readily recognised by its greater length and central position of its centromere. Numerous characteristic landmarks provide useful aid for the identification of this chromosome.

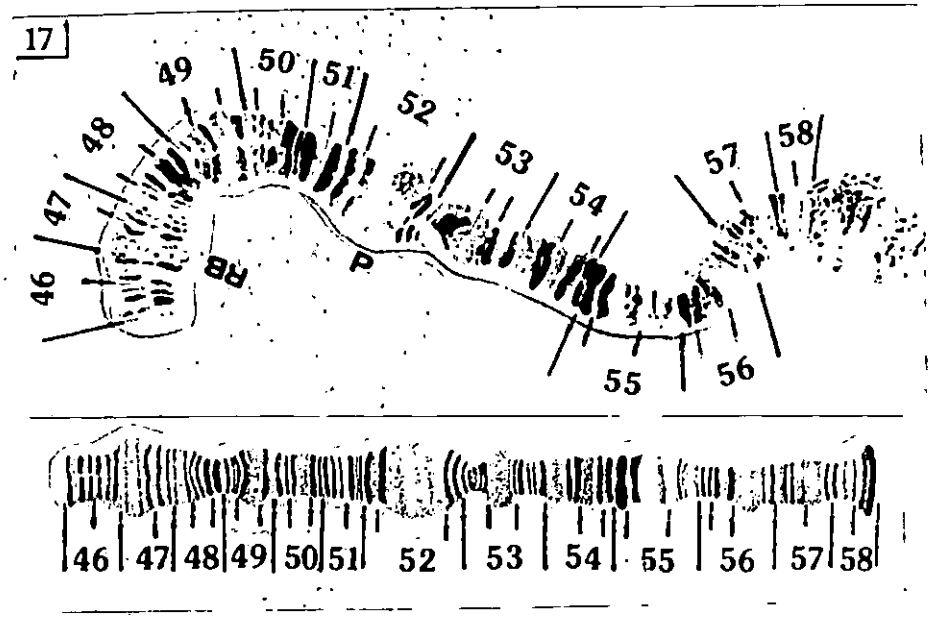
The 1S is more or less equal in diameter throughout its length. However, a distinctive constriction is present in section 8 . Deeply stained banding groups are present throughout its length. Of these, a group of dark bands in the sections (2-5) followed by another group of dark bands (7B-10) serve as an important reference point. There is a puff in section 14 followed by a region of dark bands.

The long arm (Fig.16) also have the preponderance of dark bands as in 1S. This arm is also endowed with specific characters useful as landmarks. There is a large bulbous centromere in the section 23, and is provided with some irregular thick centromeric bands at two ends. A prominent dark band in 26A is followed by a series of bands, extending from 26B to 30A. There is a shield-like pattern in section 34, while a distinctive constriction in 36A provides a convenient marker for the mid point of 1L. There lies a lightly stained bulge in sections 41-42A. A group of three heavy bands is present in section 43B,C and 44A. The tip of 1L is characterised by a group of lightly stained fine

PLATE - 9

Figs. 16 - 17 : Standard photocomposite map and free hand pencil drawing of IIS (sections 46 - 58) and IIL (sections 59 - 76) of S. (S.) singtamense female. Abbreviations used - RB, Ring of Balbiani; P. Puff; C, Centromere. (X3000 approx.).

PLATE - 9


17
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47
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49
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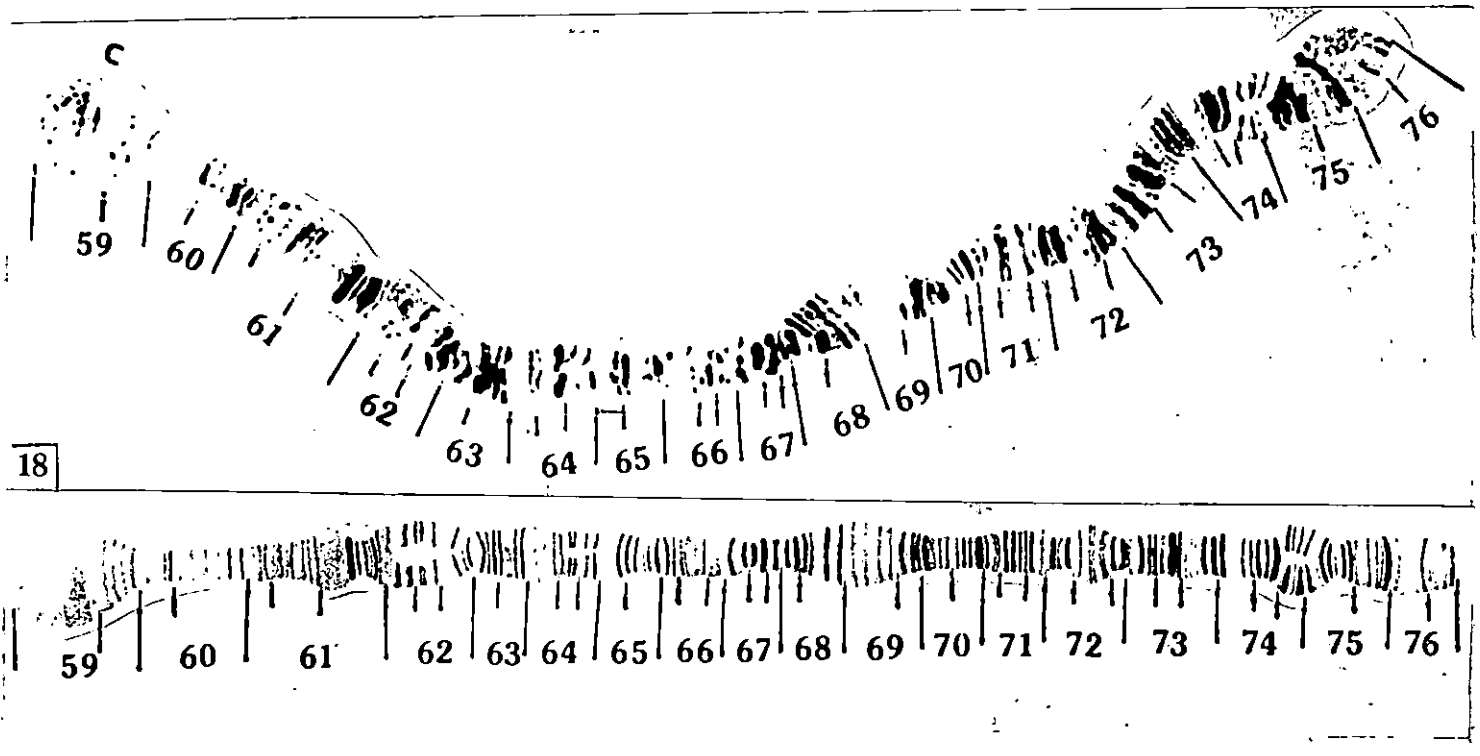
51
52
53
54
55
56
57
58
RB
P
46 47 48 49 50 51 52 53 54 55 56 57 58


C
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62
63
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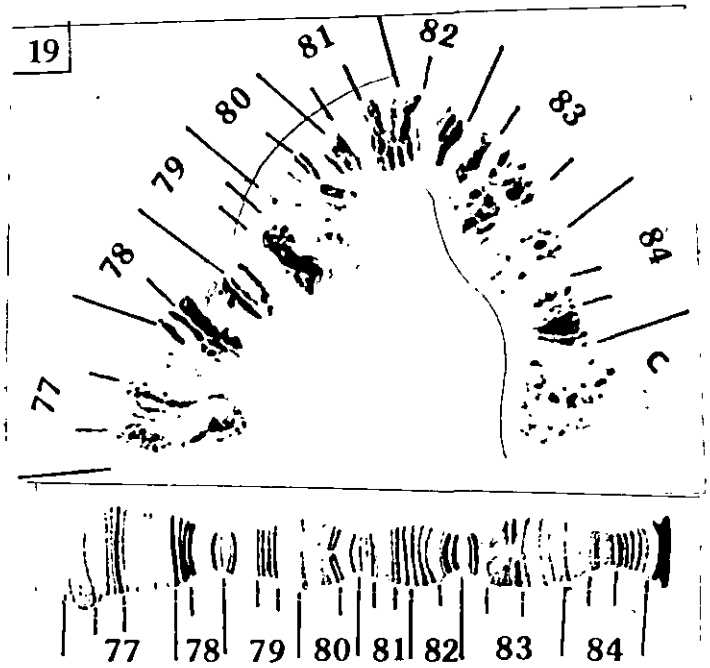
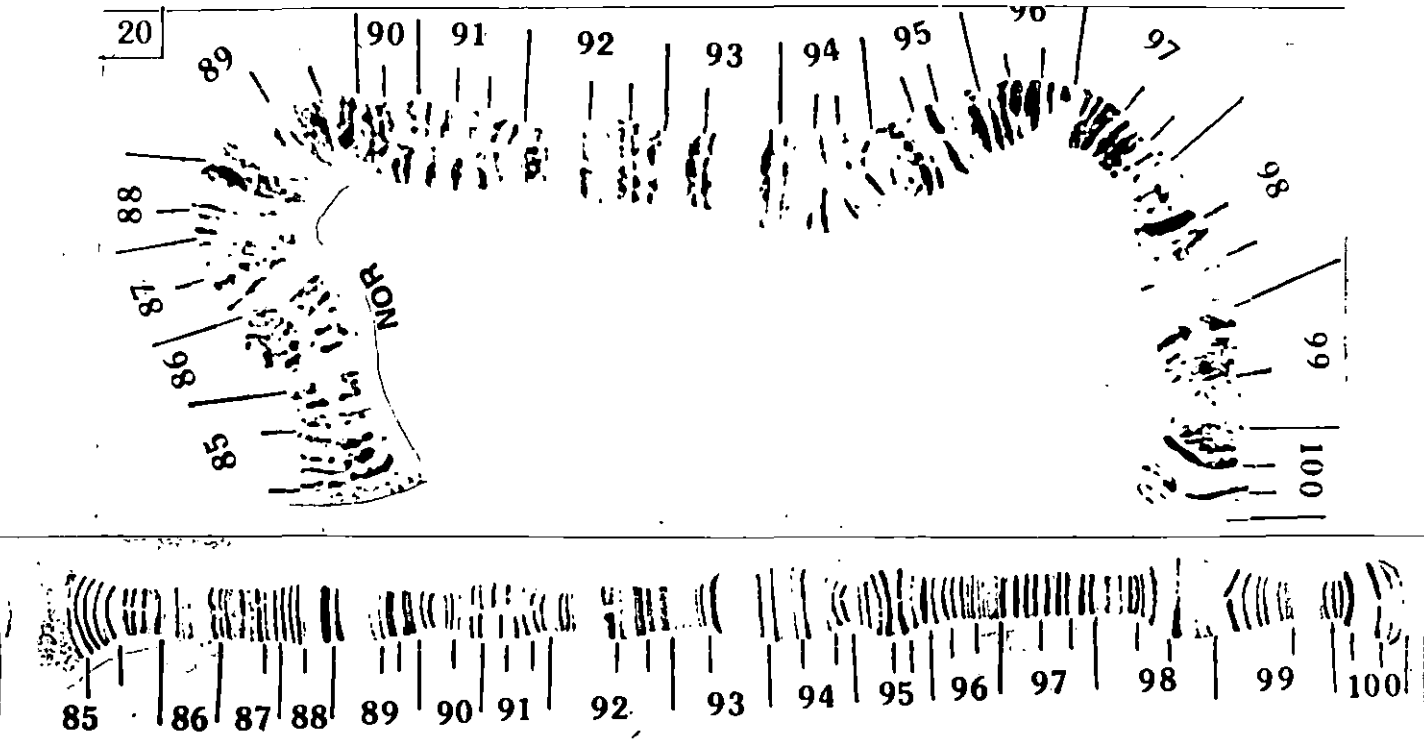
73
74
75
76
18
59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76

PLATE - 10

Figs. 19 - 20 : Standard photocomposite map and free hand pencil drawing of IIIS (sections 77 - 84) and IIIL (sections 85 - 100) of S. (S.) singtamense female. Abbreviations used - C, Centromere; NOR, Nulcleolar Organiser Rigion. (X3000 approx.).

PLATE - 10


19
77
78
79
80
81
82
83
84
C
77
78
79
80
81
82
83
84


20
85
86
87
88
89
90
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93
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95
96
97
98
99
100
NOR
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96
97
98
99
100

bands.

Chromosome II : The chromosome II (Plate 9, Figs. 17 & 18) is somewhat shorter than chromosome I, metacentric in nature and is rich in morphological characteristics.

The most striking feature of IIS (Fig.17) is the presence of Balbiani Ring near its tip in section 47. A large pale puff is found in section 52 next to a series of prominent dark bands in sections 50C - 52A. Other important characters include a group of dark bands in sections 54-55 A,B of which a large band in 55A serves as an important reference point. Moreover, the terminal end of IIS is provided with very fine lightly stained bands (section 46).

A prominent centromere is present in section 59 of IIL (Fig. 18). The banding group includes a series of dark bands in sections 70 - 73. Terminal end (section 76) of this arm is lightly stained and without any distinguishing bands. However, in 75B, a dark long band serves as an important reference point.

Chromosome III : Chromosome III (Plate 10, Figs.19,20). could be demarcated from other two chromosomes by its smaller length. But unlike chromosome I and chromosome II, the centromere is submedian in position in this chromosome. However, as in other two chromosomes, the centromere is large and prominent. This chromosome is also endowed with many specific bands useful as landmarks. (Plate 10, Figs. 19 and 20). The tip of IIIS (Fig. 19.section 77) is flared to some extent and is provided with a dark band (77B) in the

midst of the numerous lightly stained fine bands. A group of four dark bands is present at the base of the flared end in section 78.

The IIIL (Fig.20) is also provided with numerous landmarks. There is a large prominent centromere in section 85A, devoid of any remarkable bands. The centromere is followed by four large bands in 85B. There is a prominent nucleolar organiser region in section 86. Just near NOR a group of fine bands is present in section 87B and 88A. A bulge is encountered in 93B. Other characteristic band group include a group of dark bands in section 95-98A. At the tip of this arm, there are two deep bands in section 100A,B.

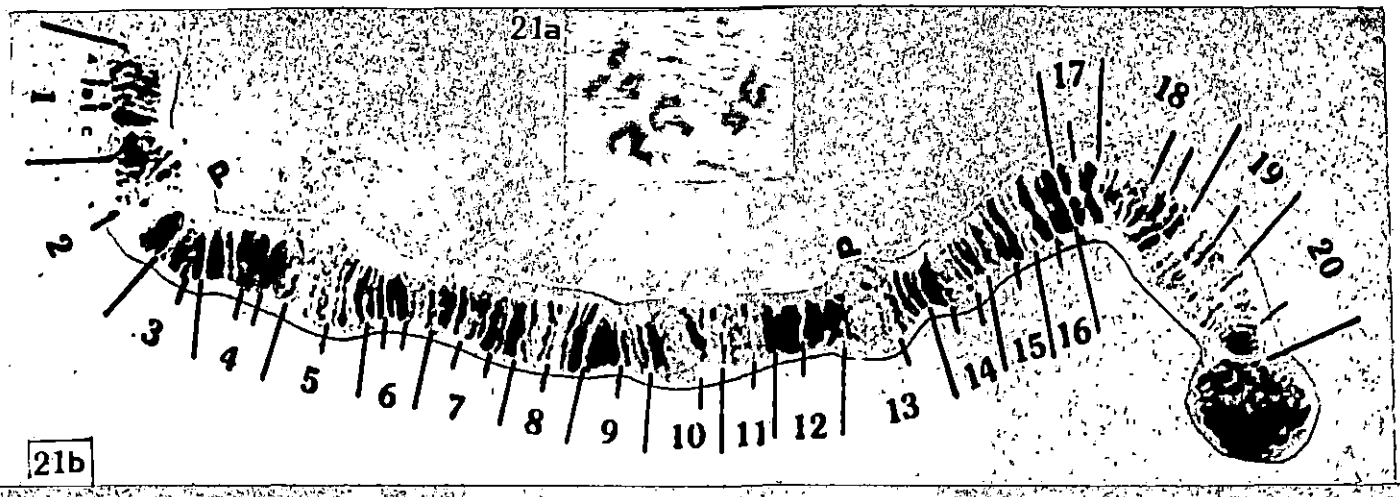
Comments The haploid count, the general banding pattern and characteristic landmarks of all three chromosomes of S. (S.) singtamense, agree broadly with those of other Simulium species reported earlier by Bedo (1975a, 1976,1977) and Rothfels and Dunbar (1953). However, S. (S.) singtamense differs from its congeneric species S. (S.) dentatum by the presence of a submetacentric third chromosome while in S. (S.) dentatum it is metacentric. There is snarp difference in length among all three chromosomes of S. (S.) singtamense though in S.(S.) dentatum the difference is less prominent among the three chromosomes.Moreover, NOR, which is present in IS in S.(S.) dentatum is present in IIIL in S. (S.) singtamense. However, in both the cases, the general banding pattern of centromere. Nevertheless, the general banding

PLATE - 11

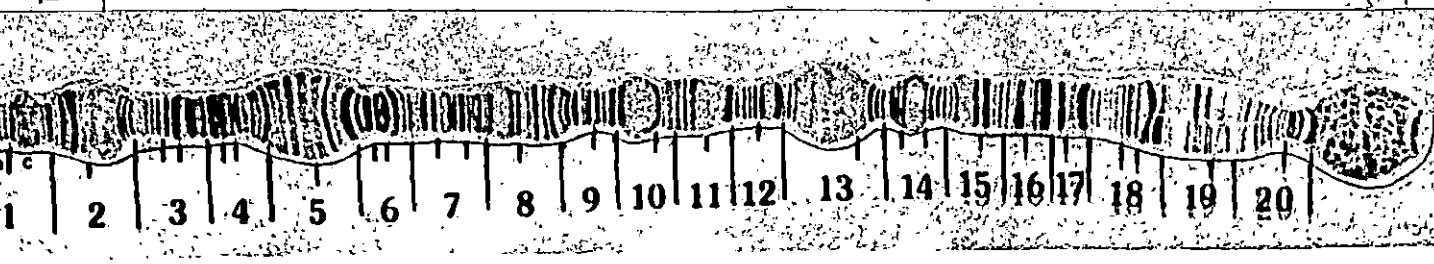
Figs. 21a : Mitotic metaphase plate from neural ganglion cells of S. (S.) himalayense female. (X2500 approx.).

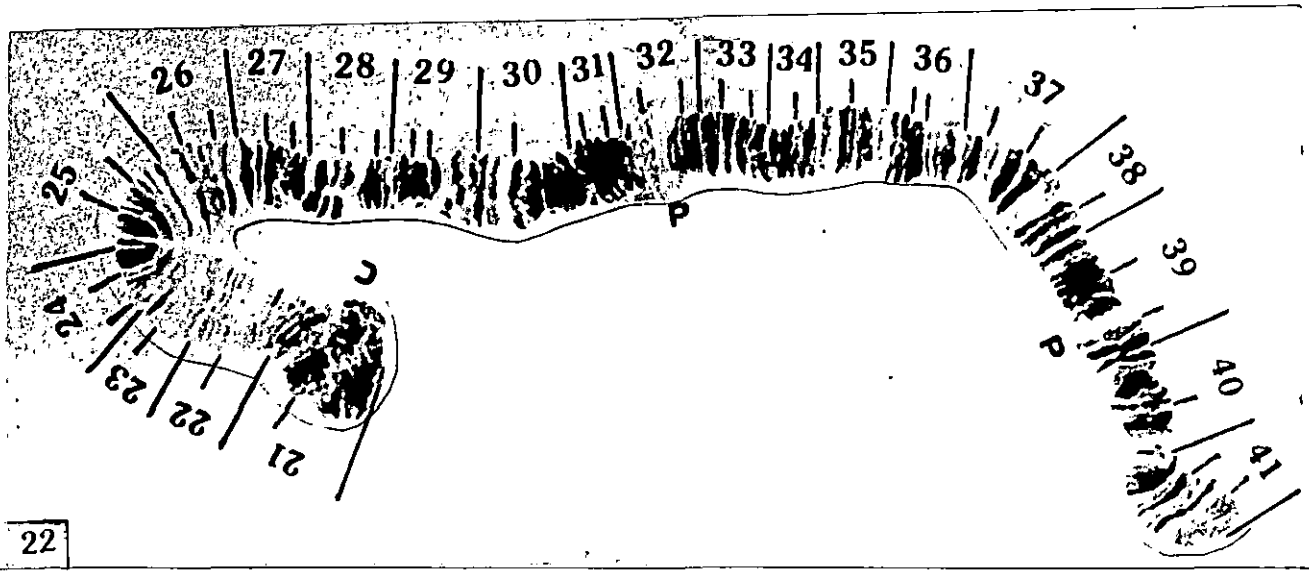
Figs. 21b - 22 : Standard photocomposite map and free hand pencil drawing of IS (sections 1 - 20) and IL (sections 21 - 41) of S. (S.) himalayense female. Abbreviations used - P, Puff; C, Centromere. (X3000 approx.).

