A COMPATIVE STUDY OF THE LARVAL. SALIVARY GLAND CHROMOSOMES OF FOUR BLACK FLY SPECIES IN NORTHERN NIGERIA.

By

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THESIS APPROVAL

This thesis by Priyadarshini Kumarakulatungam meets the regulations governing the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

Affectionately dedicated to my parents.

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ABSTRACT

The gross morphological characteristics of larval salivary gland chromosomes of four black fly species namely S.sirbanum, S **.hargreave**si. S.adersi and S .ruficorne were studied using S.sirbanum as a standard. The landmarks used for this comparison were arm ratios, the degree of pairing, the expanded region, the nucleolar organiser, the "double bubble", the Balbiani Ring, the Para Balbiani, the "blister", homologous hands and chromosome ends.

The arm ratios were found to be constant among all the four species while the TCL varied. The position and expression of the expanded region, the nucleolar organiser, the Balbiani Ring, the Para Balbiani and the "blister" were found to be characteristic of each species. The nucleolar organiser was found to occur both heterozygously and homozygously. From the position of the nucleolar organiser in **S.ruficorne** it is evident that the nucleolar organiser is involved in a shift. The occurence of a pericentric inversion is another possible explanation. Possible reasons for the position of the nucleolar organiser in S Adersi have been discussed. The expression of the Balbiani Ring, the "double bubble", the Para Balbiani and the "blister" were found to be interspecifically and intraspecifically variable. Reasons for such variations were discussed. The positions of the Balbiani

Ring and the Para Balbiani among species indicate the presence of interspecific structural rearrangements. The presence of complex interspecific heterozygous rearrangements were observed only in S .ruficorne.

Homologies were best detected at the regions adjoining the centromere and at the chromosome ends in all the four species. Reasons for the preservation of bands at these two regions were discussed. The degree of homology are found to be more extensive at the Ills end in all the four spocies despite the fact that S.ruficorne belongs to *a* separate sub-genus. This is indicative of the conservative nature of evolution in the Similiidae.

INTRODUCTION

Black flies also known as buffalo gnats are small insects of the family Simuliidae. They have a world wide distribution and are of considerable economic importance. Blackfly females are among the most voracious blood sucking insects known to bite man as well as animals. In North America and Europe large swarms bite cattle and can kill large numbers of these animals as reported by Chandler and Read (1967). In Canada Simulium venustum is known to be the vector of a severe protozoan parasite of ducks as reported by Zimring (1953). In parts of Africa, Mexico and Central America, Le Berre (1966), Duke et al (1966), Chandler et al.(1967), Crosskey (1956) and Service (1980) have reported that some blackfly species are vectors of the filarial worm, Onchocerca volvulus, which causes Onchocerciasis, a disease that cause permanent blindness in man.

All the vector species are included in a single genus, Simulium, as reported by Chandler et al (1967): S.ochraceum, S.metallicum, S.callidum in Mexico and Central America, S.neavi s.l and S.damnosum s.l.in Africa. In Nigeria, other possible vectors include S.bovis, which was found to be infected by a worm similar to Onchocerca volvulus, in limited areas of the country (Jamnback, 1976). S.yoram developed infected larvae after feeding on infected carriers (Jamnback, 1976). Of the four

species under study, S.adersi was found to develop infected larvae after feeding on infected carriers (Crosskey, 1957 cited by Jamnback, 1976). S.adersi is also known to bite man as reported by Carpenter, (1920 cited by Freeman and de Meillon 1953) who found them biting men on Kome Island, Lake Victoria and Gibbins (cited by Freeman and de Meillon 1953) who received the same species from Kome Island captured by C.C. Gowdey.

There are about 130 species recorded in Africa, out of which about twenty-one species have been described from West Africa (Freeman and de Meillon, 1953). Quite recently Vajime and Dunbar (1975) have described, on chromosomal grounds, additional eight cytospecies from West Africa. S.ruficorne, belonging to the subgenus Eusimulium, is the only species that also occurs outside the Ethiopean region i.e. it is found in the Ethiopean region and is also found in a few Indian oceanic islands and North Africa. In the Namibian desert it is found in perennial fountains. S.adersi is widely distributed throughout the Ethiopean region. S.hargreavesi is widely distributed in East Africa and some parts of West Africa. S.damnosum s.l. is widely distributed throughout the Ethiopean region.