PLATE - II

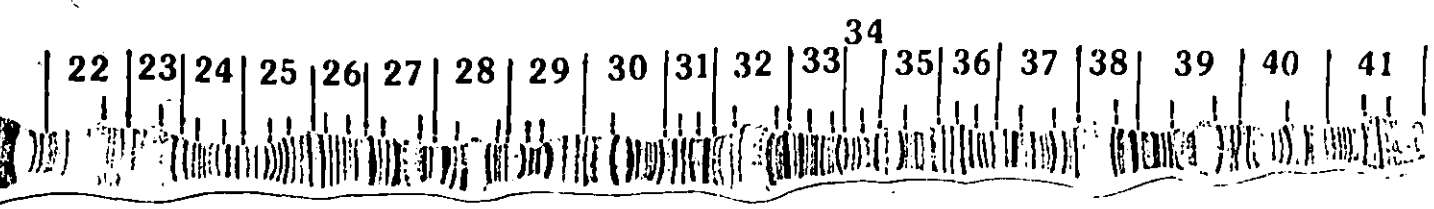


21b





22



pattern of both the species is found to be more or less similar.

Family – Simuliidae

Subfamily – Simuliinae

Tribe – Simuliini

Simulium (Simulium) himalayense (Female) Puri,1932

Neuroblast metaphase cells (Plate 11,Fig.21a) revealed normal Simuliid complement of three pairs of chromosomes (2n=6). Of these, chromosome I and chromosome II are metacentric while chromosome III is submetacentric. Measurement of the mitotic chromosomes is given in the Table 3.

Table : 3 : Mitotic Chromosome measurements of two species of Himalayan black flies.

Name of the species	Chromosome number	Mean length (µm) S.E.M. (±)		Total length	Arm Ratio	Centromeric index	Relative %length	Nature of chromosome
		Short arm	Long arm	(S + l)	r=l/s	i		
Simulium (Simulium) himalayense	I	2.31 0.01	2.7 0.14	5.01	1.17	46.2	45.58	Nearly median (M)
	II	1.2 0.32	1.87 0.03	3.07	1.55	39.00	27.93	Nearly median (M)
	III	1.04 0.01	1.87 0.06	2.91	1.79	26.3	26.47	Nearly sub-median (SM)
Simulium (Eusimulium) praelarqum	I	2.39 0.05	2.6 0.29	4.99	1.08	47.89	42.87	Nearly median (M)
	II	1.35 0.16	2.18 0.04	3.53	1.61	38.24	30.32	Nearly median (M)
	III	1.04 0.01	2.08 0.10	3.12	2.00	33.34	26.87	Nearly submedian (SM)

This species also showed three polytene chromosomes (n=3) each one is tightly paired,and identifiable centromeres are present in all the three chromosomes. The measurement of the polytene chromosomes is summarised in the Table 2. The length of the longest chromosome was 41.28% of TCL while second and third chromosomes measured 30.61% and 28.11% respectively. Therefore, individual chromosomes could be demarcated on the basis of their total length. The photocomposite maps of each chromosome were prepared on the basis of sections assigned per arm.

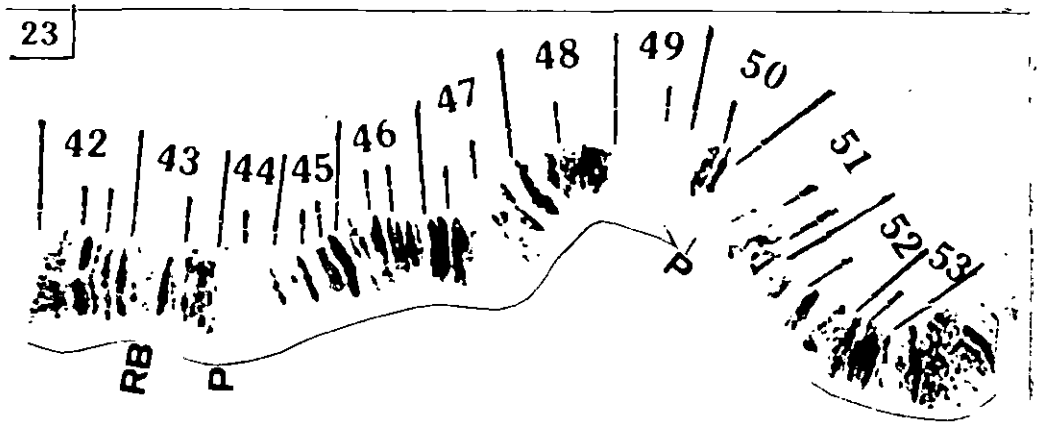
Chromosome I :This chromosome (Plate 11, Figs.21b,22) is distinguished by its greater length and median centromere. The short arm (IS) (Fig.21b) is divided into 20 sections. The terminal end (Section 1) showed some fine bands and one heavy band in 1C. A puff was encountered in section 2B, followed by dark heavy band in 4A. Other identifying landmarks include a capsule in Section 10,a pale puff in section 13A. A marker region consists of a group of 5 darkly stained bands in Sections 16 and 17.

In the long arm (IL) (Fig.22), centromere is present in the Section 21 and is characterised by dark stained zones at its two ends, while the center is lightly stained. In some individuals, in good preparation, some fine bands were seen in the region 22-23. Other distinguishing landmarks include a heavy band in Section 24C, a glazed band in section 30, a large pale puff in 32 and a group of four bands in sections 38-39A followed by a smaller puff in Section 39B. Some

PLATE - 12

Figs. 23 - 24 : Standard photocomposite map and free hand pencil drawing of IIS (sections 42 - 53) and IIL (sections 54 - 72) of S. (S.) himalayense female. Abbreviations used - RB, Ring of Balbiani; P, Puff; C, Centromere. (X3000 approx.).

PLATE - 12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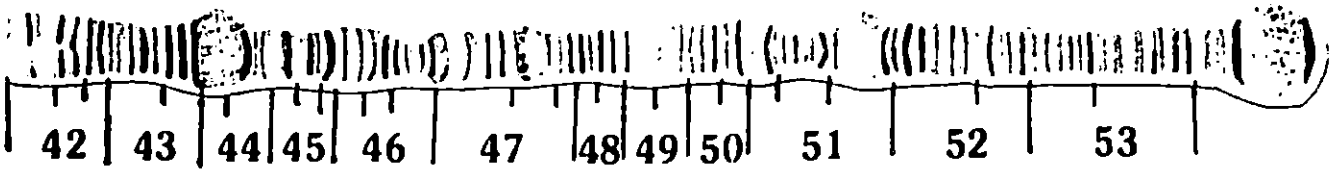
53
RB
P
P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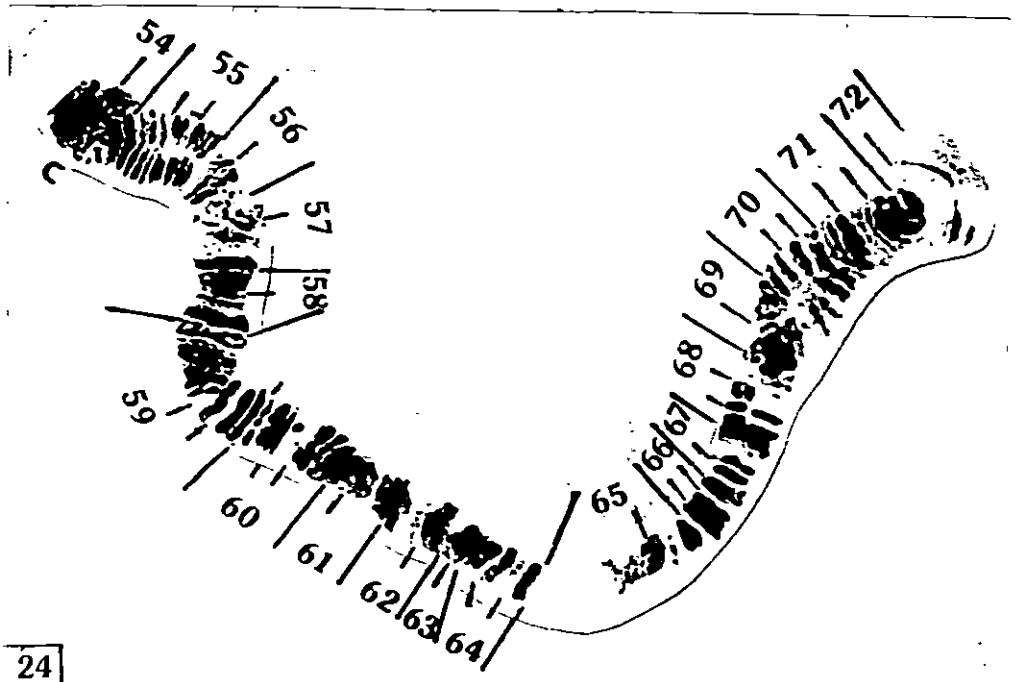
54
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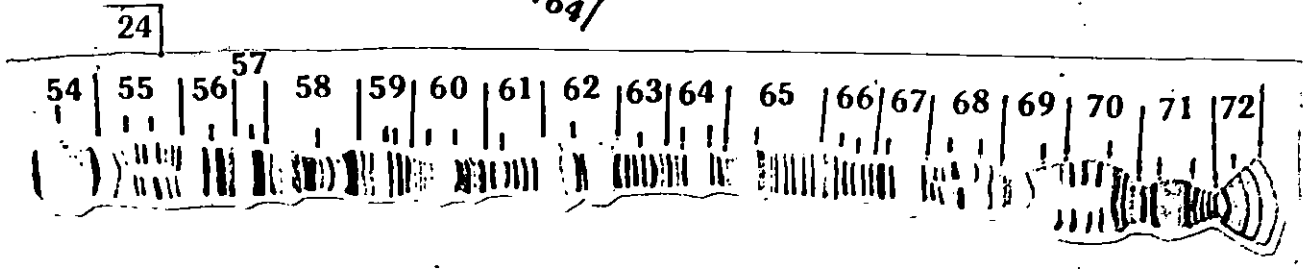
24
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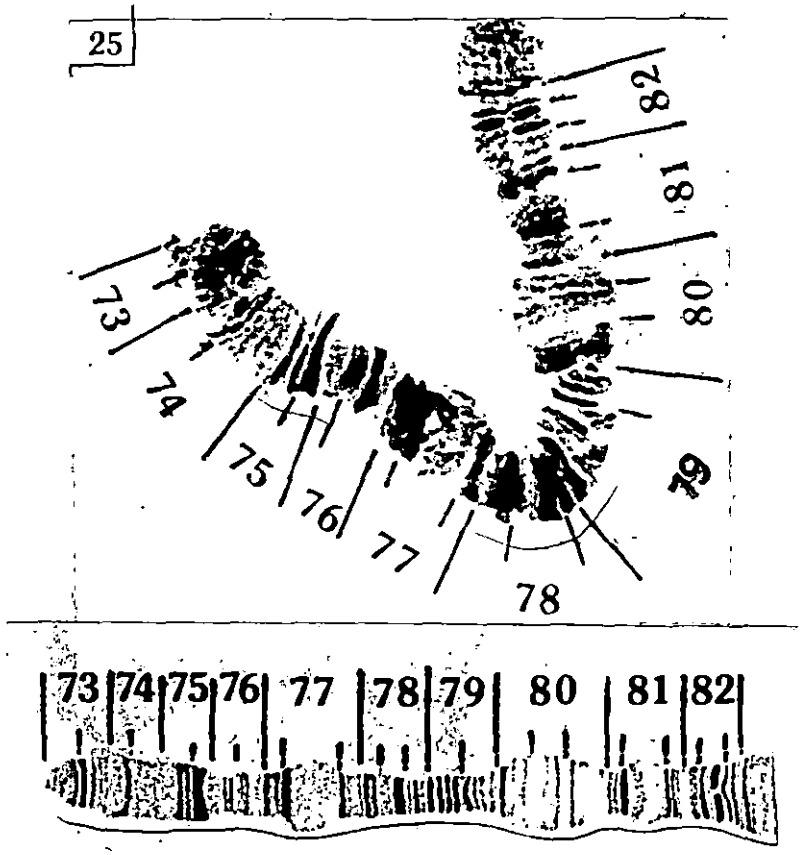
PLATE - 13

Figs. 25 - 26 : Standard photocomposite map and free hand pencil drawing of IIIS (sections 73 - 82) and IIIL (sections 83 - 100) of S. (S.) himalayense female. Abbreviations used - C, Centromere; NOR, Nucleolar Organiser Rigion; P, Puff; PBR, Parabalbiani Ring. (X3000 approx.).

PLATE - 13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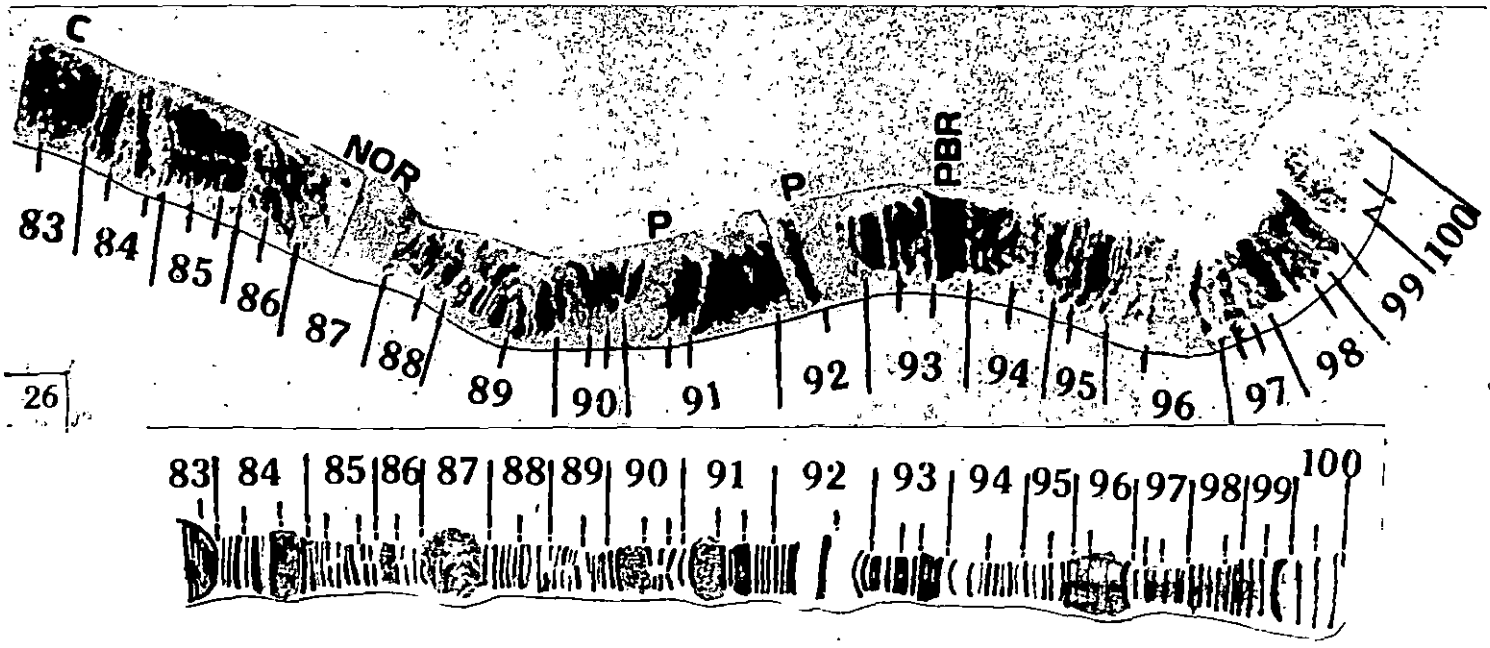
73 74 75 76 77 78 79 80 81 82


C
NOR
P
P
PBR
26
83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

lightly stained bands were encountered in the tip of IL in Section 41.

Chromosome II: Chromosome II (Plate 12,Figs.23,24) is somewhat shorter than chromosome I. It is metacentric in nature with a prominent centromere and is distinguished by the presence of Balbiani Ring. The short arm (IIS) (Fig.23) is characterised by the presence of a Balbiani Ring in Section 43 followed by a pale puff in Section 44, centromeric landmarks include a dark band in section 45C and a pair of heavy dark bands in 47A. Pale puffs are also found in Sections 49 and 51.

The long arm (IIL) (Fig.24) is subdivided into 19 Sections. The prominent centromere is easily identifiable in Section 54. It is characterised by a sharp band at two ends and granular deeply stained central region. Other characteristics include a group of fine post centric bands in section 55A, and preponderance of heavy bands throughout the length of the chromosome. The terminal end (Section 72) is flared and lightly stained, showing fine bands in some good preparations.

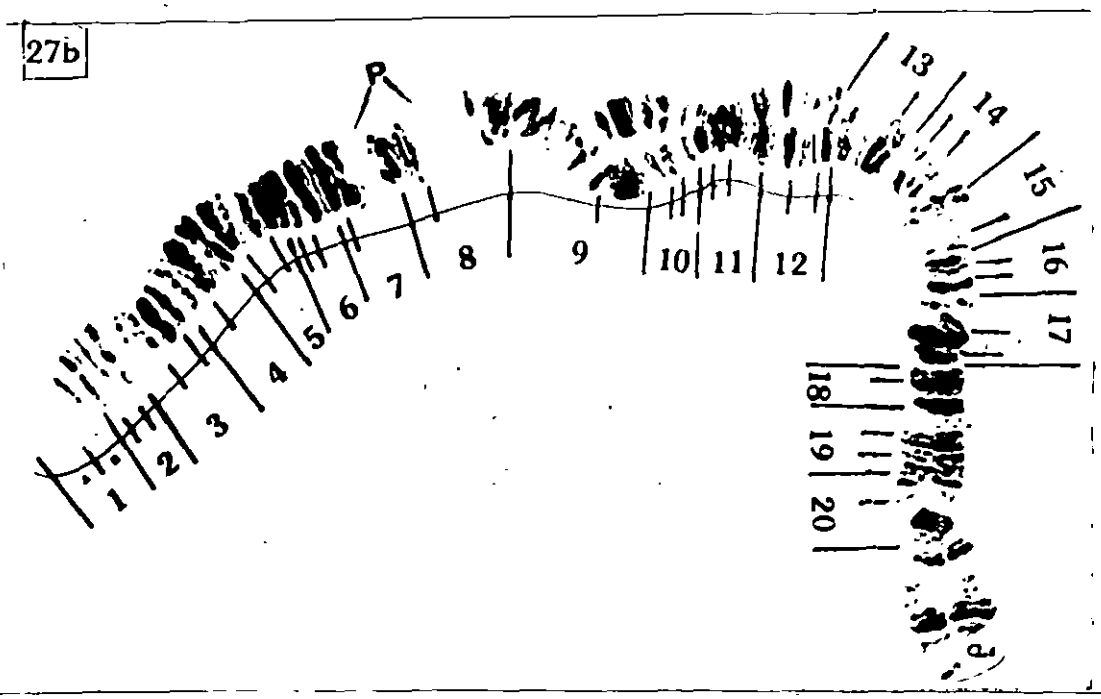
Chromosome III: The smaller length and submedian centromere serve to distinguish chromosome III (Plate 13,Figs. 25,26) from other two chromosomes. This chromosome is characterised by the presence of nucleolar organising region. The short arm (IIIS) (Fig.25) is divided into 10 sections and is characterised by the presence of a group of dark bands in Section 75, a group of heavy bands in 78 and a

PLATE - 14

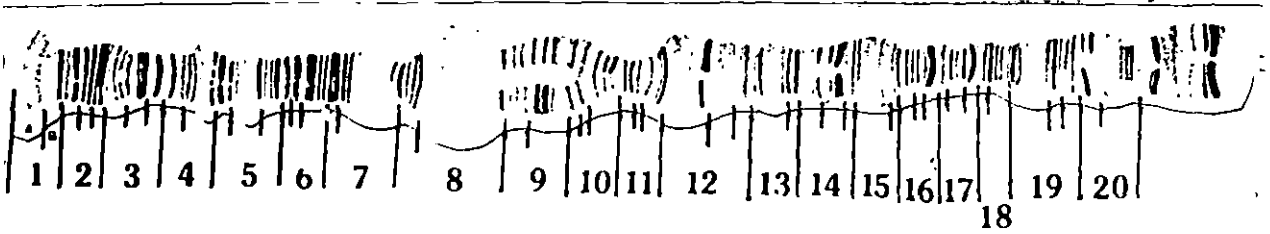
Fig. 27a : Neural ganglion mitotic metaphase plate of S. (E.) praelargum male. (X2500).