TAXONOMIC BACKGROUND.

Difficulties have been encountered in classifying the blackfly

population in Africa. An account of the classification and problems encountered are summarised below. For details on this subject the reader is referred to Freeman and de Meillon (1953) and Crosskey (1960, 1969).

A classification of the Ethiopean Simuliids based on adult and pupal characters was given by Freeman and de Meillon (1953). They accepted only two simulium genera Simulium Latreille and Cnephia Enderlein.

From West Africa 21 species and 28 pupal forms of Simuliidae were placed by Freeman and de Meillon under Simulium Latreille. They criticized Edward's (1931 cited by Freeman and de Meillon 1953) classification which consisted of two subgenera Simulium and Eusimulium because the former authors could not separate the two subgenera using morphological features. Freeman and de Meillon grouped the blackfly species into Division A and Division B: these divisions were based on the structure of the male genitalia and the pupal respiratory histoblasts. Species belonging to each division were organised into smaller species groups. They admitted that they did not use good key characters for the grouping of species under each division. Thus, to achieve a satisfactory grouping some species groups were defined on characters of the female and others on pupal or male characters. Since Division A

is more primitive, it is considered to correspond to Eusimulium of Edwards. (Freeman and de Meillon 1953). Freeman and de Meillon rejected the generic names Simulium and Eusimulium, based on male genital characters.

Division 'A':

Freeman and de Meillon recognised only four species groups under Division A since it was difficult to clearly distinguish the different species groups or to define individual species because of the apparent uniformity of the features of the species.

Group I: S.alcocki group:

According to Freeman and de Meillon, Group I probably needs further division since they did not use substantial characters in defining the species groups. Sixteen species are included here. S.alcocki, S.johannae, S.impukane, S.mcmahoni, S.bequarti, S.speculiventre etc.

Group II: S.cervicornutum group:

Group II is distinguished from Group I by the formation of the pupal respiratory filaments. Three species are included: S.cervicornutum, S.unicornutum and S.aureliani.

Group III: S.ruficorne group:

Male style as in Group I, that is, even width and gradually tapering. Species included: S.ruficorne, S.nigritaris etc.

Group IV: S.hirsutum group:

"Male style sharply narrowed for its apical half or third". Pupal respiratory filaments branched or filamentous type. Species included: S.hirsutum, S.adersi, S.neavi, S.woodi etc.

Division 'B':

Three species groups are included here.

Group V: S.griseicolle group:

Contains only S.griseicolle species. This is the only Ethiopean species without hairs on the basal section of the radius.

Group VI: S.dentulosum group:

This is the only species where subgroups can be recognised: debegene and its allies with hair on the katepisternite and four pupal filaments; rutherfoodi and its allies with reduced parameral hooks. Eighteen species are included here: S.dentulosum, S.rutherfoordi, S.loutetense, S.debegene etc.

Group VII: S.medusaeforme group:

This group, as pointed out by Rubtzov (1907, 1940 cited by Freeman and de Meillon, 1953) corresponds to Enderlein's genus Wilhelmia. Pupal respiratory filaments are compound, consisting of large, thin walled primaries and small secondaries arising from them. Species included: S.medusaeforme, S.vorax, S.damnosum, S.colasbelcouri, and S.bovis.

Crosskey (1960) contributed a supplementary scheme to that by Freeman and de Meillon. Unlike Freeman and de Meillon who used pupal, male and female characters Crosskey used larval characters to define Division A and B omitting the neavei group even though he encountered difficulties in distinguishing Division A from Division B.