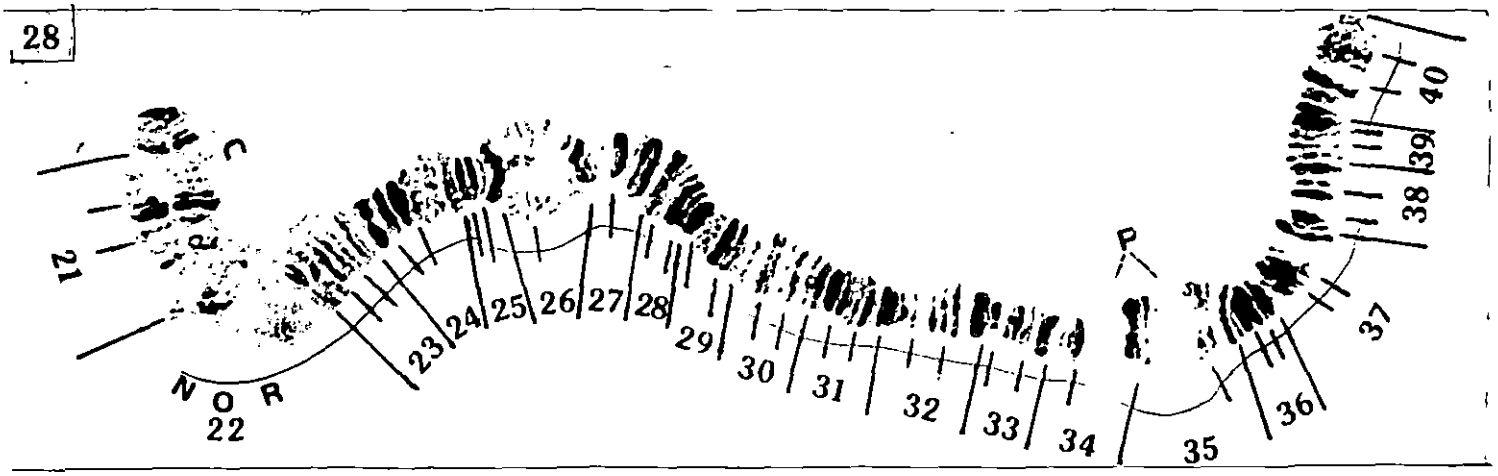
Figs. 27b - 28 : Standard photocomposite map and free hand pencil drawing of IS (sections 1 - 20) and IIL (sections 21 - 40) of S. (E.) praelargum male. Abbreviations used - C, Centromere; NOR, Nucleolar Organiser Region; P, Puff. (X3000 approx.).

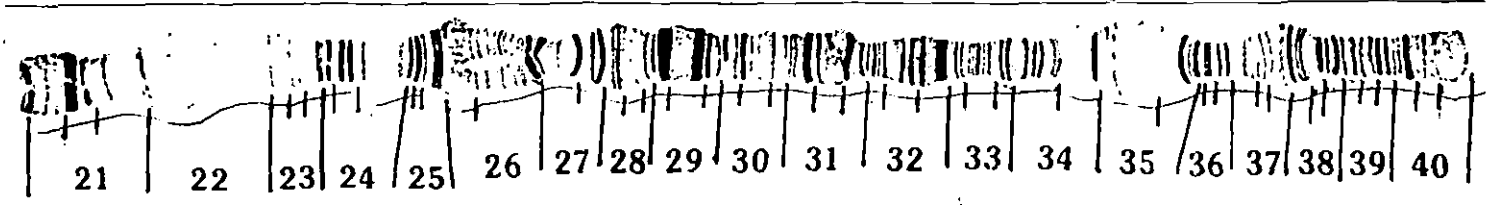
PLATE - 14


27b
P
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
P


27a


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20


28
C
21
22
NOR
23
24
25
26
27
28
29
30
31
32
33
34
P
35
36
37
38
39
40


21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40

bulge in section 80. On the other hand, the long arm (IIIL) (Fig.26) is divided into 18 sections and centromere is present in section 83. The centromeric region is homogeneously stained without any characteristic band. Important landmarks,include nucleolar organiser in section 87, pale puffs in 91 and 92, Parabalbiani in 93C and one heavy band each in sections 98A and 99A.

Comment: The diploid count and the general banding pattern of S. (S.) himalayense is in accordance with those of its congeneric species namely,S. (S.) dentatum and S. (S.) singtamense. However, it differs from its congeneric species in the location of important landmarks such as BR, PBR, NOR etc.,on the polytene chromosomes.

Family - Simuliidae

Subfamily - Simuliinae

Tribe - Simuliini

Simulium (Eusimulium) praelargum (male) Datta, 1973

Neuroblast metaphase cells (Plate - 14, Fig.27a) revealed normal black fly complement of three pairs of chromosomes. Of these, first and second chromosomes are metacentric while the third one is submetacentric. Measurement of the mitotic chromosomes is given in Table 3. The second and third chromosome could be demarcated on the basis of their arm ratios, though the difference between their length was very little. As in other black flies, this species also revealed

three polytene chromosomes each one is tightly paired and identifiable centromeres are present in all three chromosomes. The percentage of TCL of each chromosome arm is presented in Table 2. The length of the largest chromosome was 39.90 % of TCL, while II and III chromosomes measured 32.78 and 27.32 %, respectively. Therefore, individual chromosome could be demarcated on the basis of their total length. The standard map of each chromosomes was prepared on the basis of the sections assigned per arm.

Chromosome I: This chromosome is distinguished by its greater length, median centromere and the presence of nucleolar organising region (Plate 14, Figs, 27b,28).

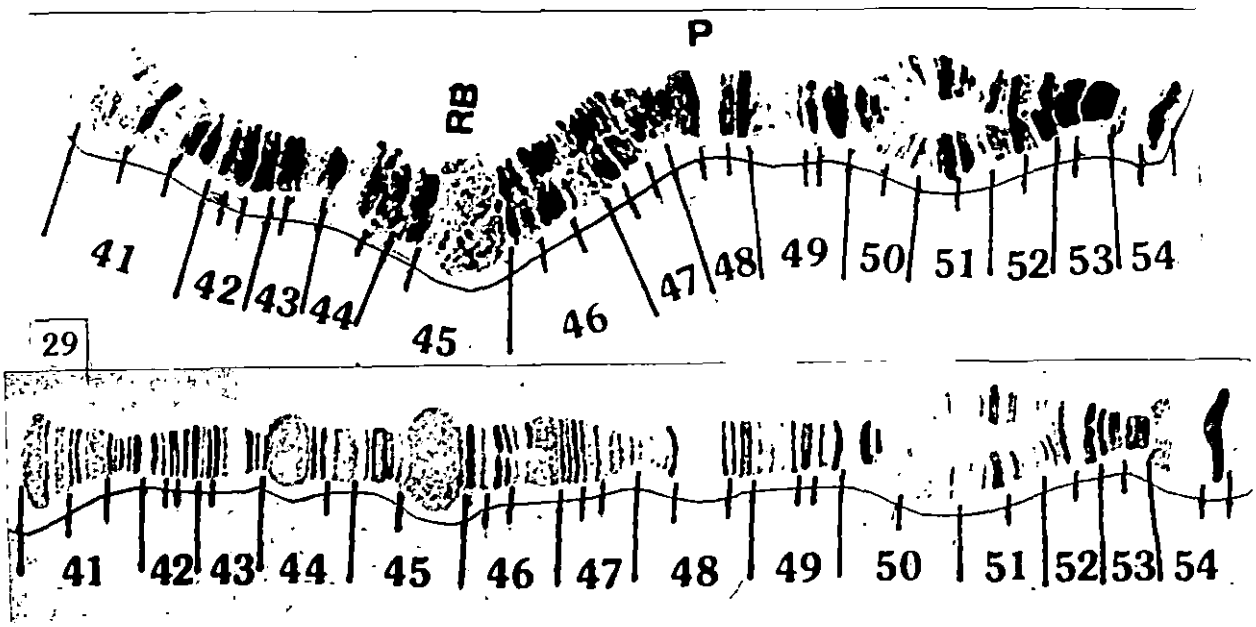
The short arm IS (Fig. 27b) is divided into 20 sections of which section 1 is lightly staining and no distinguishing bands were encountered. However, a series of dark bands in the sections 3B-7A and 17-18 serve as an important reference point. Other characteristic features of this chromosome include a small pale puff in section 7B followed by another large puff in section 8B. There is a distinctive constriction in the section 11B.

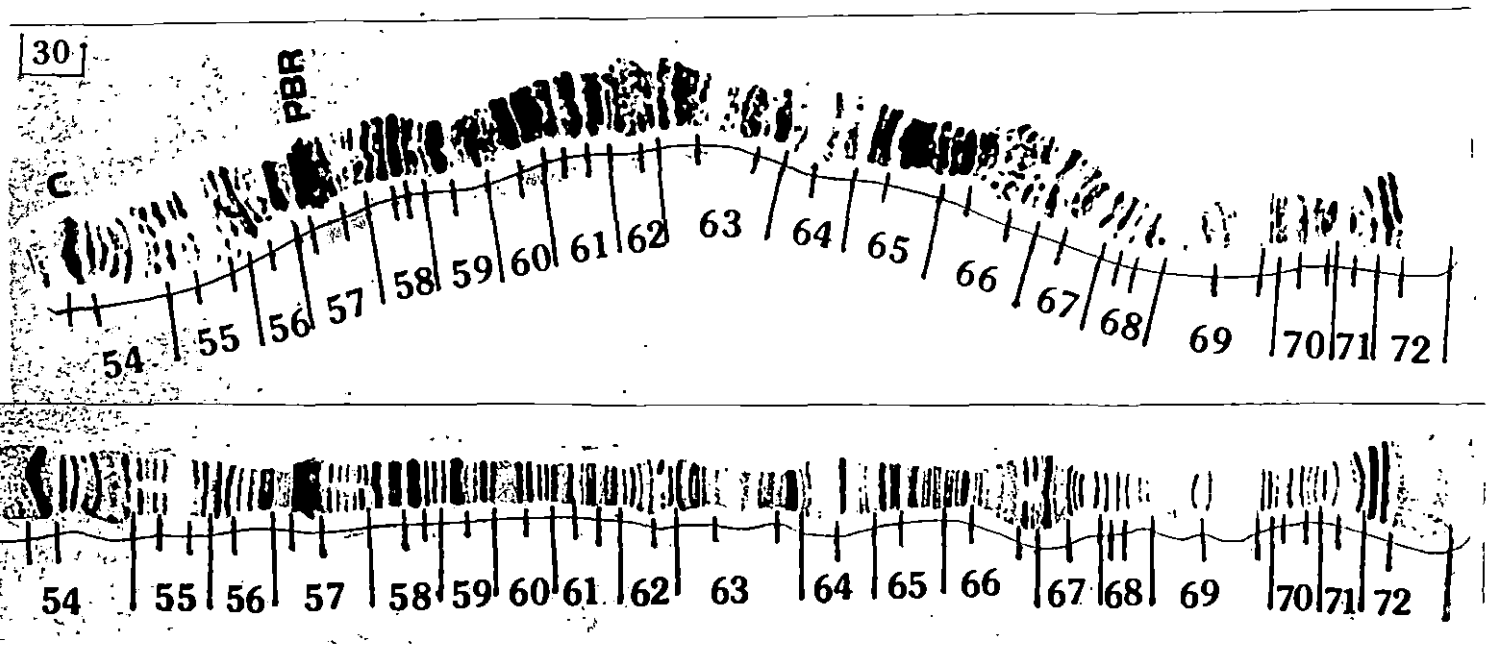
The long arm (IL) (Fig.28) is also divided into 20 sections. Centromere in the section 21A,B is remarkable by the presence of two pairs of bands at its two ends, of which, the bands of 21B are more prominent. However, the centromeric region (21A) does not have any defined centromere bands. A nucleolus of variable expression is always produced by the organising region in section 22 and

PLATE - 15

Figs. 29 - 30 : Standard photocomposite map and free hand pencil drawing of IIS (sections 41 - 53) and IIL (sections 54 - 72) of S. (E.) praelargum male. Abbreviations used - C, Centromere; PBR, Parabalbiani Ring; P, Puff; RB, Ring of Balbiani. (X3000 approx.).

PLATE - 15


P
RB
41
42
43
44
45
46
47
48
49
50
51
52
53
54
29
41
42
43
44
45
46
47
48
49
50
51
52
53
54


30
C
PBR
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72

it has typical irregular appearance. The remarkable banding groups include a group of three deeply stained bands in the sections 24A,B, bulge in section 24C, a marker band in 25C and a shield-like pattern in section 29. A neck/constriction is also encountered in section 32A followed by two pale puffs; a smaller one in section 34B while the larger one is present in the section 35A. The tip of the IL (section 40) is diffusedly stained and is devoid of any remarkable banding pattern. However, a sharp band in the section 40A serves as an important landmark to identify the terminal end of IL.

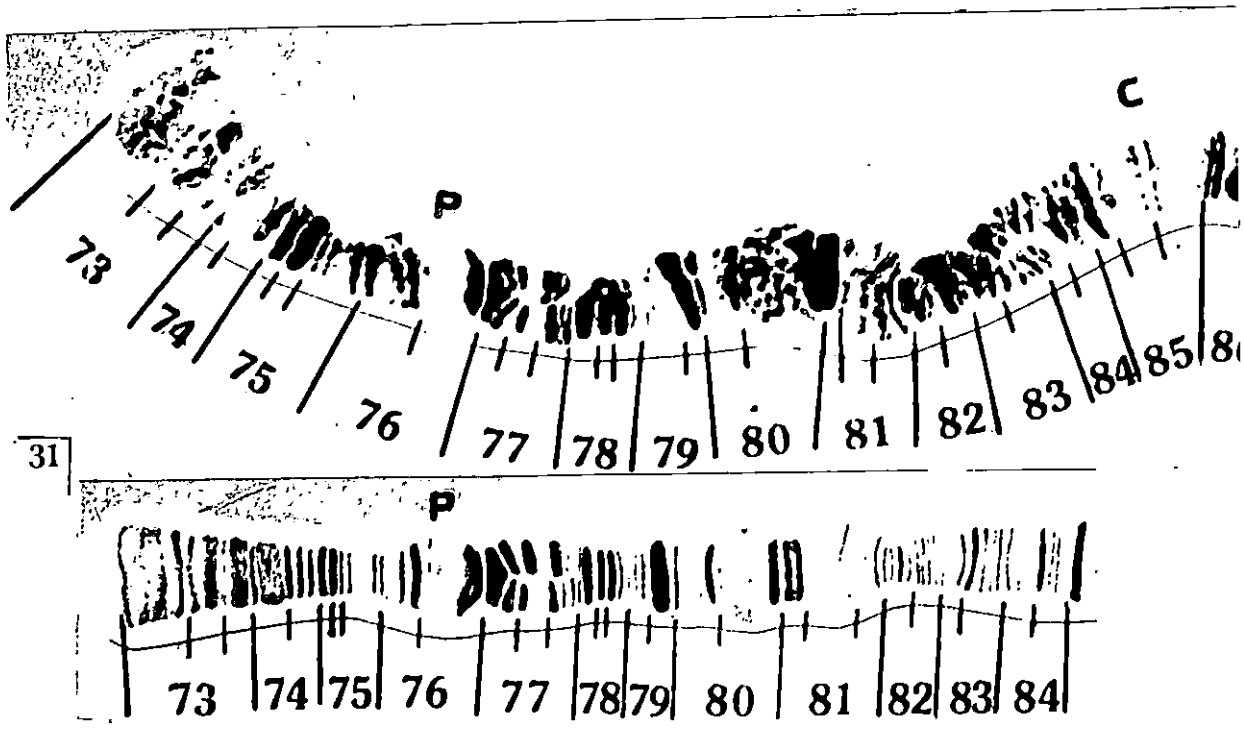
Chromosome II : The chromosome II (Plate 15, Figs. 29,30) is somewhat shorter than chromosome I and is metacentric in nature. This chromosome is distinguished by the presence of Balbiani Ring in section 45B. The short arm (IIS) (Fig.29) is rich in morphological characteristics. Of particular importance are the presence of two groups of bands designated as group I and group II in the sections 41C - 43A and 52B - 53 respectively. A constriction is present in the middle of the IIS (section 48A) which is followed by a pale puff in the section 48B. There is a marker band in the section 52A and the terminal end of IIS could be identified by its bell shaped appearance. This end is remarkably characterised by a deeply stained solitary band in section 41B, while the other bands in section 41A are lightly stained and only identifiable in good preparations.

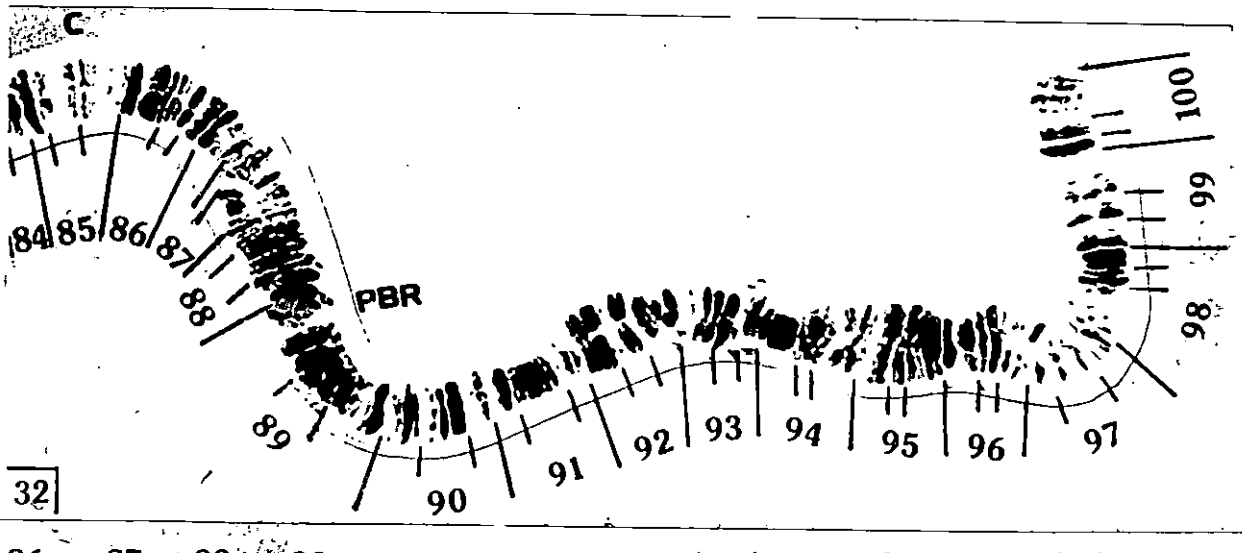
The long arm (IIL) (Fig.30) is subdivided into 19 sections. The centromere is characterized by a deeply

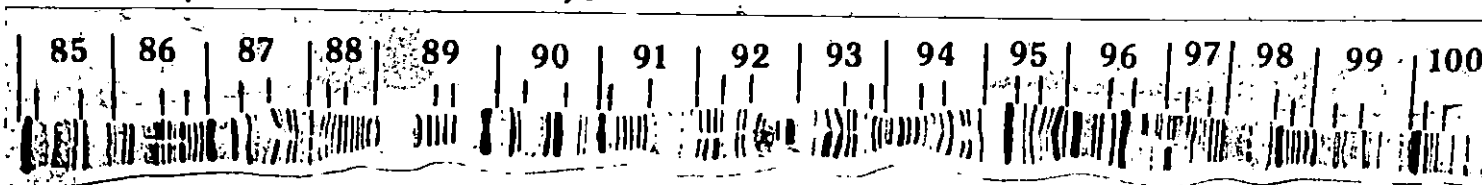
PLATE - 16

Figs. 31 - 32 : Standard photocomposite map and free hand pencil drawing of IIIS (sections 73 - 84) and IIIL (sections 85 - 100) of S. (E.) praelargum male. Abbreviations used - P, Puff; C, Centromere; PBR, Parabalbiani Ring. (X3000 approx.).

PLATE 16


C
P
73
74
75
76
77
78
79
80
81
82
83
84
85
31
P
73
74
75
76
77
78
79
80
81
82
83
84


C
84
85
86
87
88
PBR
89
90
91
92
93
94
95
96
97
98
99
100
32


85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

stained band in section 54A,B,which is followed closely by a group of fine lightly staining post centromeric bands in sections 54C and 55. Other identification points include a Parabalbiani in section 57B, a group of three heavy bands in section 58. There is a dark band marker in section 59A which serves as an important reference point. The terminal end is broad, lightly stained and characterised by two distinct bands in section 72.

Chromosome III : The smaller length and the submedian position of centromere served to distinguish the chromosome III (Plate 16, Figs,31,32) from other two chromosomes. However, unlike other two chromosomes,the centromere in this chromosome is not very prominent (section 85A).

The short arm (IIIS) (Fig. 31) is divided into 12 sections and is endowed with remarkable identifying characteristics. The club shaped terminal end, which is encountered in all the individuals studied (section 73), provides useful aid for chromosome identification. Other remarkable features include two darkly stained marker bands in section 75, a group of three bands in section 78, a large marker band in section 79B and another marker in 81A. A pale puff in section 76B and a constriction in 82A also serve as important identifying character.

The long arm (IIIL) (Fig.32) is also endowed with many characters which serves as useful aids for chromosome analysis. This arm is subdivided into 16 sections. Centromere (85) is characterised by a darkly stained band.

However, it is not so clearly defined in all the individual of this species so far studied. Remarkable banding groups include a group of dark bands in the sections 86 and 87A followed by Parabalbiani in section 89A. There is a single marker band each in sections 96B and 100A. The terminal end is diffused without any specialised distinguished characters.

COMMENT: The diploid count of S. (E.) praelargum male and general banding pattern agree well with its congeneric species, S. (S.) dentatum, S. (S.) singtamense and S. (S.) himalayense. However, it differs from the congeneric species in the position of centromeres in the chromosomes, position of Balbiani Ring , position of Parabalbiani and the position of NOR.

Family – Simuliidae

Subfamily – Simuliinae

Tribe – Simuliini

Simulium (Eusimulium) ghoomense (Female) Datta, 1975

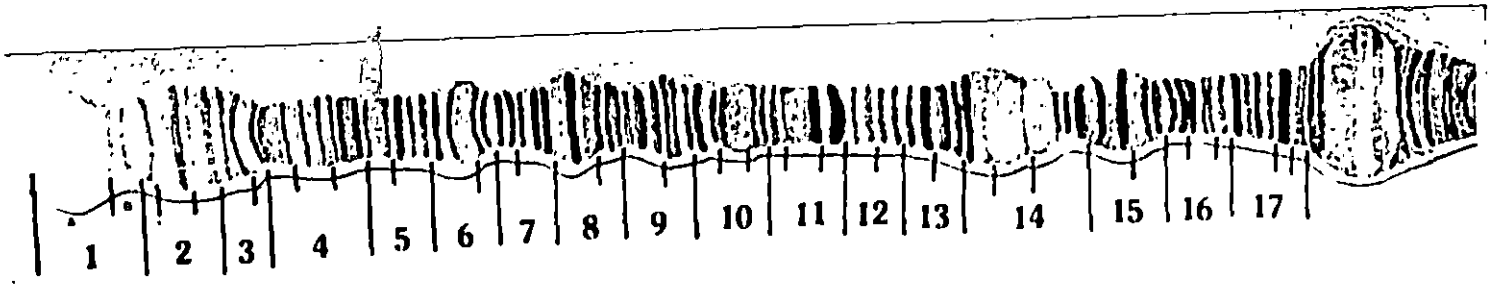
This species also revealed three polytene chromosomes n=3, each one is tightly synapsed with median and submedian centromeres. The % of total complementary length of each chromosome arm is given in the Table 2. The length of the largest chromosome was 40.00 % of total complementary length while that of II and III were 32.00 % and 28.00 % respectively. However, the difference between II and III was very less. The standard map of each chromosome was prepared

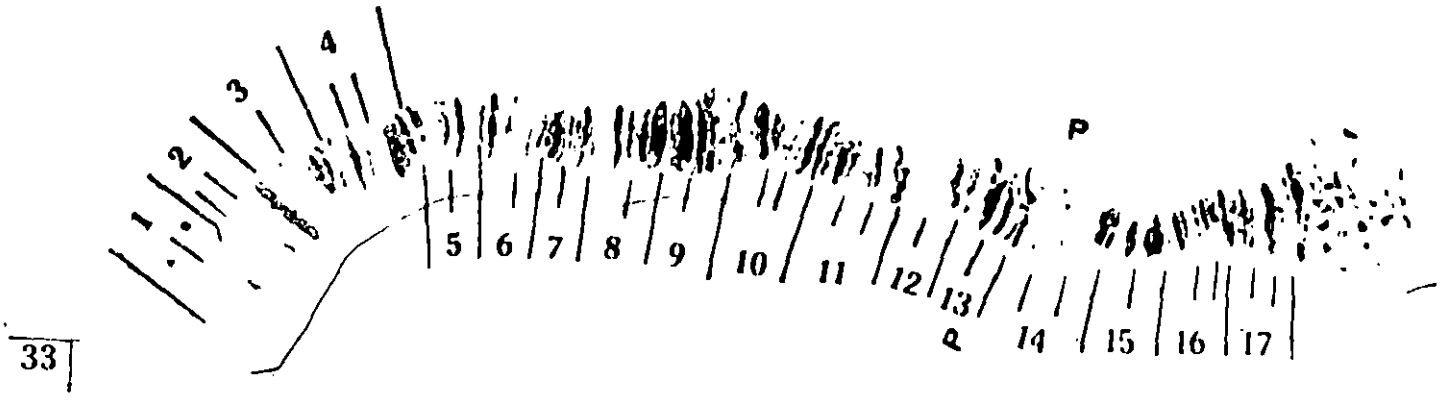
PLATE - 17

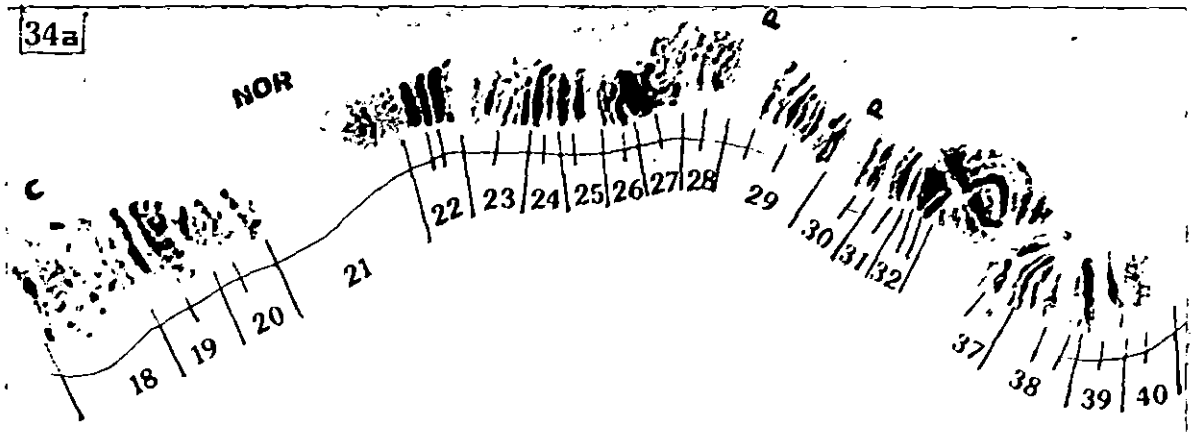
Figs. 33 - 34a : Standard photocomposite map and free hand pencil drawing of IS (sections 1 - 17) and IL (sections 18 - 40) of S. (E.) ghoomense female. Abbreviations used - P, Puff; NOR, Nucleolar Organiser Region; C, Centromere. (X3000 approx.).

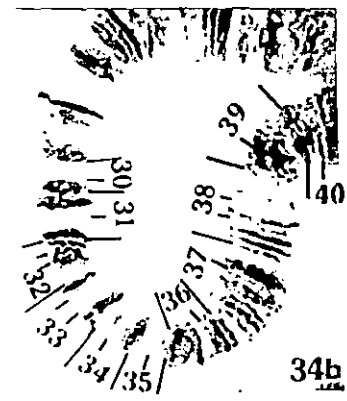
Fig. 34b : Standard photocomposite map and free hand pencil drawing of IL (sections 30 - 40) representing the normal segment of the chromosome arm of S. (E.) ghoomense female. (X3000 approx.).

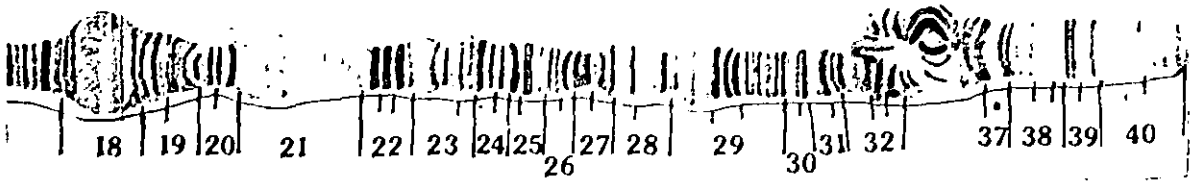
PLATE - 17

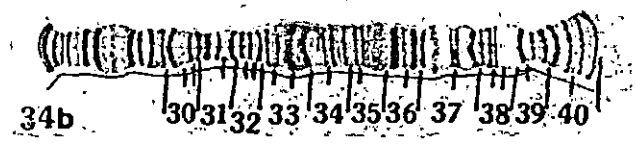

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
P
Q
33


34a
NOR
C
Q
Q
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
37
38
39
40


30
31
32
33
34
35
36
37
38
39
40
34b


18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
37
38
39
40


34b
30
31
32
33
34
35
36
37
38
39
40

on the basis of sections assigned per arm.

Chromosome I: This chromosome (Plate 17,Figs. 33 ,34a,b) is distinguished from other two chromosomes by its greater length,metacentric nature and by the presence of a prominent nucleolar organiser in the section 21 of IL.

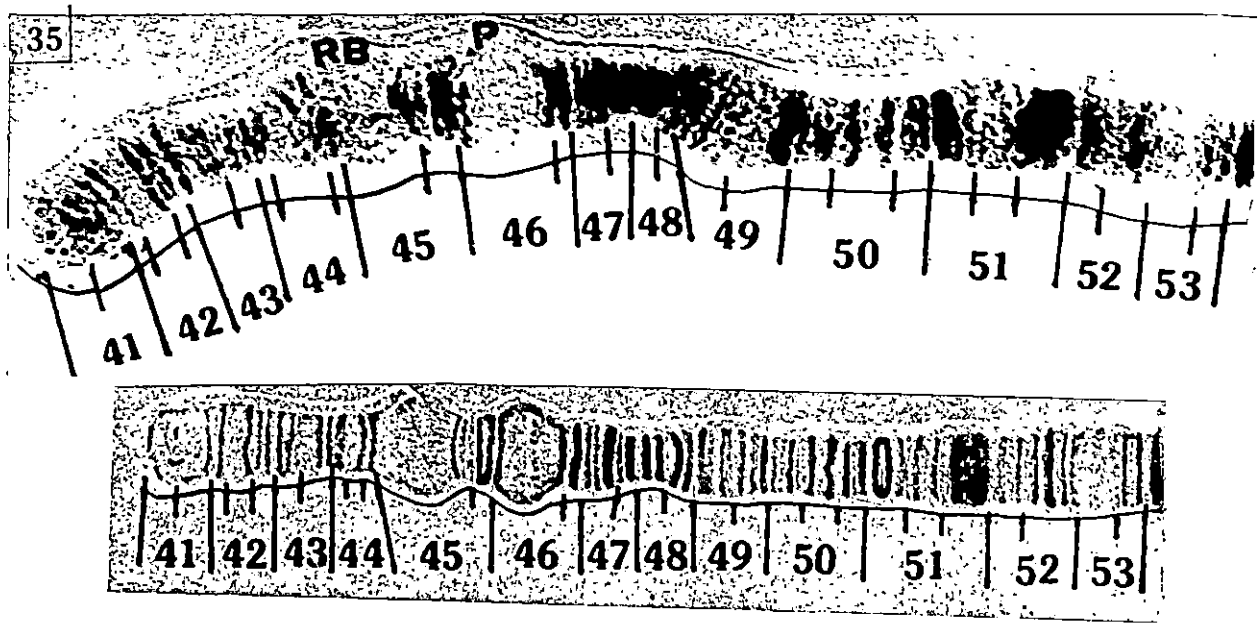
In the short arm (IS) (Fig.33), several characteristic landmarks provide useful aids for chromosomal analysis. The IS is divided into 17 sections. The terminal end of IS (Section 1) was lightly stained and did not possess any definite shape. A darkly stained band was encountered in the section 2B. There are two groups of darkly stained bands,one group in sections 8-9, while the other in 13. There are two pale puffs in section 14.

The long arm (IL) (Fig.34a,b),is subdivided into 23 sections. The centromere (section 18) is irregularly stained expanded region which is devoid of sharp centromere bands. There is a dark marker band in the section 19A followed by a nucleolar organiser region in 21. Near nucleolar organiser region there is a group of three darkly stained bands in section 22 which serves as important landmark. There is a bulge in 23A followed by a series of dark bands in sections 24,25 and 26. There are two large puffs one each in 29A and 30A interspersed by series of fine dark bands. In some individuals of this species, paracentric inversion were encountered, encompassing the sections 32-36 (Fig,34b). However,the frequency of this inversion was not estimated. Tip of IL (Sec.40) did not reveal any remarkable bands.

PLATE - 18

Figs. 35 - 36 : Standard photocomposite map and free hand pencil drawing of IIS (sections 41 - 53) and IIL (sections 54 - 72) of S. (E.) ghoomense female. Abbreviations used - C, Centromere; P, Puff; PBR, Parabalbiani Ring; RB, Ring of Balbiani. (X3000 approx.).

PLATE - 18


35
RB
P
41 42 43 44 45 46 47 48 49 50 51 52 53
41 42 43 44 45 46 47 48 49 50 51 52 53

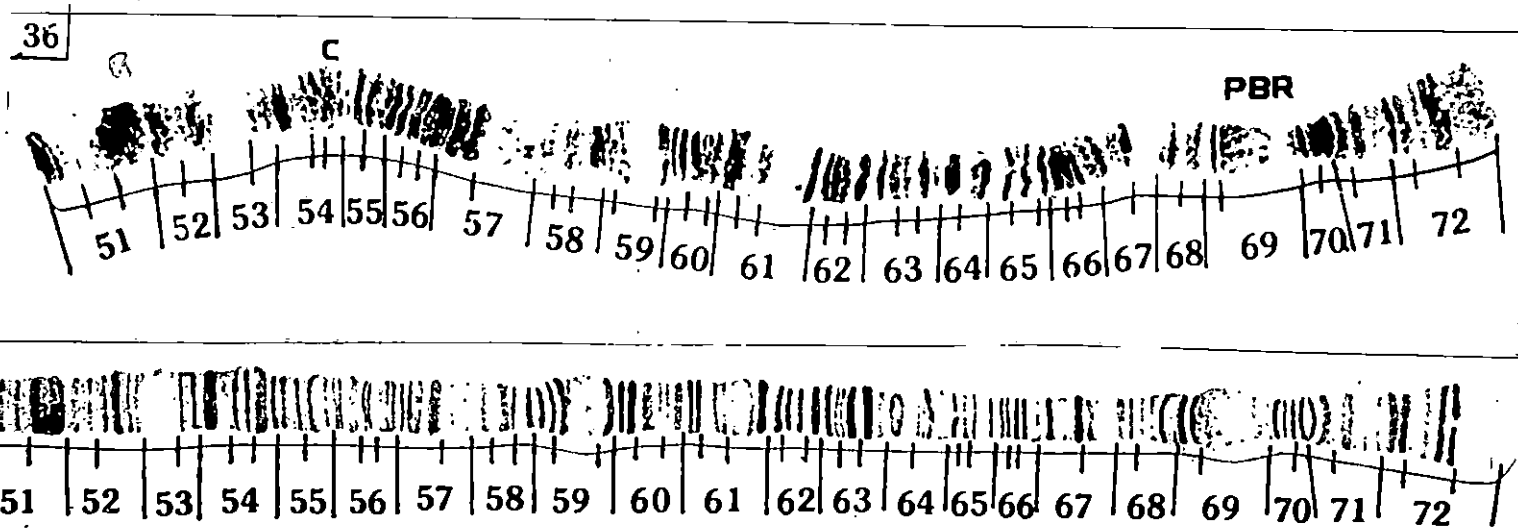
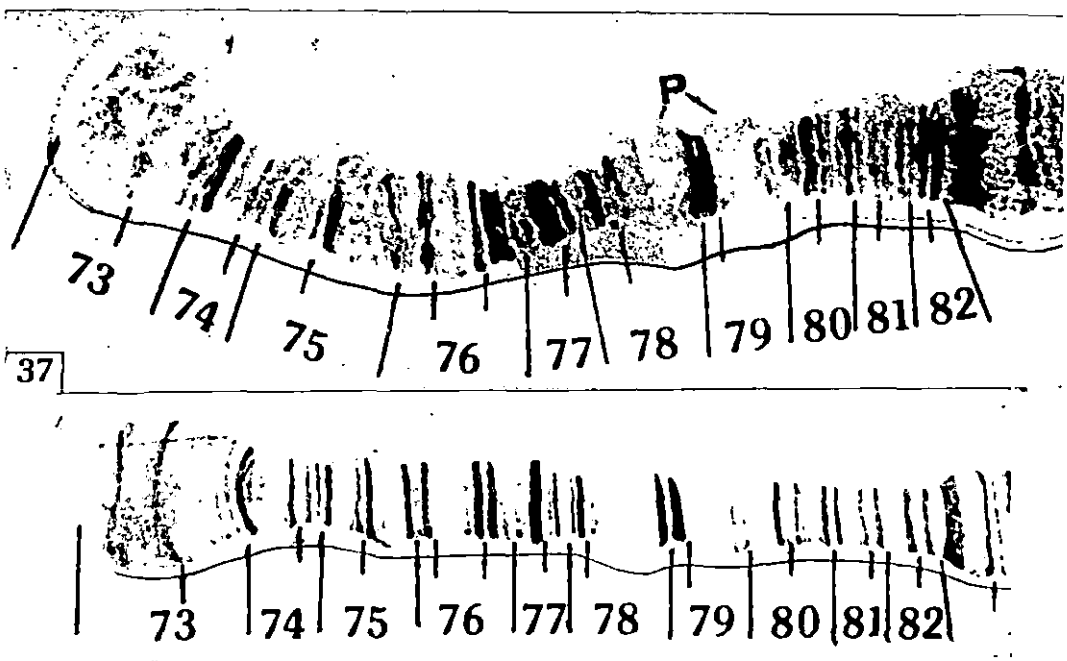
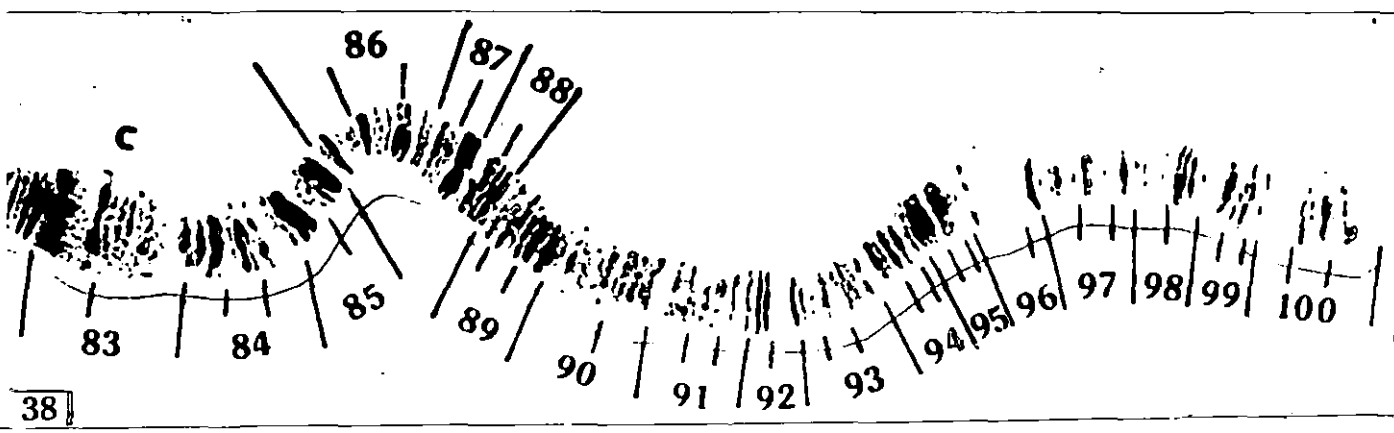

36
C
PBR
51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72

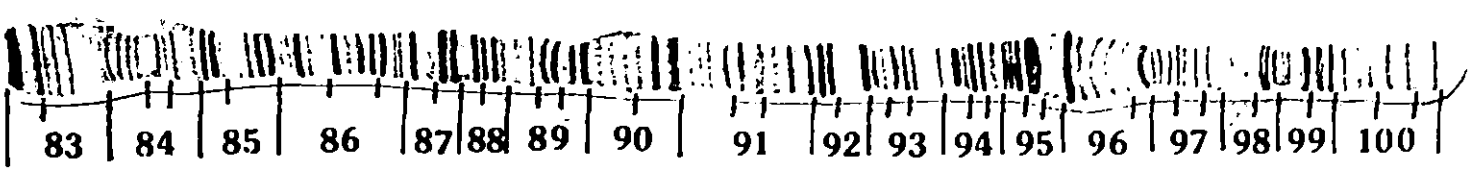
PLATE - 19

Figs. 37 - 38 : Standard photocomposite map and free hand pencil drawing of IIIS (sections 73 - 82) and IIIL (sections 83 - 100) of S. (E.) ghoomense female. Abbreviations used - P, Puff; C, Centromere. (X3000 approx.).