Freeman and de Meillon found several Ethiopean species that were represented by two or more distinct pupal forms. Crosskey regarded these pupal forms to be complexes of allied species because he observed that the characters of the larvae of different pupal forms were identical except the pupal respiratory histoblasts. But later Crosskey (1969) suggested that, since there is little difference between the larvae, the pupal forms should be treated as distinct species. He, However, tentatively considered the neavei complex and copleyi complex as a separate genus eventhough their male genetalia and pupal respiratory histoblasts are similar; he based

his consideration on the fact that throughout the simuliidae only the larvae of the two complexes (neavei/copleyi) are phoretic

Because of the difficulties involved in the classification of the Simuliidae from Africa, Crosskey's "reclassification of the African Simuliidae" utilises comprehensive morphological features of the adult, pupal and larval stages. The main points of this work which is currently used by entomologists are summarised below.

Crosskey classified all the known blackfly species under a single family Simuliidae. He recognised two subfamilies under Simuliidae in agreement with Smart (1945, cited by Crosskey 1969) namely the Parasimuliinae, containing only the single "remarkable" genus Parasimulium and the other - the Simuliinae - comprising all other known black flies.

Crosskey recognised two tribes under Simuliinae namely the Prosimuliini, containing the "more primitive forms" and the Simuliini, containing the "more specialised forms". There exists no single character for distinguishing species in one tribe from those in the other tribe.

Crosskey recognised four genera of African simuliidae (namely, Metacnephia, Prosimulium, Simulium and Afrosimulium). He placed the genus Prosimulium under Prosimuliini and the remaining genera (Metacnephia, Afrosimulium and Simulium) under

Simuliini.

He treated the tribe Prosimuliini in a broad sense, and recognised three subgeneric segregates namely Prosimulium, Procnephia and Paracnephia. Three species groups were included under Paracnephia namely the brincki species group, the muspratti species group and the terneri species group.

The tribe simuliini consists of four genera, i.e. (1) Simulium Latreille, the largest cosmopolitan genus; (2) Austrosimulium Tonnoir, the endemic Australasian genus; (3) Afrosimulium, the new monotypic genus; and (4) Metacnephia, the Holarctic genus.

Taking all characters together Crosskey regarded Austrosimulium as a valid genus although it is closely related to Simulium Latreille. The genus Simulium falls into a number of segregates which are distinguished from one another by the larval, pupal and adult characters. Some of the segregates were recognised as subgenera by Crosskey even though it is not possible to distinguish one subgenus from the other in all stages. Most of these subgenera are endemic to one or two zoogeographical regions except Eusimulium which is cosmoplitan to a certain extent.

From Africa sixteen subgenera are recognised under Simulium. Eight of these are confined to the Ethiopean region, (namely Anasolen, Edwardsellum, Freemanellum, Lewisellum, Meilloniellum,

Metomphalus, Phoretomyia and Pomeryellum), one to the Malagasy region (Xenosimulium), one is common to the Holarctic and Ethiopean region (Byssodon), one is casmopolitan (Eusimulium) and one occurs only on St. Helena Island (Dexomyia). The remaining four subgenera occur in Palearctic Africa (namely Wilhelmia, Odagmia, Simulium s.s. and Tetisimulium). A summary of the major features of the eight Ethiopean subgenera is presented below.

Crosskey included the first two divisions of Freeman and de Meillon under the subgenus Pomeroyellum. Pomeroyellum is the largest endemic subgenus consisting of about 30% of the species and forms of Simulium from the region. It is closely allied to Eusimulium. The males of Pomeroyellum and Eusimulium are very slightly distinguishable. The females evenly covered with pale scales are totally indistinguishable from those of Eusimulium. The most satisfactory means of separating the two subgenera are by studying the morphology of the larvae.

The characteristic feature of females of most Pomeroyellum species is the reduction of pale scaling dorsally on the last few abdominal segments, so that these appear rather bare, dark and shining. The species of Pomeroyellum fall into several distinct

groups principally on characters of the immature stages.

bequarti group:

This is a small group and differs from the rest as the larval cuticle is bare and thus resembling that of subgenus Eusimulium. The male, female and pupal characters are similar to Pomeroyellum. Thus it is best assigned under the subgenus Pomeroyellum.

alcocki group:

The alcocki group defined by Crosskey corresponds with the group treated by Freeman and de Meillon, except that it is slightly restricted, i.e. the bequarti group, schoutedeni group and S.(P).kenyae are excluded here. Freeman and de Meillon placed S.speculiventre from the Seychelles under alcocki group. But Crosskey showed that it is actually a species of Eusimulium since no species of Pomeroyellum are known from any islands in the Indian Ocean.

schoutedeni group:

This is a small group containing only two species. It is intermediate between the groups alcocki and cervicornutum because the pupal gills resemble the alcocki, and a larval stage (with large flat abdominal scales etc.) resembles the bequarti group.

Thus schoutedeni is placed together with alcocki and cervicornutum under the subgenus Pomeroyellum.

kenyae group:

This group is proposed for the single species S.(P).kenyae and is placed under Pomeroyellum. It cannot be satisfactorily placed under any other species groups as for its distinct characters. The male differs from all other Ethiopean black flies by having the hind basitarsus very greatly dilated. The male hypopygium closely resembles that of cervicornutum and S.(P).mcmahoni of schoutedeni group. Abdomen with compound deeply multifid setae with the branches further bifurcated is closely akin to that of S.alcocki. Thus it is placed under Pomeroyellum.

cervicornutum group:

The type species of Pomeroyellum is this species and is typical of the subgenus nomenclaturally. It is wide spread in the Ethiopean region and contains all those species of Pomeroyellum in which the pupal gills are not of the filamentous type. The large blackened and erect scales on the "posterodorsal" part of the abdomen is characteristic of cervicornutum larvae, which is a unique character among the larvae of the subgenus Simulium.

Subgenus Eusimulium:

This is the largest and most widely distributed cosmopolitan subgenus of Simulium. In the Ethiopean region they are represented by about 12% of the species. In Africa they cover a greater geographical range than any other subgenus. The subgenus contains the only species of Simuliidae known from the Sahara: Simulium (Eusimulium) ruficorne Macquart which can survive in conditions of very reduced flow or even no flow at all. Recorded from Tibesti and Tassili des Ajjer massifs in the central Sahara. Four species groups are recognised under this subgenus out of which only two species groups (ruficorne group and loutetense group) are found in the Ethiopean region.

ruficorne group:

This group was termed the ruficorne group by Freeman and de Meillon (1953) for the Ethiopean fauna. S.(E).ruficorne has the widest distribution among the black fly fauna, i.e., it occurs both in the Palearctic and the Ethiopean regions. It is absent in North America but is widely distributed throughout the Old World that includes Europe, North Africa and Canary Islands, Middle East and Southern Arabia, Central Asia, the Oriental Region, Australia, New Guinea and New Caledonia, and the whole of the Ethiopean and

Malagasy regions. It is closely related to the latipes group. The ruficorne - group has successfully colonized several of the remote islands of the Atlantic (St. Helena) and Indian Oceans (Seychelles etc.). Freeman and de Meillon (1953: 64) placed the species speculiventre in their alcocki group of Simulium before Crosskey's (1966, cited by Freeman and de Meillon, 1953) study of the immature stages. Based on the discription of the immature stages Crosskey showed that S.speculiventre belongs to the subgenus Eusimulium and not to Pomeroyellum.

S.ruficorne, the text studied species occurs in very slow running waters. They have been found in just trickling waters. Van Someren (1944, cited by Crosskey 1969) found them in the water standing in hoof prints, in Somaliland. In Northern Nigeria S.ruficorne larvae are found abundantly during the late dry season when conditions are severe (Crosskey, 1956).

loutetense group:

The three Ethiopean species placed by Freeman and de Meillon under the dentulosum group of Division B are assigned to the subgenus Eusimulium by Crosskey (1969). Freeman and de Meillon assigned these species to Division B because of the following character: multiple parameral hooks in the male hypopygium. Crosskey, considering the characters of both sexes and all stages

together, placed the species under Eusimulium. Species included: S.(E).loutetense, S.(E).rutherfoordi and the Nearctic [illegible]. S.(E).furculatum.