PLATE - 19


P
73
74
75
76
77
78
79
80
81
82
37
73
74
75
76
77
78
79
80
81
82


C
86
87
88
83
84
85
89
90
91
92
93
94
95
96
97
98
99
100
38


83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

Chromosome II: This chromosome (Plate 18,Figs 35,36) is somewhat shorter than chromosome I ,metacentric in nature and is distinguished by the Ring of Balbiani.

The short arm (IIS) (Fig.35) is rich in morphological characters and is divided into 13 sections. The terminal end of IIS was homogeneously stained and without any definite shape. There is a Ring of Balbiani in section 45A, followed by a large pale puff in 46A. Balbiani Ring and the puffs are interspersed by only few bands. There is a neck almost in the middle of IIS in 48A, a large marker band is presentin 51A and deep heterochromatic block in 51C.

The chromosome IIL (Fig.36) is further subdivided into 19 sections. There is a diffusedly stained centromere in section 54. There is a series of sharp bands in sections 55-57A. There are two small puffs one each in sections 59B and 61C separated by a group of dark bands. Other characteristic landmarks include three bands in section 66A,B, a Parabalbiani ring in 69 and a distinctive neck in section 70. The terminal end (section 72) is lightly stained and is provided with two pairs of bands.

Chromosome III: (Plate 19,Figs.37,38) This chromosome is the shortest of all the three chromosomes and is submetacentric in nature. The chromosome IIIS (Fig, 37) is subdivided into 10 sections and is characterised by the presence of two pale puffs one each in sections 78B and 79B, separated from each other by two dark bands. On the other hand,IIIL (Fig.38) is subdivided into 18 sections. The

centromere in section 83 is characterised by a prominent dark band. There is a series of dark bands in sections 94 and 95. The terminal end (section 100) of this chromosome is diffused in nature while in good preparations some fine bands were encountered.

Comment: The diploid count of S. (E.) ghoomense female and the general banding pattern is in accordance with its congeneric species S. (E.) praelargum, S. (S.) dentatum, and S. (S.) singtamense and S. (S.) himalayense. There is preponderance of puffs as observed in S. (E.) praelargum. It differs from other species in the location of NOR, centromere, Parabalbiani and Balbiani Ring.

Fig. A : Idiograms of S. (S.) dentatum , S. (S.) singtamense, S. (S.) himalayense , S. (E.) praelargum and S. (E.) ghoomense based on relative percentage length of chromosomes. C - centromere; F - flared end; NO - nucleolar organiser; RB - ring of Balbiani.

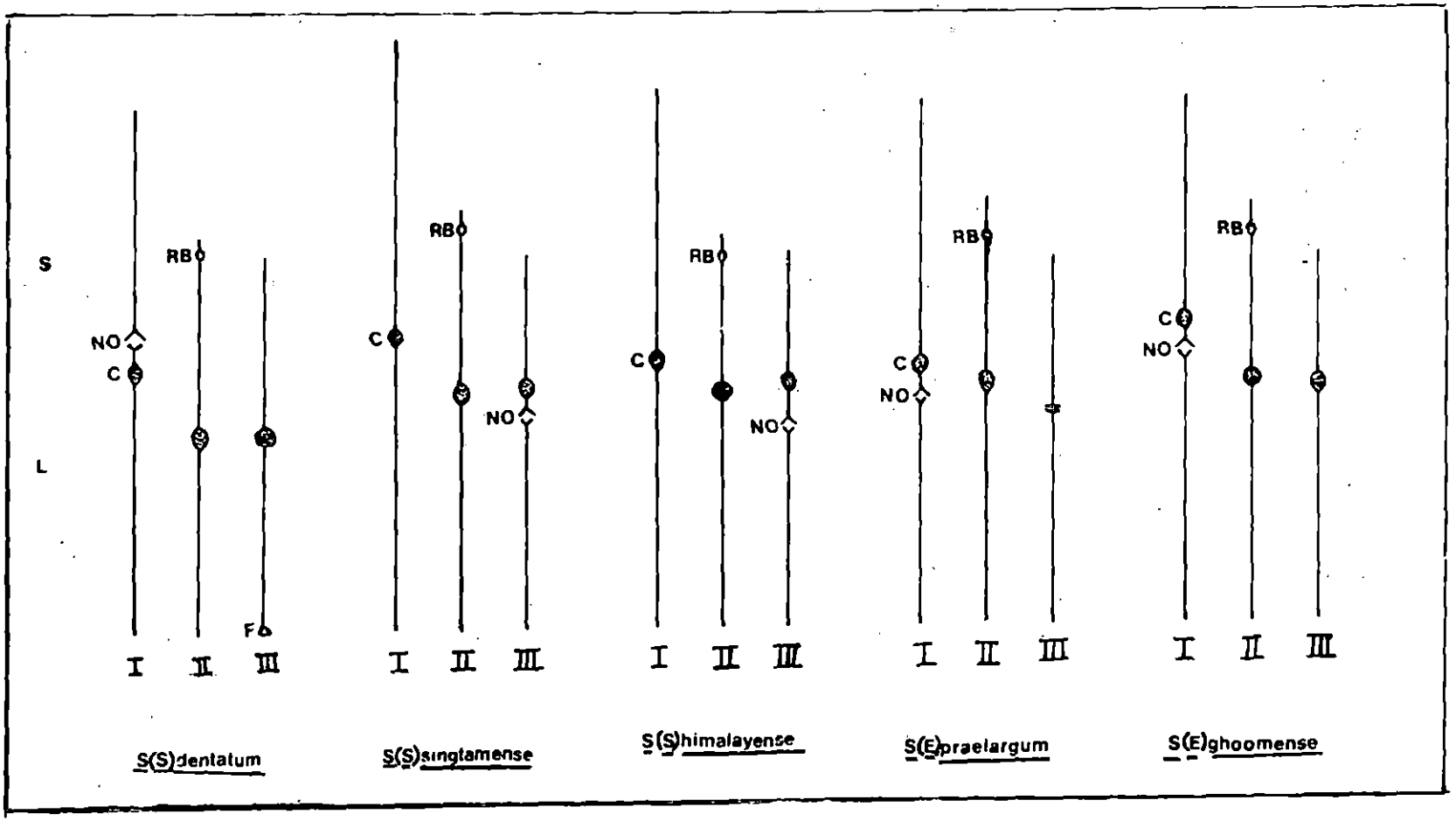

S
L
NO
C
RB
F
I
II
III
S(S)dentatum
S(S)singtamense
S(S)himalayense
S(E)praelargum
S(E)ghoomense

Fig. A:

Discussion

Chromosome number in black flies

Neuroblast cells contain 2n=6 chromosomes in all the Simulium species so far studied. Salivary gland nuclei have three polytene chromosomes with two homologues more or less intimately paired. Moreover, centromeric regions in most species form characteristic expanded regions dividing the polytene chromosome in the same proportions as the mitotic metaphase chromosomes. The major identifying landmarks of polytene chromosome of different species include Balbiani Ring, nucleolar organiser, puff, Parabalbiani and characteristic banding patterns. In the present investigation, the polytene chromosome of S. (S.) dentatum, S. (S.) singtamense, S. (S.) himalayense, S. (E.) praelargum and S. (E.) ghoomense and the ganglion chromosomes of S. (S.) himalayense and S. (E.) praelargum have been studied .

Idiograms (Fig. A) of five species were constructed from % TCL of each polytene chromosome. The position of centromere, Ring of Balbiani, nucleolar organiser region is shown in each of the idiograms. Inspection of idiograms of five species revealed that all the chromosomes are tightly paired and without any asynapsis. The chromosome I of S. (S.) singtamense is longer than that of other four species though the chromosome II and chromosome III of all the five species are more or less equal in length. In all the five

species, the Balbiani Ring is present in the terminal region of IIS chromosome. However, the position of NOR was found to be variable in those species. It was present in the IIIrd chromosome of S. (S.) singtamense and S. (S.) himalayense, while in S. (S.) dentatum, S. (E.) praelargum and in S. (E.) ghoomense it was in the Ist chromosome. However, in all the five species NOR was present near the centromere. In S. (S.) dentatum, the IIIL is characterised by flared end while no such distinctive character was encountered in other four species. Therefore, the comparison of the idiograms of five species of Simulium revealed that two species of the subgenus Eusimulium, S (E.) praelargum and S. (E.) ghoomense are very closely related, while the three species of the subgenus Simulium, S. (S.) dentatum, S. (S.) singtamense and S. (S.) himalayense differs from each other in respect of location of NOR, presence of flared end and in the length of the Ist chromosome. Furthermore, chromosome count, banding patterns, major morphological characters of these five species agree well with other Simulium species reported by earlier workers (Rothfels and Dunbar, 1953; Rothfels, 1956, 1979; Rothfels et al, 1978; Rothfels and Freeman, 1966, 1977; Rothfels and Mason, 1975; Rothfels and Nambiar, 1975; Bedo, 1975a,b, 1976, 1977a).

However, there is a deviation from the basic Simuliid complement in the genus Cnephia (Procunier, 1982b). Four of the five species of this genus revealed n=3, while in Cnephia lapponica, the basic chromosome complement is

reduced from n = 3 to n = 2 metacentric chromosomes as a result of fusion of chromosomes II and III.

General morphology of polytene chromosome

Centromere:

In black fly, centromere is an expanded region of polytene chromosome usually characterised by a heavy dark band (Dunbar,1962). The expanded region correspond in position to the centromeres in mitotic chromosomes. The expanded regions were joined to form a pseudochromocenter in Simulium pictipes A (Bedo,1975a) in S. melatum (Bedo,1976). True chromocenter, which is a regular phenomenon in Drosophila (White,1973) was also reported (Rothfels and Freeman,1976) in four species of Prosimulium, P. fontanum, P. saltus, P. approximatum and P. mysticum. In the present investigation, of the five species studied, three species S. (S.) dentatum, S. (S.) singtamense and S. (S.) himalayense showed prominent centromeres while in two species, S. (E.) praelargum and S. (E.) ghoomense, centromeres were difficult to recognise. The location of centromere in the polytene chromosomes of all the five species is summarised in Table 4.

Table : 4 : Distinguishing landmarks of three chromosomes of five species of Simuliidae.

Name of the species	Position of centromere	Position of BR	Position of PBR	Position of NOR	Position of Puffs
S. (S.) dentatum (Female)	IL - 21(A) IIL - 55 IIIL - 84	IIS - 41B	IIL - 64B	IS - 19	IS - 12 IIS - 42
S. (S.) singlamense (Female)	IL - 23 IIL - 59 IIIL - 85A	IIS - 47	---	IIIL - 86	IS - 14 IIS - 52
S. (S.) himalayense (Female)	IL - 21 IIL - 54 IIIL - 83	IIS - 43	IIIL - 93C	IIIL - 87	IS :2B,13A IL :32,39B IIS:44,49,51 IIIL :91,92
S. (E.) praelarqum (Male)	IL - 21A,B IIL - 54A,B IIIL - 85	IIS - 45B	IIL - 57B IIIL-89A	IL - 22	IS :7B,8B IL :34B,35A IIS :48B IIIS - 76B
S. (E.) ghoomense (Female)	IL - 18 IIL - 54 IIIL - 83	IIS - 45A	IIL - 69	IL - 21	IS:14.IL:29A, 30A.IIS:46A. IIL:59B,61C. IIIS:78B/79B

It has been observed that there was little variation in the location of centromeres in all the five species studied here. Dark heavy centromeric bands of S. (S.) singtamense and S. (S.) himalayense are comparable to that of the species belonging to S. pictipes group (Bedo,1975a), while the centromere of S. (S.) dentatum did not reveal any heavily stained band, but rather granular in appearance with irregular bands similar to that of the members of S. venustum/verecundum complex (Rothfels et al., 1978),S. fibrinflatum and S. luggeri (Gordon,1984). On the other hand, the centromeres of two species, S.(E.) praelargum and S. (E.) ghoomense were difficult to recognise. In the former species,the centromere was not typically expanded region though it is characterised by dark heavily stained bands. On the other hand, the centromere of chromosome I of S. (E.) ghoomense is easily identifiable while it needs a little effort to identify centromere in case of chromosomes II and III. It is interesting to note that the centromere of S. (E.) ghoomense is comparable to that of Metacnephia sp.(Procunier,1982a). Moreover, centromeres of polytene chromosomes could also be identified by C and fluorescent banding techniques. The centromeric regions of the members of S. pictipes group displayed bright fluorescence with Quinacrine (Bedo,1975 a),while polytene chromosome of S. ornatipes and S. melatum(Bedo,1975b) found to be C band positive. Therefore, banding technique is very useful in the identification of centromere when it was not a typical expanded region.

Nucleolar organising region:

The nucleolar organiser appears as a gap in the polytene chromosomes surrounded by nucleolus. The bands in the immediate vicinity of NOR are somewhat disrupted and the chromonemata on either side of the organiser appear to extend like a complex branching system with roots into the actual nucleolus. Moreover, study of nucleolar relations in Simuliidae promises to be diagnostically useful, because of the intra-specifically constant and inter-specifically variable attachment of the main nucleolus. In Simulium, the terminal nucleolus which was reported in the members of S. pictipes group (Bedo, 1975 a) was difficult to identify without counterstaining by light green. However, nucleolar organiser of interstitial region could easily be identified without subjecting it to any specialised staining technique. In all the five species investigated here, NOR was located in the interstitial region. In S. (S.) dentatum, S. (E.) praelargum and S. (E.) ghoomense, NOR was located in IS 19, IL 22 and IL 21 respectively, while in S. (S.) singtamense and S. (S.) himalayense it was found in IIIL 86 and IIIL 87 respectively (Table 4). Therefore, except S. (S.) dentatum, where NOR is located in IS arm, the location of NOR is similar in other two species of the subgenus Simulium. Furthermore, in two species of the subgenus Eusimulium, the NOR is also found in the same arm. Hence, the location of NOR could be used effectively in the characterisation at subgeneric level. Interstitial NOR was also reported earlier

in S. venustum and verecundum complex (Rothfels et al.,1978),Cnephia(Procunier,1982b), species of Prosimulium (Rothfels and Freeman,1977),and Simulium (Bedo, 1977; Gordon,1984). Besides primary nucleolus, a secondary nucleolus was also reported among the members of S. ornatipes(Bedo, 1977). The secondary nucleolus frequently fused with the main nucleolus in S. ornatipes. However, in the species investigated here, no such secondary nucleolus was observed. Among other dipteran species, the NOR is found to be located in the pairing segment of sex chromosome in different species of Drosophila, while in some species of Chironomus, more than one nucleolus was observed (White,1973). Furthermore, nucleolus is present in all the tissues in all stages of development and its location in the karyotype is the same in both polytene and mitotic nucleoli. Therefore, nucleolar organiser region naturally serves as an important character for cytotaxonomic study.

The Ring of Balbiani (RB):

Since the site of RB or Balbiani Ring is species specific, therefore, this structure also serves as an important landmark for the identification of Simulium species. It is identifiable in all the species studied in the present investigation. In all the five species, the RB is present in IIS chromosome. In S. (S.) dentatum, S. (S.) singtamense and S. (S.) himalayense, they were encountered in IIS 41B, IIS 47 and IIS 43 respectively, while in S. (E.) praelargum and S. (E.) ghoomense, it was observed in IIS 45B

and IIS 45A respectively (Table 4). Therefore, it seems that the location of RB is species specific in three species of the subgenus Simulium, while in two species of Eusimulium no such specificity was perceptible. Furthermore, a comparison with other Simulium species revealed that the RB is present in the section IIS 42 in all the members of the Simulium pictipes group (Bedo, 1975a), while in species of Simulium ornatipes and S. ruficorne, it was reported in section IIS 43 (Bedo, 1977,1989), comparable to that of S. (S.) himalayense. On the other hand, RB is located in IIS 47 in S. venustum / verecundum (Rothfels et al.,1978), in S. jenningsi, S. fibrinflatum and S. luggeri,(Gordon, 1984) similar to that of S. (S.) singtamense.

Moreover, in the genus Cnephia, the RB was also located in IIS 47 (Procunier, 1982b), indicating a close relationship between Simulium and Cnephia with respect to the position of Balbiani Ring. Therefore, the present study clearly shows that BR could be used as an important landmark for the characterisation at generic and subgeneric levels.

Puffing in polytene chromosome At particular stages of development, some of the genetic loci in the polytene chromosome undergo a spectacular change in appearance. They become converted into large swellings or puffs. It has been generally believed that a process of puffing is due to the biosynthetic activity of a particular loci concerned. Therefore, study of the puffing pattern is of great

importance for understanding gene action. Puffs were encountered in various members in all the species under present investigation. Position of puffs in polytene chromosomes of different species is summarized in Table 4.

In S.(S.) dentatum and S. (S.) singtamense two large and clear puffs were seen in IS and IIS respectively. On the other hand, in S. (S.) himalayense, S. (E.) praelargum and in S. (E.) ghoomense, a large number of puffs were encountered in Ist. 2nd. and 3rd. chromosomes. Puffs were also reported in Simulium pictipes (Bedo, 1975a), S. ornatipes (Bedo, 1977) and in members of S. venustum/verecundum complex (Rothfels et al.,1978). However, no attempt has yet been made to study the detailed puffing pattern of different species of Simulium. However, in other dipterans, puffing patterns have been extensively studied. In Chironomus tentans and C. pallidivittatus, puffing patterns have been studied (Grossbach,1968,1969) in detail in relation to the synthesis of specific silk like proteins which are produced in large amount in the cells of salivary gland. In Drosophila melanogaster, the puffing pattern of X chromosome loci is similar in both the sexes. But a group of puffs were active for a longer time in male than in female (Ashburner,1967,1969a). While studying the puffing patterns of D. simulans and D. melanogaster, Ashburner (1969a,b) observed that X chromosome of D. simulans form two puffs which were absent in D. melanogaster. Moreover,D. melanogaster has one autosomal puff (46A) which was not

present in D. simulans. It is also interesting to note that hybrid between these species show a heterozygous puff. Furthermore, differences in time of puffing and size of puff also exists between different strains of D. melanogaster and certain puffs are active in some strains,but are not seen in others (Ashburner,1969 c). Therefore,the study of puffing patterns may be a helpful guide in demarcating congeneric as well as sibling species. Studies of the puffs in chromosomes of five species under present investigation also revealed the difference in location and number of puffs between congeneric species. Hence,it seems that, in the Simuliidae,the study of the puffing pattern could also be used as an important landmark for the comparison of the polytene chromosome of different species.

Parabalbiani Ring (PBR) Parabalbiani is darkly stained band with one sharply defined and one diffused edge. This structure serves as an useful polarised marker. PBR is unique to the Simuliidae and readily recognised in the species of different genera. Of the five species studied in the present investigation ,PB was encountered in all the species except S. (S.) singtamense. In S. (S.) dentatum, the PB was found in IIL 64B in both male and female individuals while in S. (S.) himalayense and S. (E.) praelargum, it was located in IIIL 93C and IIIL 89A, IIL 57B respectively. It was found in IIL 69 in S. (E.) ghoomense (Table 4). Therefore, the distribution of PB in all the four species indicates that this landmark could be effectively used in

distinguishing the species. PB, comparable to the above named species, was also reported in other species of Simuliidae. In each species, it serves as an important landmark such as in S. pictipes (Bedo,1975a), S. ornatipes(Bedo 1976) ,S. venustum/verecundum complex (Rothfels et al.,1978) and in Metacnephia (Procunier,1982a).