Subgenus Meilloniellum:

It is an endemic Ethiopean subgenus. Rubtzov (1962, cited by Crosskey 1969) erected this genus for the species placed by Freeman and de Meillon (1953) in their hirsutum group. Crosskey recognised the segregate as a separate subgenus, containing only the small free living forms, previously included by Freeman and de Meillon under the hirsutum - group, and excludes all those Ethiopean Simulium in which the larval and pupal stages live in phoretic association with crab and mayfly nymphs. A unique species of the subgenus is Simulium adersi, in having the pleural membrane haired; all other characters confirms the position of S.adersi in Meilloniellum. S.adersi occurs in large and small streams both in fast and slow flowing waters. They are found in abundance during the late dry season (Crosskey, 1956) and early rains. They are widely distributed in most parts of the Ethiopean region.

Subgenus Lewisellum:

This subgenus contains the most remarkable and only Simulium

species of tropical Africa, in which the larval and pupal stages live in phoretic association with river crabs. Since these forms comprise "a distinctive, morphologically homogenous, and ecologically clearly defined segregate" (Crosskey, 1969) in Simulium s.l. they are treated as a seperate subgenus by Crosskey. This subgenus includes the type species S.(L).neavei. This species was placed by Freeman and de Meillon (1953) in their hirsutum group.

Subgenus: Phoretomyia:

The adults of this subgenus are uniform in character. The larvae however live phoretically upon mayfly larvae and their larval, pupal characters are heterogenous when compared to those of other typical free-living Simulium aquatic stages. The characters such as; the pupal abdomen often bearing numerous additional hooks, larval head broad and bulbous sometimes with the cephalic apotome widest near the middle and a few other characters confirm that Phoretomyia deviates from the norm. This is regarded by Crosskey, as an adaptation to phoretic mode of life. He found it difficult to signify a few structural modifications in the larvae. The species placed under this subgenus are classified into three species groups based on the large difference in larvae and pupae. Each species group is associated with a particular genus of mayfly and are morphologically different from each other.

berneri group:

This is a small group in which the larvae are associated with the nymphs of unidentified species of Blossoneuris. These species are recorded from Ghana, Angola, and Uganda. Only two species have so far been identified S.berneri by Freeman and S.kumboense by Grenier et al. (1965 cited by Crosskey 1969). Freeman and Grenier regarded the latter as a subspecies of berneri group since they are very closely allied but Germain et al. (1965 cited by Crosskey 1969) regarded this as a separate species.

lumbwanum group:

These are known to be associated phoretically with the genus Afronurus. This group contains only a single species S.lumbwanum which is regarded by Crosskey to be a complex of two sibling species. This was assigned by Crosskey in a separate species group for its diversion from other Simulium species including the species coplevi and beneri, and in regard of the extraordinary pupal abdominal "onchotaxy". It is regarded to be intermediate between the coplevi and beneri group.

coplevi group:

The species of this group live in association with species of Afronurus and Beatis, and are also recorded to be found on an

unidentified species of Beatidae. The cephalic fan plays a very important role in distinguishing this species. Crosskey assigned S.diceras under this species group even though its position lays uncertain when comparing some larval characters. If adult characters are taken into consideration S. diceras is better stated under S.berneri group but since they have the same phoretic partner and a few similar pupal characters they are assigned to the coplevi group.

Subgenus:freemanellum:

This subgenus was proposed by Crosskey for a small group of species that were placed in the dentulosum group by Freeman and de Meillon (1953) or in the genus Anasolen by Rubtzov (1962, cited by Crosskey 1969). They have several morphological characters and ecological preferences in common with dentulosum. Since they bare several characters that are different from other Simulium species, they are recognised as a separate subgenus. The most notable character of the subgenus Freemanellum is the presence of conspicuous hairing on either side of the katepisternal region of the adult thorax, which is absent in other Ethiopean subgenera. In the Ethiopean fauna Freemanellum is most nearly related to the subgenus Anasolen.