Banding pattern

The pattern of dense bands and less dense interbands that characterise the most polytene chromosomes is a feature that has been of the utmost value to geneticists, particularly since the demonstrations by Bridges (1937) that the genetic and polytene chromosome maps are colinear. In the present investigation, of all the five species studied, the distribution of heavily and lightly stained bands in the polytene chromosome complement is not random, but reflects some overall organisation. In S. (S.) dentatum, both in male and female sexes, there was a preponderance of dark bands in all the three chromosomes. There were some fine bands near the nucleolar organiser region in IS while in IL, series of dark bands distributed throughout the chromosomes. Though the diameter of chromosome I was uniform, constricted necks were encountered in IIL 59 and in IIIL 95. Fine lightly stained bands were found near the centromeres of chromosome I, II and III. Unlike the former species, all three chromosomes of S .(S.) singtamense are of uniform diameter and are characterised by some specific banding patterns such

as groups of heavy bands in IS 2-5; IS 7-10. Moreover,there was a shield - like band in IL 34. There were two groups of darkly stained bands, IIS 50C - 52A and IIS 54-55A. In all three chromosomes, fine lightly stained bands were found near the centromeric regions. In S. (S.) himalayense,the distribution of light and deeply stained bands were more or less uniform. However, as in other two species,lightly stained bands were present near the centromeric regions in all the three chromosomes. The specific banding character of this species includes band in IS 4A, a group of deeply stained bands in IS- 16 and 17; heavy band in IL- 24C, a group of four bands in IL- 38 and 39A. Dark marker bands were also present in IIS 45C - 47A. In the IIIrd chromosome, there was a group of heavy bands in IIIS- 78 and a group of deeply stained bands in IIIS- 75. In S. (E.) praelargum, on the other hand, dark and light bands were distributed uniformly throughout the length of the chromosome as in S. (S.) himalayense. The diameter of the chromosome was not uniform. Constricted neck, one each in IS- 11, IL- 32 and IIIS- 82 was also encountered in S. (E.) praelargum which were comparable to those of IIL- 59 and IIIL- 95 in S. (S.) dentatum. Moreover,in S.(E.) praelargum, deeply stained group of bands were found in all the three chromosomes. Besides the marker band, shield -like band was also encountered in IL- 29. Comparable shield - like band was also found in IL- 34 of S. (S.) singtamense. In S. (E.)

ghoomense, on the other hand,there was a preponderance of lightly stained bands as in S. (E.) praelargum and S. (S.) dentatum. Moreover,specific group of marker bands were also found in all the three chromosomes, especially in chromosome I. In some individuals of this species, an inversion involving the sections 33-37A was encountered in IL. However, no such inversion was found in other species under present investigation. Therefore, similarity and dissimilarity between the banding patterns of these five species suggest that there is an interrelationship between all these five species and each species is characterised by specific marker bands.

Sex Chromosome :

Sex chromosome heteromorphy is known from many groups of animals and plants, and in most cases it is the Y chromosome which is the smaller element. Presumably such systems evolve from morphologically identical and freely recombining sex chromosomes. Size difference between the sex chromosomes of Simuliidae is virtually unknown and in most species they are indistinguishable in the polytene nucleus (Post, 1985). Therefore, sex is determined primarily by a single locus or by very short length of chromosome (Rothfels and Mason, 1975). However, many studies have revealed species in which the sex chromosomes have become differentiated by the linkage of inversions as observed in Simulium erythrocephalum (Post, 1985). Such inversions may be fixed or polymorphic on either of the sex chromosomes or occur on

both but at different frequencies (Post, 1982a). All the five spcies namely, S. (S.) dentatum, S. (S.) singtamense, S. (S.) himalayense, S. (E.) praelargum and S. (E.) ghoomense under present investigation did not reveal any morphologically distinguishable sex chromosomes in male or female and only larval sex was identified from the structure of the gonads. Except S. (E.) ghoomense, where an inversion was encountered in IL of some of the individuals, no inversion or sex specific heteroband was encountered in any of these species. Therefore, detail study of the population of each species might throw some light on the sex chromosome in these species which is not within the scope of present investigation. However, the presence of sex chromosomes was reported in other members of Simuliidae. Typically, in black flies, sex chromosome become differentiated by linkage of chromosomal rearrangements to either the genetic X of genetic Y chromosome. Species of Prosimulium (Ottonen, 1966; Rothfels, 1956) show increase in differentiation of sex chromosomes by addition of inversions resulting in complex configuration involving whole arms. Inversion played an important role in the differentiation of Y chromosome in S. longistylatum while S. pictipes revealed 'heteroband' in IIL (Bedo, 1975a). Inversions also played an important role in the determination of sex in the species of S. jenningsi group (Gordon, 1984). This type of sex chromosome system was also found in Chironomidae (Beermann, 1955). A partial linkage of inversion to the X or Y chromosome was reported

in Chironomus tentans (Acton, 1957) and in C. intertinctus (Martin, 1962). The simplest kind of sex chromosome differentiation involves a failure of polytene chromosome pairing between specific sites of the X and Y chromosomes, the inclusion of heterochromatic supernumerary bands (Basrur, 1959) or alteration in size and composition of existing bands (Bedo, 1975a). Bedo (1977) also reviewed the distribution of para- and pericentric inversions in 65 black fly species from five genera namely, Prosimulium, Twinnia, Cnephia, Eusimulium and Simulium and found significantly higher frequency of pericentric inversions in the sex chromosome systems. Therefore, inversions play an important role in the differentiation of sex chromosomes in Simuliidae.

Cytophylogeny in black flies

Detailed studies of the polytene chromosome of numerous species of black flies now mainly confined to Canadian and European species, and little work has been done on the chromosome of Oriental black flies. Dey and Wangdi(1984 a) reported the mitotic chromosomes of four species namely, Simulium (Simulium) dentatum, S. (S.) ramosum, S. (E.) aureohirtum and S. (E.) purii from the Darjeeling and adjoining hill areas which is a part of Eastern Himalayas. They have found the diploid count of 2n=6 in each of the four species and chromosomes are metacentric in nature .Moreover, Dey and Wangdi (1984 b) have also reported

the presence of supernumerary chromosomes in the neuroblast metaphase chromosomes of two other species, S. (E.) gracilis and S. (E.) ghoomense. This work has been further extended by studying the polytene chromosomes of five species, reported in the present investigation. A comparison of the polytene chromosomes of these five species with those of European and North American species did not reveal any remarkable difference. However, further studies are required on the species of Eastern Himalayan region to make any meaningful linkage between the species of these continents. Rothfels et al., (1978) constructed an outline chromosome phylogenies that extended through the Prosimuliinae and encompassed most of the known species in Gymnopais, Twinnia, Heloden and Prosimulium. Less extensive phylogenies was also reported in Cnephia, Metacnephia, Eusimulium (Bedo 1977; 1979 a,b,c; Dunbar, 1965, 1967) and Simulium (Rothfels et al., 1978; Bedo, 1977, 1989). However, no serious effort has been made to link up those phylogenies though certain chromosome ends (IS) and middles are shared by members of all genera of the family. For the IIS arm, Dunbar (1967) claimed that the closest members of Cnephia, and Eusimulium, C. dacotensis and Eusimulium anatinum differ in but a single inversion. Furthermore, it has been suggested (Dunbar, 1967) that the IIS sequence of some Cnephia species can probably be successfully compared to that of of some Prosimulium, and that of Eusimulium to that of some Simulium. Therefore, it seems that there is

possibilities in linking up different phylogenies. Moreover, the chromosomal rearrangements like inversion plays an important role in the speciation process. Fixation of inversions and acquisition of unique floating inversions suppose to be one of the important processes in the evolution of Simuliidae (Bedo,1977). Chubareva (1977) also proposed a chromosome phylogeny for Simuliidae where Gymnopais has been considered as base on the basis of morphological dogma and not by cytological analysis. Rothfels (1979) also assumed that Gymnopais is the most primitive of all cytologically studied black flies, though Helodon, Gymnopais, Twinnia and Prosimulium are cytologically closely related genera. However, nucleic acid hybridisation studies (Sohn et al.1975,Teshima,1972) suggested that some species within Simulium appear to be as remote from each other as they are from species of the genus Cnephia. Moreover, at an interfamily level this type of study hold the promise of allowing the identification of most closely related genera of different families and thereby providing clues to ancestry. Therefore, integration of the cytological data from different areas and continuous interaction among cytologists,molecular biologists and taxonomists will certainly contribute towards elucidating phylogeny in Simuliidae.

SUMMARY

1. The salivary gland chromosomes have been studied in five species of blackflies, belonging to the family Simuliidae. Of these, males and females of Simulium (Simulium) dentatum and only males of Simulium (Eusimulium) praelargum and only females of S. (S.) singtamense, S. (S.) himalayense and S. (E.) ghoomense were studied. Besides salivary gland chromosomes, the neuroblast metaphase chromosomes of S. (S.) himalayense and S. (E.) praelargum were also studied.

2. The present investigation was carried out to obtain the cytological information on the black fly species of Eastern Himalayan region to prepare standard map and to correlate the information with that of black fly species of other regions.

3. In each species the chromosome count is found to be n=3. The measurement of the salivary gland chromosomes was made and idiogram for each species was prepared. The neuroblast metaphase revealed 2n=6 chromosomes.

4. The photocomposite chromosome map of each species was constructed and compared among them. Each species could be distinguished from others by the location of characteristic landmarks, namely, centromere,Balbiani Ring, puffs, Parabalbiani and of NOR in the polytene chromosome.

5. Analysis of the chromosome data revealed that the general morphological characters and banding patterns of polytene chromosomes agree broadly with those of European and North American species of black flies.

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Addendum to Ph.D. thesis entitled 'studies on the salivary gland chromosomes of some species of black flies'

In the light of the examiner's report, some sections of the thesis have been rearranged and rectified and this addendum has been prepared with amended portions of the thesis. In this context, I wish to mention that in the present work attempt has been made to present the chromosomal profile of five species of Simuliidae hitherto unknown cytologically. While compiling the work, I have mainly followed the classification suggested by Rothfels (1979) and Datta(1975). However, I could not include the recent groupings of simuliid species from West Bengal(Datta 1992) in my thesis since the Journal of Bengal Natural History Society where the paper was published came out of press after submission of my thesis. Moreover, to maintain consistency with our recent publication (Dey *et al.* 1993), all correction and discussion have been made with reference to earlier classification. Nevertheless, I would include the recent species groupings of Simuliidae from West Bengal by Datta (1992) in our forthcoming publications. In this addendum, besides incorporating all the suggestion made by the examiner, a note has been added on the systematic treatment of the species studied in the section of discussion.

The section of literature review (page 10-18) has been rearranged as follows:

Tribe : Simuliini: This tribe includes six cytologically studied groups namely, *Simulium*, *Eusimulium*, *Edwardsellum*, *Inseliellum*, *Psilozia*, and *Shewellomyia*. Except *Simulium* and *Eusimulium*, cytological studies are piecemeal in other groups.

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Genus : Simulium:This genus is better known cytologically than the other groups of Simuliidae. *S.tuberosum* contains four to five siblings (Landau 1962 ;Rothfels 1981). All major species specific inversions were found on IIS and all are sex related. The banding sequence of other arms were identical in all the siblings except for a total of 83 floating inversions. The AB sibling has been found in Europe and North America and is thought to be true *S.tuberosum* (Rothfels 1981). The geographical distribution of sibling of *S.tuberosum* was further studied by Mason (1982). He observed four new siblings which like originals have fixed differences in chromosome arm IIS. One of these,FGI distinguished by a high degree of polymorphism and the presence of fixed differences from the *tuberosom* standard in arms IS,IL and IIIL occurred both in Alaska and Norway. A comparison of the standard *tuberosum*, the *S. venustum* and the FGI sibling chromosome pattern revealed that the FGI sibling to be much closer to the *venustum* standard than any other *tuberosum* sibling (Mason 1982).Sex chromosome polymorphism in *S.tuverosum*complex has also been studied by Mason (1984). He observed that the closely related sibling species could be distinguished by the banding pattern on their sex chromosome.*Simulium tuberosum* differs from the *venustum* standard by a total of 16 inversions and from all other *simulium* species by having the NO in the base of IIIL in other species(Rothfels 1979). *Moreover, Simulium vittatum* was found to be composed of 3 sibling species two of which are defined as IIIL-1 and one as IS-7 cytospecies (Rothfels and Featherston 1981). There are several polymorphisms may of which are shared by both siblings and which vary in their frequencies within each

sibling. Further studies (Adler and Kim 1984) revealed ecological differences between IIIL-1 and IS-7 siblings. The species *S. decorum* which is a member of *Simulium argyreatum/decorum* complex also have sibling species. The *decorum* sibling is distinguished from the other two by the presence of a heavy band at the base of IL and by chromosome III being the sex chromosome (Rothfels 1981). Furthermore, one of the largest complexes in North America is that of the *Simulium Venustum/S. verecundum* which includes the principal noxious biter of man. Study of polytene chromosomes show that both *S. venustum* and *S. verecundum* include a minimum of seven sibling species designated by their IIS sequence (Rothfels *et al.*1978). The basic chromosome complement of *S. venustum/verecundum* (n=3), in arm association and arm ratios, is same as in *S. tuberosum*, except that the NO is in the base of IIIL rather than IIIS. This change in position of NO is common to all other members of *Simulium* so far studied (Rothfels *et. al.* 1978). Moreover, *S. verecundum* lineage differs from its *venustum* counterparts by 10 fixed inversions (Rothfels 1981). Limnological features are also found to be associated with the distribution of of cytotypes of this complex (Gordon and Cupp 1980).

However, no sibling species were found in *jennings*i group (Gordon 1984). Three species, *S. jennings*i, *S.fibrinflatum* and *S. luggeri*,differ by 6 fixed inversions and by 19 floating inversions, 4 of which are related to sex determination in *S.jennings*i.

The study of the polytene chromosome banding patterns of 11 members of *S. metallicum* complex revealed that, as in other

members of *Simulium*, the chromosome complement consists of n=3 (Conn *et al.*1989). For chromosome I, the puffed region is followed by three heavy bands in section 12 and 13 in the IS which serves to distinguish IS from IL. Arms IS and IL may also be separated by the banding patterns in their ends. Chromosomes II and III are characterised by standard land marks namely, Balbiani ring and the double bubble in IIS, Parabalbiani and grey band in IIL, the blister and the capsule in IIIS and the basal marker in IIIL.Centromere regions in chromosome II and III of all members of the *S.metallicum* complex examined so far are rather bulbous and uniform in expression. Bands are discernible in the centromeres, but they do not stain darkly. Construction of cytophylogeny separates the 11 members of *S. metallicum* complex into three lineages. Elsen and Post (1989) found a new subspecies within the *Simulium damnosum* complex on the basis of analysis of polytene chromosome and the larval morphology. It is named *S. (Edwardsellum) squamosum kitettense* ssp.n. Chromosomally, it is most similar to *S. squamosum* from which it differs by 8 new fixed inversions.

Subgenus <u>**Eusimulium**</u>:Our cytological knowledge of *Eusimulium (=Nevermannia)* is still fragmentary. This subgenus is composed of the following species groups:*aureum*, *vernum*, *feurborni* and *ruficorne* (Datta 1992). Though the present tendency is to assign subgeneric rank to *Eusimulium* (within *Simulium*),cytological workers continue to treat *Fusiumlium* as a genus for reasons of convenience and consistency with earlier publications. (Hunter & Connolly 1986). Dunbar (1962) divided this taxon into several species groups of which *aureum* and *vernum* groups have been

studied in detail. *Eusimulium aureum* group has n=2 chromosomes. The very long dicentric first chromosome is due to the fusion of chromosomes II and III that is I of *aureum*= II and III of other simuliids;II of *aureum*=I of other simuliids (Dunbar 1958). Chubareva (1974) also reported n=2 for *Eusimulium brachyantherum*.Therefore, this group would be a promising one in which to search for reduction of the chromosome complement to the theoretical level of one (Rothfels 1979). On the other hand, *Eusimilium vernum* group has a chromosome number of n=3 in common with most black flies. In general the pairing of homologues is loose, although the degree of pairing varies among 12 cytotypes within *Eusimiluim vernum* group. The NO is in the base of IS arm throughout the *vernum* complex. Two of the cytotypes have a chromocenter while other four cytotypes carry supernumerary chromosomes. Moreover, five of the total six chromosome arms are involved in sex determination in the various members of this complex (Brockhouse 1984,1985). Study of the cytotaxonomy of seven North American species in the *E.vernum* group (Hunter and Connolley 1986) further revealed that there exist two cytological lineages within *E.vernum* group.*E.aestivum*, *E.impar*, *E.pugetense*and *E.quebecense* belong to one lineage while *E.gouldingi*, *E.croxtoni*and *Simuliums*p to the other. The former lineage is characterised by the fixed inversions IIIS-1 and by IIIL-19 (fixed and floating);the latter lineage by fixed inversions IL-1,2,3 & 4;IIL-4 and III-4,5 and by III-6 (fixed and floating).Further study on the species of *E.Vernum* group from Alaskan region, however,revealed that only fixed inversions

distinguishes *E.vernum*, *E.decolletum* and *E.pugetense* complex from one another (Allison & Shields 1989).

Besides fixed inversions, differentiated sex chromosomes and chromosomal polymorphism also distinguishes sibling species of *S.ornatipes* and *S.neornatipes* (Bedo 1977). The same author (Bedo 1984) while studying the population of *S.neornatipes* observed that sibling species of *S.neornatipes* are characterized by fixed inversions and differentiated sex chromosome, and *S.neornatipes* distinguishes from *S.ornatipes* by five fixed inversions. Moreover,a comparison of standard map of *S.ornatipes-neornatipes*species complex with that of *S.ruficorne*revealed a striking similarity which indicates a close taxonomic relationship between two species(Bedo 1989). on the other hand, *S.loerchae* Adler, a new species in *S.vernum* group is characterised by n=3 chromosomes, fixed inversions at 1L-2 and primitive $X_OY_O$sex chromosome system. It has been suggested that this species has been derived from the *Vernum* standard by three inversions (Adler 1987). Furthermore, a comparison of the polytene chromosomes of *S.furculatum* and the *S.vernum*standard revealed that the former does not belong to the *S.vernum* species group (Hunter 1989).

Subgenus Edwardsellum: Species of this taxon received considerable cytotaxonomic attention since this subgenus includes the vectors of human onchocerciasis in Africa (Dunbar 1966;Vajime & Dunbar 1975). Twenty-five sibling species of *Edwardsellum dampsum*have been described so far (Quillevere *et al*.1976). The cytotaxonomy of *S.Sanctipauli* and *S.Soubrense* has been described by Post (1986). It has been noted that in addition to inversion 2L-7,two

other newly recognised inversions 1L-A and 2L-A can be used in combination to identify *S.sanctipauli*, *S.soubrense* and a new species *S. soubrense* B.

Subgenus Inseliellum: Only two species of this taxon namely, *Inseliellum tahitiense* and *I.oviceps* have been studied cytologically (Rothfels 1979). Chromosomally these two species are extremely close and four of the six arms are identical while only four inversions distinguishes these two species. In both the species, females are heterogametic and are more closely related to each other than to any other *Eusimulium* so far studied. However, *I.tahitiense* is male chiasmate whereas *I.oviceps* is male achiasmate.

Subgenus Psilozia: *Simulium (Psilozia) vittatum* was the first blackfly species to be mapped (Rothfels and Dunbar 1953). The congeneric species *S.(P.)argus* also showed the same gross feature as *S.(P.)vittatum* with identical IL,IIS and IIIS. However, IIL differs by two fixed inversions while IS is homologous upto the centromeric region (Pasternak 1964).

Subgenus Shewellomyia: The species in *Simulium (Shewellomyia)* have been examined by conventional and quinacrine fluorescence staining method (Bedo 1975). *Simulium (Sh) pictipes* Hagen consists of three siblings, *pictipes* A, *pictipes* B, and *S.(Sh.) longistylatum*. In all three siblings, the haploid chromosome number is n=3. Specific differences include a simple and a complex inversions, a shift of basal bands between the short arm of the second and third chromosome , details of the sex chromosome and the amount of DNA in certain individual bands and

expanded centromeric regions. However, the unique situation is that the chromosome markers are located in a different element of the complement in each of the three species. Thus *S.pictipes* A has heteroband in IIL while *S.pictipes* B and *S.(Sh.) longistylatum* have Y chromosome inversions in the IIIL and IS respectively (Bedo 1975).