Subgenus Anasolen:

This subgenus is unique when considering its ecological

requirements. Even though it is related to the subgenera Freemanellum and Xenosimulium it does not show any affinities towards other subgenera. Hence it is regarded as a separate subgenus.

Subgenus: Byssodon:

Crosskey placed all those species common to the Palearctic and Ethiopean regions under a separate subgenus, Byssodon. It is the only Ethiopean subgenus that has the basal section of the radius bare. Based on this character and a few others this subgenus is isolated from other endemic Ethiopean Simulium subgenera and their ecological requirements. Thus is regarded by Crosskey as a separate subgenus.

Freeman and de Meillon included S.damnosum s.l. and S.medusaeforme under a single species group. But Crosskey recognized both species as different species groups and included them under separate subgenera. He gave his valid reasons for doing so. The subgenus Metomphalus corresponds to the medusaeforme group of Freeman and de Meillon except that the species S.damnosum s.l. excluded in Crosskey's classification is included under a separate subgenus. He included all those species whose larvae were distinctive due to the stricking covering of scales and paired swellings or tubercles on the first five segments of

the abdomen under the subgenus Edwardsellum.

Subgenus Metomphalus:

This is a large endemic Ethiopean subgenus which has no affinity with any South American or the Orienta - Astralasian segregates of the Simulium fauna. Since it is closely allied to the Palearctic subgenus Wilhelmis, one of its segregates S.medusaeforme was included under Wilhelmia by Rubtzov (1940, cited by Crosskey 1969) which was later placed in the species group of medusaeforme by Freeman and de Meillon (1953) which in turn was placed in a separate subgenus by Crosskey (1969). Three distinctive species groups are included under this subgenus based on larval and pupal characters.

albivirgulatum group:

This group contains a single species S.albivirgulatum which is remarkably distinct from all other African Simulium fauna except for those of Edwardsellum, since both have a covering of large scales over the thorax and abdomen which is absent in other African Simulium. It also has other larval characters which enable it to be classified under a separate subgenus. But considering the close similarity in both adults and pupal characters to that of Metomphalus, it is better assigned under

the subgenus Metomphalus.

bovis group:

About six species are included in this group whose immature stages are most uniform. S.wellmanni is unique among other African Simulium since it possesses multiple spinules on the style of the male genitalia. It is in close affinity to bovis based on female, pupal and larval characters. The position of this species in this group is affirmed by the presence of an intermediate species, S.jangi.

medusaeforme group:

This is the largest species group of Metomphalus occuring in high altitude mountain streams in swift cold cascades. The species are rather uniform in character in the adults and the larvae. They differ in the pupae because the pupal gills have deviated from the two distinct pupal filaments to a single thin walled filament. A remarkable feature of this group is the presence of small distinct teeth in the female cibarium. The larvae closely resemble Anasolen larvae, except for the small post genal cleft in Anasolen larvae that distinguishes the two. A stricking larval feature of medusaeforme which is also seen in the vorax group is the unusual elongation of the distal one of

the pair of serrations. This is probably one of the main reasons for classifying yorax and medusaeforme under the same subgenus. S.medusaeforme larvae are found in both large and small rivers in swift broken waters. They are also found in waterfalls attached to rock surfaces in such abundance that they blacken the surface of the rocks. They can also be found on grass, streams and leaves in thick masses. They are usually mistaken for S.dammosum s.l. at first glance. S.medusaeforme can be collected in all months of the year but they are most abundance in the months, June to September.

Subgenus: Edwardsellum:

The main reason for classifying S.dammosum and some other species under a separate subgenus has already been mentioned. The larvae of these species are unique among the Ethiopean Simulium fauna. They resemble Thoretomyia in having the fore tarsi dilated, but they can be distinguished by the bold black and silver grey pattern on the scutum of the males. The presence of dilated fore tarsi in both subgenera does not indicate that they are related. This is a character which had developed independently in the two subgenera. Edwardsellum is known to resemble Metomphalus closely, in the character of the pupa, larval head, the male genitalia and the gonopophyses of female terminalia. But since they differ

largely in their larval and adult stages from that of Metomphalus they are considered as a separate subgenus.