Table : 1 : List of Black fly species used as material with some associated ecological data

Taxon	Place of Collection	Date of Collection	Temp. of water at the time collection (°C)	Altitude of the collection site (in metre)	Sex of the specimen	Relative abundance	No. of larvae analyzed
Family : Simuliidae Sub Family : Simuliinae Tribe : Simuliini							
i. Simulium (Simulium) dentatum	Lebong (Stream)	24:10:1989	15	1650	male & female	Abundant (15 - 25 %)	25
ii. Simulium (Simulium) singtamense	Victoria Falls	17:10:1988	16	2132	female	Rare (below 5 %)	15
iii. Simulium (Simulium) himalayense	Lebong (Stream)	24:10:1989	15	1650	female	Dominant (over 25 %)	30
iv. Simulium (Eusimulium) praelarqum	Happy Valley (Stream)	01:08:1990	18	1500	male	Abundant (15 - 25 %)	35
v. Simulium (Eusimulium) ghoomense	Victoria Falls	17:10:1988	16	2132	female	Rare (below 5 %)	10

All the species were identified by the author by following the species identification key by Datta, 1973; 1974a,b, 1975; Datta & Pal, 1975.

Table : 2 : Measurement of polytene chromosomes from 10 nuclei of each of the five species

Name of the species		IS	IL	IIS	IIL	IIIS	IIIL
S.(S.) dentatum	% TCL of area	19.22 ±0.26	21.33 ±0.19	14.28 ±0.25	15.34 ±0.12	13.23 ±0.24	16.60 ±0.21
(Female)	% TCL of chromosome	40.55		29.62		29.83	
	Secs. Assigned per arm	20	20	14	16	13	17
	Arm ratio	1		1.14		1.3	
S. (S.) singlamense	% TCL of arms	21.70 ±0.37	22.50 ±0.16	13.23 ±0.26	18.22 ±0,25	8.19 ±0.27	16.15 ±0.18
(Female)	% TCL of chromosome	44.20		31.45		24.34	
	Secs. Assigned per arm	22	23	13	18	8	16
	Arm ratio	1.04		1.38		2.0	
S.(S.) himalayense	% TCL of arms	20.12 ±0.45	21.16 ±0.30	11.81 ±0.21	18.80 ±0.30	10.09 ±0.10	18.02 ±0.28
(Female)	% TCL of chromosome	41.28		30.61		28.11	
	Secs. Assigned per arm	20	21	12	19	10	18
	Arm ratio	1.05		1.58		1.8	
S.(E.) praelargum	% TCL of arms	19.93 ±0.19	19.97 ±0.18	13.37 ±0.13	19.41 ±0.30	11.70 ±0.18	15.62 ±0.08
(Male)	% TCL of chromosome	39.90		32.78		27,32	
	Secs. Assigned per arm	20	20	13	19	12	16
	Arm ratio	1		1.46		1.33	
S.(E.) ghoomense	% TCL of arm	16.83 ±0.17	19.17 ±0.14	12.81 ±0.06	23.09 ±0.31	9.97 ±0.06	18.03 ±0.13
(Female)	% TCL of chromosome	40.00		32.00		28.00	
	Secs. Assigned per arm	17	23	13	19	10	18
	Arm ratio	1.35		1.38		1.8	

± Standard error of mean

% TCL - Percentage of total complement length.

In the section of materials and methods (queries 2&3), Tables 1&2 are reconstructed as follows:

Total number of larvae used in the study have been mentioned in Table 1 while standard Error of Mean is shown in Table 2 (Encls. Table 1&2).

Regarding construction of map from one sex except *S. dentatum* is due to the non availability of sufficient number of larvae belong to both sexes. However, attempt has been made since 1988 to collect as many larvae as possible (Table 1), and chromosome maps have been constructed from three years collection.

The constant depletion of larval population of Simuliidae in the Himalayan region (Darjeeling and adjoining hill areas) is due to the deforestation and urbanization which is ultimately destroying the natural habitat of larvae. Springs and small hill streams are drying up due to prolonged drought in this region. Datta(1992) also express his concern from the destruction of ecological balance in this region and has commented that the days are numbered for Simuliids in this region. Therefore, inadequate number of larva limits our chromosomal study and inspite of our best effort, we could not construct maps from both the sexes.

In the section of discussion (query nos. 1&4), necessary corrections have been named and as per suggestion of the examiner, a note has been added on the recent classification of five species studied.

Systematic treatment of five species studied

While studying the polytene chromosomes of Simuliidae from Darjeeling and adjoining hill areas, the classifications as suggested by Datta & his collaborators (Datta 1973,1974,Datta & Pal 1975) has been followed. Recently, the same author (Datta 1992) has assigned 27 species and 7 unnamed taxa of West Bengal to 5 subgenera, viz. *Nevermannia* Enderlein, *Montisimulium* Rubtzov, *Gomphostilbia* Enderlein, *Himalayum* Lewis and *Simulium* Letreille(s str) under the genus *Simulium* Latreille(s.l) of the tribe Simuliini in the subfamily Simuliinae. These species are typical for the Oriental region though some of the species are spread out to the adjoining palearctica region.

Furthermore, some species-groups have been created within some subgenera by Datta(1992). The five species under present investigation are also placed under some species groups namely,*S.(S.) dentatum* belongs to *multistriatum* group in subgenus *Simulium* while *S.(S.) singtamese* belongs to unknown group in the same subgenus. On the other hand,*S.(S.) himalayense* belongs to *variegatum* group in subgenus *Simulium*. Furthermore, *S.(E.) praelargum* belongs to *feuerborni* - group in subgenus *nevermannia* while *S.(E.) ghoomense* belongs to subgenus *Montisimulium*. Therefore, in the recent classification, *S.(E.) praelargum* and *S.(E.) ghoomense* are placed under two new subgenera *Nevermannia* and *Montisimulium* respectively.

However, in the present thesis, earlier classification has been followed for reasons of convenience and consistency with our earlier work. Nevertheless, the recent species grouping will be followed in our forthcoming publications.

Chromosome number in black flies:

Neuroblast cells contain 2n=6 chromosomes in all the species of*Simulium* so far studied. Salivary gland nuclei have three polytene chromosomes with two homologues more or less intimately paired. Moreover, centromeric regions in most species form characteristic expanded regions dividing the polytene chromosomes in the same proportions as the mitotic metaphase chromosomes. The major identifying landmarks of the polytene chromosome of different species include Balbiani ring, nucleolar organiser, puff, Parabalbiani and characteristic banding patterns. In the present investigation, the polytene chromosome of*S.(S.) dentatum*, *S.(S.) singtamense*, *S.(S.) himalayense*, *S.(E.) praelargum* and *S.(E.) ghoomense*, and the ganglion chromosomes of *S.(S.) himalayense* and*S.(E.) praelargum* have been studied.

Idiograms (Fig.A) of five species were constructed from % TCL of each polytene chromosome. The position of centromere, Ring of Balbiani, nucleolar organiser region is shown in each of the idiograms. Inspection of five species revealed that all the chromosomes are tightly paired and without any asynapsis. The chromosome I of*S.(S.) singtamense* is longer than that of other four species though the chromosome II and III of all the five species are more or less equal in length. In all the five species, the Balbiani Ring is present in the terminal region of IIS chromosome. However, the position of NOR was found to be variable in those species. It was present in the IIIrd chromosome of*S.(S.) singtamense* and *S.(S.) himalayense*, while in *S.(S.) dentatum*, *S.(E.) praelargum* and*S.(E.) ghoomense* it was in the Ist

chromosome. However, in all the five species NOR was present near the centromere. In*S.(S.) dentatum*, the IIIL is characterised by flared end while no such distinctive character was encountered in other four species. Therefore, the comparison of the idiograms of five species of *Simulium* revealed that two species of the subgenus *Eusimulium*,*S.(E.) praelargum* and *S.(E.) ghoomense* are very closely related, while the three species of the subgenus *Simulium*, *S.(S.) dentatum* , *S.(S.) singtamense* and *S.(S.) himalayense* differ from each other in respect of location of NOR, presence of flared end and in the length of the 1st chromosome. Furthermore, chromosome count, banding patterns and characteristic landmarks of these five species agree well with other species of *Simulium* reported from elsewhere (Rothfels and Dunbar 1953; Rothfels 1956,1979;Rothfels *et al*.1978; Rothfels and Freeman 1966,1977;Rothfels and Mason 1975;Rothfels and Nambiar 1975;Bedo 1975a,b, 1976,1977a).

However,there is a deviation from the basic Simuliid complement in the genus *Cnephia* (Procunier 1982b.).Four of the five species of this genus revealed n=3, while in *Cnephia lapponica*,the basic chromosome complement is reduced from n=3 to n=2 metacentric chromosomes as a result of fusion of chromosomes II and III.

General morphology of polytene chromosome

Centromere :In black fly centromere is an expanded region of polytene chromosome usually characterised by a heavy dark band(Dunbar 1962). The expanded region correspond in position to the centromeres in mitotic chromosomes. The expanded regions were

joined to form a pseudochromocenter in *Simulium pictipes* A (Bedo1975a) and *S.melatum* (Bedo 1976). True chromocenter, which is a regular phenomenon in *Drosophila* (White 1973),was also reported (Rothfels and Freeman 1976) in four species of *Prosimulium* namely, *P. fontanum, P. saltus,P. approximatum* and *P.mysticum*.In the present investigation,of the five species studied,three species,*S.(S.) dentatum, S.(S.) singtamense* and *S.(S.) himalayense* showed prominent centromeres.On the other hand, centromeres were difficult to recognise in two other *S.(E.) praelargum* and *S.(E.) ghoomense*.The location of centromeres in the polytene chromosomes of all the five species is summarised in Table 4.It has been observed that there was little variation in the location of centromeres in all the five species studied here.Dark heavy centromeric bands of *S.(S.) singtamense* and *S.(S.) himalayense* are comparable to those of the species belonging to *S.pictipes* grpup (Bedo 1975a),while the centromere of*S.(S.) dentatum* did not reveal any heavily stained band , but rather granular in appearance with irregular bands similar to those of the members of *S. venustum/verecundum* complex (Rothfels *et al.* 1978),*S.fibrinflatum* and *S. luggeri* (Gordon 1984). On the other hand,the centromeres of two species,*S.(E.) praelargum* and *S.(E.) ghoomense* were difficult to recognise.In the former species,the centromere was not a typically expanded region though it was characterised by dark heavily stained bands.On the other hand,the centromere of chromosome I of *S.(E.) ghoomense* is easily identifiable while it needs a little effort to identify centromere in case of chromosomes II and III. Moreover, the application of C- and fluorescent banding techniques

facilitated the identification of centromeres in polytene chromosome.The centromeric region of the members of *S.pictipes* group displayed bright fluorescence with Quinacrine(Bedo 1975a), while polytene chromosomes of *S.ornatipes* and *S. melatum* (Bedo 1975b) found to be C-positive.

Nucleolar organising region: The nucleolar organising region appears as a gap in the polytene chromosome surrounded by nucleolus and it serves as as an important landmark for the analysis of chromosome. The bands in the immediate vicinity of NOR are somewhat disrupted and the chromonemata on either side of the organiser appear to extend like a complex branching system with roots into the actual nucleolus. Morepver,study of nucleolar relations in Simuliidae promises to be diagnostically useful, because of the intra-specifically constant and interspecifically variable attachment of the main nucleolus (Rothfels 1953). In *Simulium*, the terminal nucleolus which was reported in the members of *S. pictipes* group (Bedo 1975a) was difficult to identify without counterstaining by light green. However, nucleolar organiser of interstitial region could easily be identified without subjecting it to any specialised staining technique. In all the five species investigated here,NOR was located in the interstitial region. In *S.(S) dentatum*, *S.(E.) praelargum* and *S.(E.) ghoomense*, NOR was located in IS 19,IL 22 and IL 21 respectively while in *S.(E.) singtamense* and *S.(S.) himalayense*, it was in IIIL 87 respectively (Table 4). Therefore, except *S.(S.) dentatum*, where NOR was located in IS arm, the location of NOR was similar in other two species of the subgenus

Simulium. Furthermore, in two species of the subgenus *Eusimulium*, the NOR was also found in the same arm. Interstitial NOR was also reported earlier in *S. venustum* and *verecundum* complex (Rothfels and Freeman 1977), *Cnephia* (Procunier 1982b), species of *Prosimulium* (Rothfels and Freeman 1977), and *Simulium* (Bedo 1977; Gordon 1984). Besides primary nucleolus, a secondary nucleolus was also reported among the members of *S. ornatipes* (Bedo 1977). The secondary nucleolus frequently fused with the main nucleolus in *S. ornatipes*. However, in the species investigated here no such secondary nucleolus was observed. Among other dipteran species, the NOR is found to be located in the pairing segment of sex chromosome in different species of *Drosophila*, while in some species of *Chironomus*, more than one nucleolus was observed (White 1973).

The Ring of Balbiani (RB)::Since the site of RB or Balbiani Ring is species specific, therefore, this structure also serves as an important landmark in salivary idiogram (Rothfels and Dunbar 1953). It is identifiable in all the species studied in the present investigation. In all the five species, the RB was present in IIS chromosome. In *S.(S.) dentatum*, *S.(S.) singtamense* and *S.(S.) himalayense*, RB was encountered in IIS 41B, IIS47 and IIS43 respectively, while in *S.(E.) praelargum* and *S.(E.) ghoomense* it was observed in IIS 45B and IIS 45A respectively (Table 4). Bedo(1975a) reported the presence of RB in IIS 42 in all the members of *Simulium pictipes* group, while in *S.ornatipes* and *S. ruficorne*, it was observed in IIS 43 (Bedo 1977,1989). On the other hand, in *S.venustum/verecundum* (Rothfels et al 1978), *S. jenningsi*, *S. fibrinflatum* and in *S. luggeri* (Gordon 1984) the RB

was located in IIS 47. Moreover, in the genus *Cnephia*, the RB was encountered in IIS 47 (Procunier 1982 b). Therefore, it seems that RB is an important landmark for the identification of IIS chromosome.

Puffing in polytene chromosome: At particular stages of development, some of the genetic loci in the polytene chromosome undergo a spectacular change in appearance. They become converted into large swellings or puffs. It has been generally believed that a process of puffing is due to the biosynthetic activity of a particular loci concerned (White 1973). Therefore, study of the puffing pattern is of great importance in understanding gene action. Puffs were encountered in various members in all the species under present investigation. Position of puffs in polytene chromosomes of different species in summarized in Table 4.

In *S.(S.) dentatum* and *S.(E.)singtamense*, two large and clear puffs were seen in IS and IIS respectively. On the other hand, in *S.(S.) himalayense*, *S.(E.)praelargum* and *S.(E.) ghoomense*, a large number of puffs were encountered in 1st ,2nd and 3rd chromosomes. Puffs were also reported in *Simulium pictipes* (Bedo 1975a),*S.ornatipes* (Bedo 1977) and in members of *S.venustum/verecundum* complex (Rothfels *et al.* 1978). However, no attempt has yet been made to study the detailed puffing pattern of different species of *Simulium*. However, in other dipterans, puffing patterns have been extensively studied. In *Chironomus tentans* and *C. pallidivittatus*, puffing patterns have been studied in detail (Grossbach 1968,1969),in relation to the

synthesis of specific silk like proteins which are produced in large amount in the cells of salivary gland. In *Drosophila melanogaster*, the puffing pattern of X chrbmosome loci is similar in both the sexes. But a group of puffs were active for a longer time in male than in female (Ashburner 1967,1969a). While studying the puffing patterns of *D. simulans* and *D.melanogaster*, Ashburner (1969a,b) observed that the X chromosome of *D.simulans* forms two puffs which were absent in *D. melanogaster*. Moreover, *D.melanodgaster* has one autosomal puff (46A) which was not present in *D.simulans*. It is also interesting to note that the hybrid between these species show a heterozygous puff. Furthermore, differences in time of puffing and size of puff also exists between different strains of *D.melanogaster* and certain puffs were active in some strains, but were not seen in other (Ashburner 1969c). Therefore, the similar studies in Simuliidae will be helpful in understanding the mode of gene action.

Parabalbiani Ring (PBR): Parabalbiani is unique to the Simuliidae and readily recognised in the species of different genera. It isa darkly stained band with one sharply defined and one diffused edge. This structure serves as an useful polarised marker (Bedo 1977) of II L. Of the five species studied in the present investigation PBR was encountered in all the species except *S.(S.) singtamense*. In *S.(S) dentatum*, the PBR was found in III 64B in both male and female individuals while in *S.(S.) himalayense* and *S.(E.) praelargum*, it was located in IIIL 93C and IIIL 89A , IIIL 57B respectively. In *S.(E.) ghoomense*, the PBR was, however, located in IIL 69 (Table 4). The PBR was also reported in other members of Simuliidae namely, *S.pictipes* (Bedo 1975a), *S.ornatipes* (Bedo

1977), *S.venustum / verecund-um* complex (Rothfels *et al* 1978) and *Metacnephia* (Procunier 1982 a).

Banding pattern: The pattern of dense bands and less dense interbands that characterise the most polytene chromosomes is a feature that has been of the utmost value to geneticists, particularly since the demonstration by Bridges (1937) that the genetic and polytene chromosome maps are colinear. In the present investigation , of all the five species studied, the distribution of heavily and lightly stained bands in the polytene chromosome compliment is not random, but reflects some overall organisation. In *S. (S.) dentatum*, both in male and female sexes, there was a preponderance of dark bands in all the three chromosomes. There were some fine bands near the nucleolar organiser region in IS while in IL, Series of dark bands distributed throughout the chromosomes. Though the diameter of chromosome I was uniform, constricted necks were encountered in IIL 59 and IIIL 95. Fine lightly stained bands were found near the centromeres of chromosome I, II and III. Unlike the former species, all three chromosome of *S.(E.)singtamense* are of uniform diameter and are characterised by some specific banding patterns such as groups of heavy bands in IS 2-5, IS 7-10. Moreover, there was a shield-like band in IL 34. There were two groups of darkly stained bands IIS 50C-52A and IIS 54-55A. In all three chromosomes, fine lightly stained bands were found near the centromeric regions. In *S.(S.) himalayense*, the distribution of light and deeply stained bands were more or less uniform. However, as in other two species, lightly stained bands

were present near the centromeric regions of all three chromosomes. The specific banding character of this species includes band in IS 4A, a group of deeply stained bands in IS-16-&17, heavy band in IL-24C, a group of four bands in IL-38 and 39A. Dark marker bands were also present in IIS 45C-47A. In the IIIrd chromosome, there was a groups of heavy bands in IIIS-78 and a group of deeply stained bands in IIIS-75. In *S.(E.) praelargum*, on the other hand dark and light bands were distributed uniformly throughout the length of the chromosome as in *S.(S.) himalayense*. However, diameter of the chromosome was not uniform. in *S.(E.) praelargum* and constricted necks one each in IS-11, IL-32 and IIIS-82 were also encountered in this which were comparable to those of IIL-59 and IIIL-95 in *S.(S.) dentatum*. moreover, in *S.(E.) praelargum* deeply stained group of bands were found in all three chromosomes. Besides the marker band, shield-like band was also encountered in IL-29 comparable shield-like band was also observed in IL-34 of *S.(S.) singtamense*. In *S.(E.)ghoomense*, on the other hand there was a preponderance of lightly stained bands as in *S.(E.)praelargum* and *S.(S.) dentatum*. Moreover, specific group of marker bands were also found in all the three chromosomes, especially in chromosome I.A group of three dark bands in IL 22, a series of dark bands in IIS 47-48, a pair of dark bands in IIIS 78-79, serve as an important marker. Moreover, in some individuals of *S.(E.) ghoomense*, an inversion involving sections 33-37A was encountered in IL. Therefore, the study of the banding pattern clearly indicates that the marker bands of these species are useful as landmarks for the identification of chromosome arms.

Sex chromosome: Sex chromosome heteromorphy is known from many groups of animals and plants, and in most cases it is the Y chromosome which is the smaller element. Presumably such system evolve from morphologically identical and freely recombining sex chromosomes. Size difference between the sex chromosome of Simuliidae is virtually unknown and in most species they are indistinguishable in the polytene nucleus (Post 1985). Therefore, sex is determined primarily by a single locus or by very short length of chromosome (Rothfels and Mason 1975). The differentiation of sex chromosome by linkage of inversions which were either fixed or polymorphic was reported in *Simulium erythrocephalum* (Post 1982a, 1985). In the present investigation, male and female individuals of *S.(S.) dentatum*, females of *S.(E.) singtamense*, *S.(S.)himalayense* and *S. (E.) ghoomense* and males of *S.(E.) praelargum* did not reveal any morphologically distinguishable sex chromosome. Except *S. (E.) ghoomense*, where an inversion was encountered in IL of some of the individuals,no inversion or sex specific heteroband was encountered in any individuals of these species studied. However, detail study of the large number of individuals of both sexes might throw some light on sex chromosome in these species. As mentioned earlier, constant destruction of habitats of these species has made collection of large number of larvae from field virtually impossible.

However, the presence of sex chromosome in Simuliidae was reported from elsewhere. Typically, in black flies, sex chromosome become differentiated by linkage of chromosomal rearrangements to either the genetic X or genetic Y chromosome Species of *Prosimulium* (Ottonen 1966; Rothfels 1956) show increase in differentiation of sex chromosomes by addition of

inversions resulting in complex configuration involving whole arm. Inversion played an important role in the differentiation of Y chromosome in *S. longistylatum*, while *S. pictipes* revealed "heteroband" in IIL (Bedo 1975). Inversions also played an important role in the determination of sex in the species of *S.jenningsi* group (Gordon 1984). Similar sex determining mechanism was also reported in chironomidae (Beerman 1955). A partial linkage of inversion to the X or Y chromosome was reported in *Chironomus tentans* (Acton 1957) and *C. intertinctus* (Martin 1962). The simplest kind of sex chrompsome differentiation involves a failure of polytene chromosome pairing between specific sites of the X and Y chromosomes, the inclusion of heterochromatic supernumerary bands (Basrur 1959) or alteration in size and composition of existing bands (Bedo 1975a). Bedo (1977) reviewed the distribution of para-and pericentric inversions in 65 black fly species from five genera namely, *Prosimulium, Twinnia, Cnephia, Eusimulium* and Simulium and found significantly higher frequency of pericentric inversions in sex determination. Therefore. inversion play an important role in the differentiation of sex chrpmosome in Simuliidae.

Cytophylogeny in black flies :Detailed studies on the polytene chromosome of numerous species of black flies now mainly confined to Canadian and European species, and very little work as been carried out on the chromosome of Oriental black flies. Dey and wangdi (1984a) reported the mitotic chromosomes of four species namely,*Simulium (Simulium) dentatum, S.(S.)rapposum, S.(E.)*

aureohirtum and *S.(E.) purii* from Darjeeling and adjoining hill areas which is a part of Eastern Himalayas. They found the diploid count of 2n=6 in each of the four species and chromosomes were metacentric in nature. Moreover, Dey and Wahgdi (1984b) also reported the presence of supernumerary chromosomes in the neuroblast metaphase chromosome of two other species , *S.(E.) gracilis* and *S.(E.) ghoomense*. The polytene chromosomes of five species which has been reported in the present investigation is a further extension of the study of Himalayan Simuliidae. The present data are, however, inadequate to compare with those of European and North American species and further studies on the chromosomes of Eastern Himalayan Simuliidae are required to make any meaningful linkage between the species of these continents.

Rothfels *et al.* (1978) constructed and outline chromosome phylogenies that extended through the prosimuliinae and encompassed most of the known species in *Gymnopais, Twinnia, Heloden,* and *Prosimulium* Less extensive phylogenies were also reported in *Cnephia, Metacnephia, Eusimulium* (Bedo 1977,1979 a,b,c; Dunbar 1965, 1967) and *Simulium* (Rothfels *et al.* 1978; Bedo 1977, 1989). However, no serious effort has been made to link up those phylogenies though certain chromosome ends (IS) and middles are shared by members of all genera of the family. For the IIS arm, Dunbar (1967) claimed that closest members of *Cnephia* and *Eusimulium, C. dacotensis* and *E. apatinum* differ in but single inversion. Furthermore, it has been suggested (Dunbar 1967) that the IIS sequence of some *Cnephia* species can probably be successfully compared to that of some *Prosimulium* and IIS of *Eusimulium* to that of some *Simulium*. Therefore, it seems that

there is possibilities in linking up different phylogenies. Moreover, the chromosomal rearrangements like inversion plays an important role in the speciation process. Fixation of inversions and acquisition of unique floating inversions suppose to be one of the important processes in the evolution of Simuliidae (Bedo 1977) Chubareva (1977) proposed a chromosome phylogeny for Simuliidae where *Gymnopais* has been considered as base on the basis of morphological dogma and not cytological analysis. Rothfels (1979) also assumed that *Gymnopais* is most primitive of all cytologically studied black flies, though *Helodon*, *Gymonopais*, *Twinnia* and *Prosimulium* are cytologically closely related genera. However, nucleic acid hybridization studies (Bohn *et al.* 1975; Teshima 1972) suggested that some species within *Simulium* appear to be as remote from each other as they are from species of the genus *Cnephia*. Moreover, at an interfamily level, this type of study holds the promise of allowing the identification of most closely related genera of different families and thereby providing clues to ancestry. Therefore, integration of the cytological data from different areas and continuous interaction among cytologists, molecular biologists and taxonomists will certainly contribute towards elucidating phylogeny in Simuliidae.

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The references which are newly added on this addendum are only listed hereunder. Please see the main thesis for other references.

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The Salivary Gland Chromosomes of the Black fly *Simulium* (*Simulium*) *singtamense* (Diptera: Simuliidae)

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Analysis of polytene salivary gland chromosomes of larvae has contributed significantly towards evolutionary and cytotaxonomical research in Simuliidae. The substantial contributions made by earlier workers in the formative years of black fly cytology have been reviewed extensively by Rothfels (1979). Recently, efforts have been made to identify sibling species and to establish cytophylogeny (Bedo 1975, 1976, 1989, Rothfels *et al.* 1978, Gordon 1984, Hunter 1989) in black flies since such studies will help to develop effective vector control strategy. However, very little is known on the chromosomes of Himalayan black flies (Dey and Wangdi 1984a, b). The present investigation has been undertaken to extend our knowledge on the chromosomes of black flies and to prepare standard polytene chromosome maps of each species.

Materials and methods

The penultimate instar larvae of *Simulium* (*Simulium*) *singtamense* Datta and Pal constitute the material for the present investigation. A total of 15 larvae were collected from stones and vegetation of small streams present in and around Darjeeling and adjoining hill areas during the months of June to October. The larvae were fixed in aceto-alcohol and salivary glands were dissected out and stained in 1% orcein in equal parts of 25% lactic acid and propionic acids for about 10 min. Orcein squash preparations were then sealed in DPX mounting medium and all observations and photography were made on temporary preparations. The sex of the larvae was determined by staining the gonads of carcasses in Feulgen stain or in mixture of Picric acid-acetic acid-formalin-ethanol. The testes are spherical while ovaries are elongated. Composite photographic and hand drawing maps of polytene chromosomes were prepared following the conventions established by earlier workers (Rothfels 1956, Rothfels and Dunbar 1953, Dunbar 1959, Bedo 1975). NOR (nucleolar organiser region) was detected following the criteria of Bedo (1979) and Rothfels *et al.* (1978).

In our account of the polytene chromosomes we have not described every band or every major divisions since they are adequately demonstrated in maps. Our aim has been to present an overall picture of each chromosome and to identify their characteristic landmarks.

Results

Of the 15 larvae analysed, 10 were female and 5 were male. However, good preparations were obtained from female individuals only and standard chromosome maps were prepared from the study of 10 individuals. *Simulium* (*Simulium*) *singtamense* (female) revealed normal simuliid complement of n=3 chromosomes. Two homologues of each chromosomes are

tightly synapsed and they have prominent expanded regions. Measurement of the chromosomes was made from 10 nuclei. The length of the longest chromosome was 44.20% of total complement length while those of IInd and IIIrd chromosomes were 31.45% and 24.34% respectively. Therefore, there is a remarkable difference between the lengths of three chromosomes.

Chromosome I: This chromosome (Figs. 1–4) is readily recognised by its greater length and median position of the centromere. Numerous characteristic landmarks provide useful aid for the identification of this chromosome.

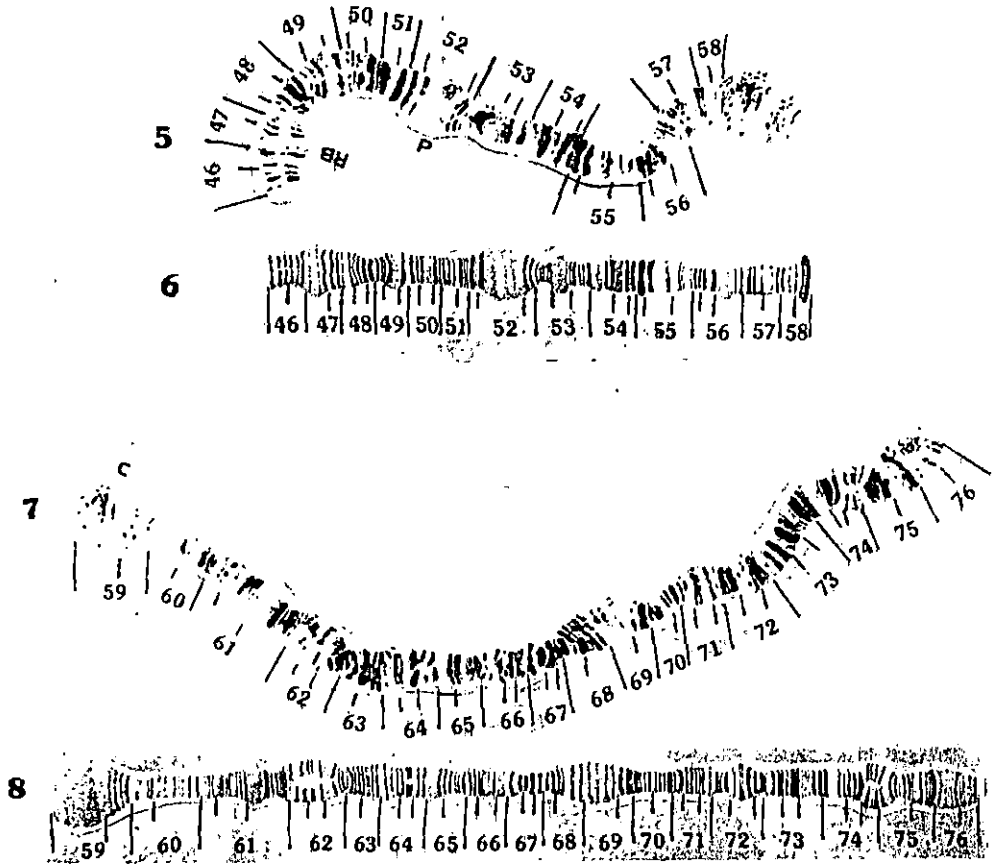
Figs. 1–4. The standard maps for chromosome I of *S.* (*S*). *singtamense*. 1, Photocomposite map of IS. 2, Hand drawing map of IS. 3, Photocomposite map of IL. 4, Hand drawing map of IL. C-centromere; P-puff.

IS (Figs. 1, 2) is characterised by a group of dark bands 2–5 followed by another group of dark bands in section 7B-10 serve as an important reference point. There is a distinctive constriction in section 8 and a puff in 14 followed by a region of dark bands.

IL (Figs. 3, 4) also have the preponderance of dark bands as in IS. This arm is also endowed with specific characters useful as landmarks. Prominent centromere with irregular dark bands is present in section 23. A prominent dark band in 26A is followed by series of

bands, extending from 26B to 30A. There is a shield-like pattern in section 34 while a distinctive constriction in 36A provides a convenient marker for the mid point of IL. There lies a lightly stained bulge in sections 41–42A. The tip of this arm is characterised by some fine bands.

Chromosome II: This chromosome (Figs. 5–8) is somewhat shorter than chromosome I and is metacentric in nature. It is distinguished by the presence of Ring of Balbiani and prominent centromere.



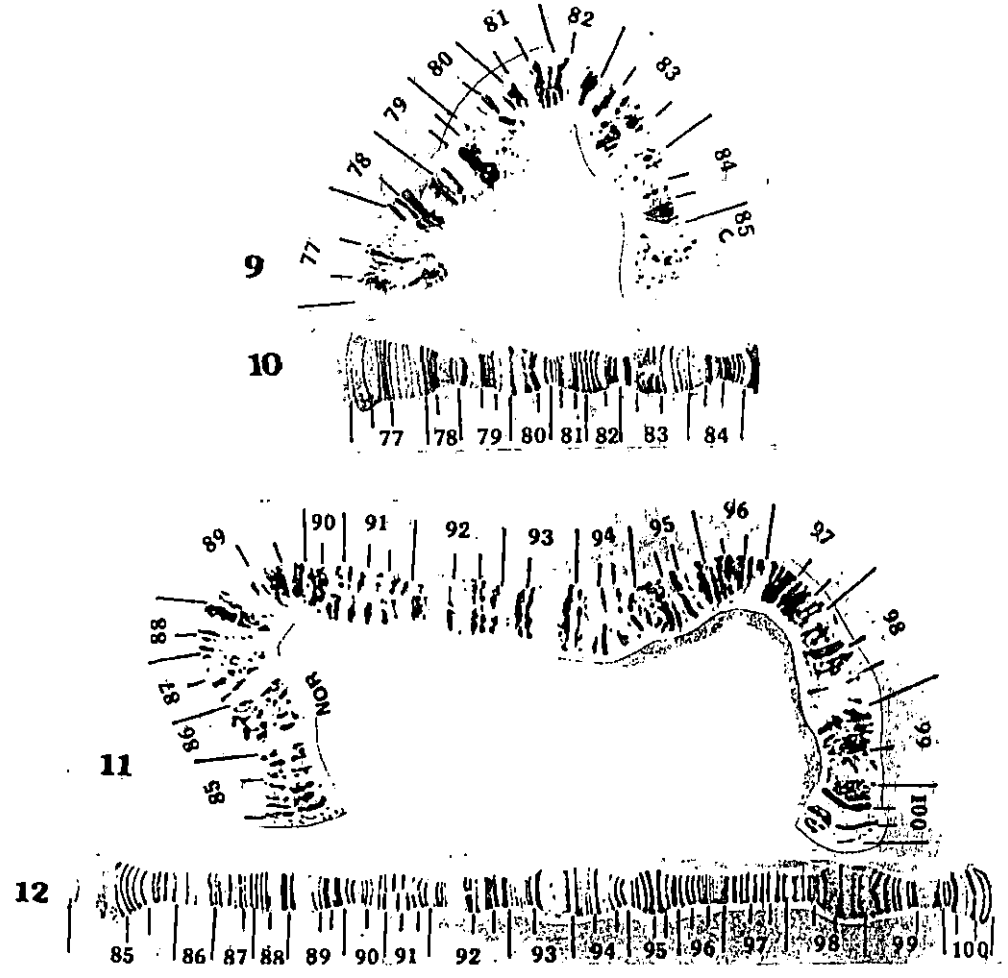
Figs. 5–8. The standard maps for chromosome II of *S.* (*S*). *singtamense*. 5, Photocomposite map of IIS. 6, Hand drawing map of IIS. 7, Photocomposite map of IIL. 8, Hand drawing map of IIL. BR-Balbiani Ring.

IIS (Figs. 5, 6): This arm is readily identified by the presence of Ring of Balbiani near its tip in section 47. There is a large pale puff in section 52 next to a series of prominent dark bands in 50C–52B. Other important features include a group of dark bands in section 54–55A, B of which a large band in 55A serves as an important reference point. The terminal end is provided with six fine bands (section 46).

IIL (Figs. 7, 8) is provided with a faintly stained expanded centromeric region without any characteristic heavy bands in section 59. The banding group includes a series of dark bands in sections 70–73. There are small asynaptic regions in sections 62 and 74–75. Terminal end (section 76) of this arm is lightly stained and without any distinguishing bands. A dark band in 75B serves as an important reference point.

Chromosome III: This chromosome (Figs. 9–12) could be demarcated from other two chromosomes by its smaller length and submedian position of the centromere. This chromosome is readily recognized by the location of nucleolous organising region.

IIIS (Figs. 9, 10) is endowed with many important characters useful as landmarks. The tip (section 77) is flared to some extent and is provided with a lone dark band (77B). A group of four dark bands is present at the base of the flared end in section 78. There is a faintly stained centromere in section 85A (Figs. 8, 10).



Figs. 9–12. The standard maps for chromosome III of *S.* (*S*). *singtamense*. 9, Photocomposite map of IIIS. 10, Hand drawing map of IIIS. 11, Photocomposite map of IIIL. 12, Hand drawing map of IIIL. NOR-nucleolar organising region.

IIIL (Figs. 11, 12) is also distinguished by numerous landmarks. NOR is present in section 86. However, its expression is not maximum in the individuals studied. Other important features include a bulge in 93B and a group of dark bands in 95–98A. The tip of this arm is partly flared and is provided with two dark bands in section 100A, B.

Discussion

The basic chromosome complement of n=3 chromosomes, intimate pairing of homolo-

gues, the general banding patterns and characteristic landmarks of *Simulium* (*Simulium*) *singtamense* agree broadly with those of congeneric species reported from elsewhere (Bedo 1975, 1976, 1977, 1989, Rothfels and Dunbar 1953, Rothfels *et al.* 1978, Gordon 1984, Hunter 1989). Two homologues in each of three polytene chromosomes are usually remain intimately paired in *S.* (*S*) *singtamense* as in most of the *Simulium* species. However, asynapsis was reported in members of *S. venustum/verecundum* complex (Rothfels *et al.* 1978), species of *S. jenningsi* groups (Gordon 1984) and in *S. furculatum* (Hunter 1989). As in other dipterans, the major identifying landmarks of *Simulium* include Balbiani Ring, nucleolar organiser, puff and characteristic banding pattern. An inspection of polytene chromosome map of *S.* (*S*) *singtamense* revealed the presence of prominent centromere with characteristic dark bands in Ist chromosome comparable to that of *Simulium pictipes* (Bedo 1975). On the other hand, the faintly stained diffuse centromeres of IInd and IIIrd chromosomes are comparable to those of the members of *S. venustum/verecundum* complex (Rothfels *et al.* 1978). The chromocenter, which is a regular phenomenon in *Drosophila* (White 1973), was lacking in Simuliidae. However, pseudochromocenter was reported in *S. melatum* (Bedo 1976) and in some species of *Prosimulium* (Rothfels and Freeman 1977). The ring of Balbiani is located near the tip of IIS in *S.* (*S*) *singtamense* as in other congeneric species. However, the tip of IIIS which is flared to some extent but does not reach the spectacular proportions as seen in the North American species, *Simulium pictipes* (Bedo 1975). On the other hand, since the location of nucleolus organising region (NOR) is intraspecifically constant and interspecifically variable, study of nucleolar relations is diagnostically useful in simuliids (Rothfels 1979). It is in the base of IIIL in *S.* (*S*) *singtamense* and in *S. venustum/verecundum* complex (Rothfels *et al.* 1978) rather than in the base of IIIS as in *S. vittatum* (Rothfels and Dunbar 1953); while in *S. pictipes* (Bedo 1975) and in *S. ornatipes* (Bedo 1977), it is located near the tip and near the base of IL respectively. The size of nucleolar organiser varies considerably in different species of *Simulium* (Bedo 1979) and maximal expression produced marked discontinuity in the chromosome as found in the members of *S. venustum/verecundum* complex (Rothfels *et al.* 1978). In *S.* (*S*) *singtamense*, on the other hand, the NOR is with subdued expression and is comparable to those in *S. ornatipes* (Bedo 1977). Study of the puffing pattern in the polytene chromosome has contributed significantly towards differentiating congeneric species of *Drosophila* (White 1973) though no such attempt has been made in Simuliidae (Rothfels 1979). In *S.* (*S*) *singtamense* two puffs are encountered one each in IS and IIS while in *S. jenningsi* (Gordon 1984) and in *S. ornatipes* (Bedo 1977), IS and IIS have one puff each respectively.

The pattern and distribution of dark and light bands are of utmost importance in the study of polytene chromosomes in Diptera. All three chromosomes of *S.* (*S*) *singtamense* are uniform in diameter and are characterised by some specific banding pattern such as a group of heavy bands in IS2-5, IS7B-10; shield-like pattern in IL34. Similar shield-like pattern was also reported in IL34-35 of *S. ornatipes* (Bedo 1977). There are two groups of darkly stained bands in IIS50C-52B and IIS54-55A, B; a series of dark bands in IIL 70-73. Furthermore, IIIrd chromosome is distinguished by three groups of bands such as IIIS78, IIIL85B and 95-98A. However, the general banding pattern of *S.* (*S*) *singtamense* is in agreement with that of other *Simulium* species reported so far (Bedo 1975, 1976, 1989, Rothfels *et al.* 1978, Gordon 1984, Hunter 1989).

In the Simuliidae sex determination is of XY type with male constituting the heterogametic sex except *S. tahitense* (Bedo 1976) where female is heterogametic. However, in *S.* (*S*) *singtamense*, female sex chromosome differentiation was not observed.

Summary

Standard maps of polytene salivary gland chromosomes of black fly species *Simulium* (*Simulium*) *singtamense* have been prepared. It revealed normal simuliid complement of n= 3 chromosomes. Two homologues of each polytene chromosomes are tightly synapsed and are provided with identifiable centromeres. First two chromosomes are metacentric while the third one is submetacentric. Characteristic landmarks of polytene chromosomes such as Balbiani Ring, nucleolar organiser, puff and characteristic banding patterns have been described and compared with those of congeneric species.

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