The two subgenera Metomphalus and Edwardsellum are closely related to Wilhelmia which is a Palearctic subgenus. Thus Stone (1963 cited by Crosskey 1969) treated all the three names as synonymous and Rubtzov (1962 cited by Crosskey 1969) treated Edwardsellum as a full genus including the Metomphalus species and the Edwardsellum species. But Crosskey (1969) recognized the three as separate segregates of equal subgeneric status which is the accepted classification so far.

CYTOLOGICAL BACKGROUND.

Cytological studies of larval salivary gland polytene chromosomes have been of major importance in the taxonomy of Dipteran species complexes particularly species groups with morphologically indistinguishable species, that is, sibling species.

Dunbar (1966) was the first to study the larval salivary gland chromosomes of an African Simuliid species. He described four sibling species of the S.damnosum complex. Later in 1969 he reported nine cytological segregates in the S.damnosum complex. A detailed band to band analysis of eight cytospecies of the S.damnosum species group was given by Vajime and Dunbar (1975). An account on the chromosomal identification of Simulium nengense

from the Menge River in Cameroon was also given by Vajime and Dunbar (1977).

The above mentioned workers used a band to band analysis in their investigations. Even though a band to band analysis has proved to be the most reliable means of describing segregates, the gross morphological features have also been useful in distinguishing species in the Simuliidae (Zimring, 1953). These features are also useful to a certain extent in detecting siblings and in grouping of species (Rothfels and Dunbar, 1953). Kunze (1953 cited by Rothfels 1979) also found that larvae collected near Vienna could be distinguished as to species on the basis of the gross features of the salivary gland chromosomes.

Zimring used the following micromorphological features to characterise species: chromosome number and size, the degree of pairing, the expanded region, the nucleolar organiser, the Balbiani Ring, the "double bubble", the Para Balbiani, the "blister", homologous bands and chromosome ends. These features were suggested by Rothfels and Dunbar (1953) to be diagnostically useful in distinguishing segregates.

Cytotaxonomic studies of nearly two hundred species from both primitive and modern genera of this world wide family of perhaps one thousand two hundred species have shown a constancy in the

ratio of the arm about the centromere (Anonymous, 1968). Of the three haploid chromosomes the longest is metacentric and the two shorter chromosomes are acrocentric (Rothfels and Dunbar, 1953).

Degree of pairing of homologues has been suggested by Rothfels and Dunbar (1953) to be useful in species grouping. Zimring (1953) grouped some North American species on the basis of the degree of pairing; all the six species reported by Zimring (1953) had loose pairing.

The usefulness of the expanded region as a gross morphological feature in distinguishing species was suggested by Rothfels and Dunbar (1953). This feature gave satisfactory results in Zimring's study. Of the two types of expanded region, that is, the cottony and the compact type, the compact type was observed in all the species studied in this text. The cottony type was first reported by Painter and Griffen (1951 cited by Rothfels and Dunbar 1953) for S.virgatum. The expanded region was found to be species specific and constant within species. Where a species lacks an expanded region, a remarkably thick and prominent centromere is observed as reported by Zimring (1953). In some species the centromeres are involved in a centric fusion as was reported by Rothfels and Dunbar (1953) in two blackfly species, Prosimulium decemarticulatum Twinn and P.multidentatum Twinn, and in others they are involved in

ectopic pairing which was first reported in Drosophila (Slizynski 1945; Kaufmann and Iddes 1963; cited by White 1973).

Rothfels and Dunbar (1953) reported the main nucleolus to be sufficiently useful in differentiating closely related species. In support of this view Zimring (1953) showed that the nucleolar organiser is of taxonomic value among Simuliidae because they are interspecifically variable and intraspecifically constant.

Rothfels and Dunbar (1953) and Zimring (1953) encountered difficulty in identifying the Balbiani Ring in the species they studied because the expression of this landmark varied among species and within species. Rothfels and Dunbar (1953) termed the largest puff to be the Balbiani Ring and Dunbar (1966) described three other puffs termed the "double bubble", the Para Balbiani and the "blister".

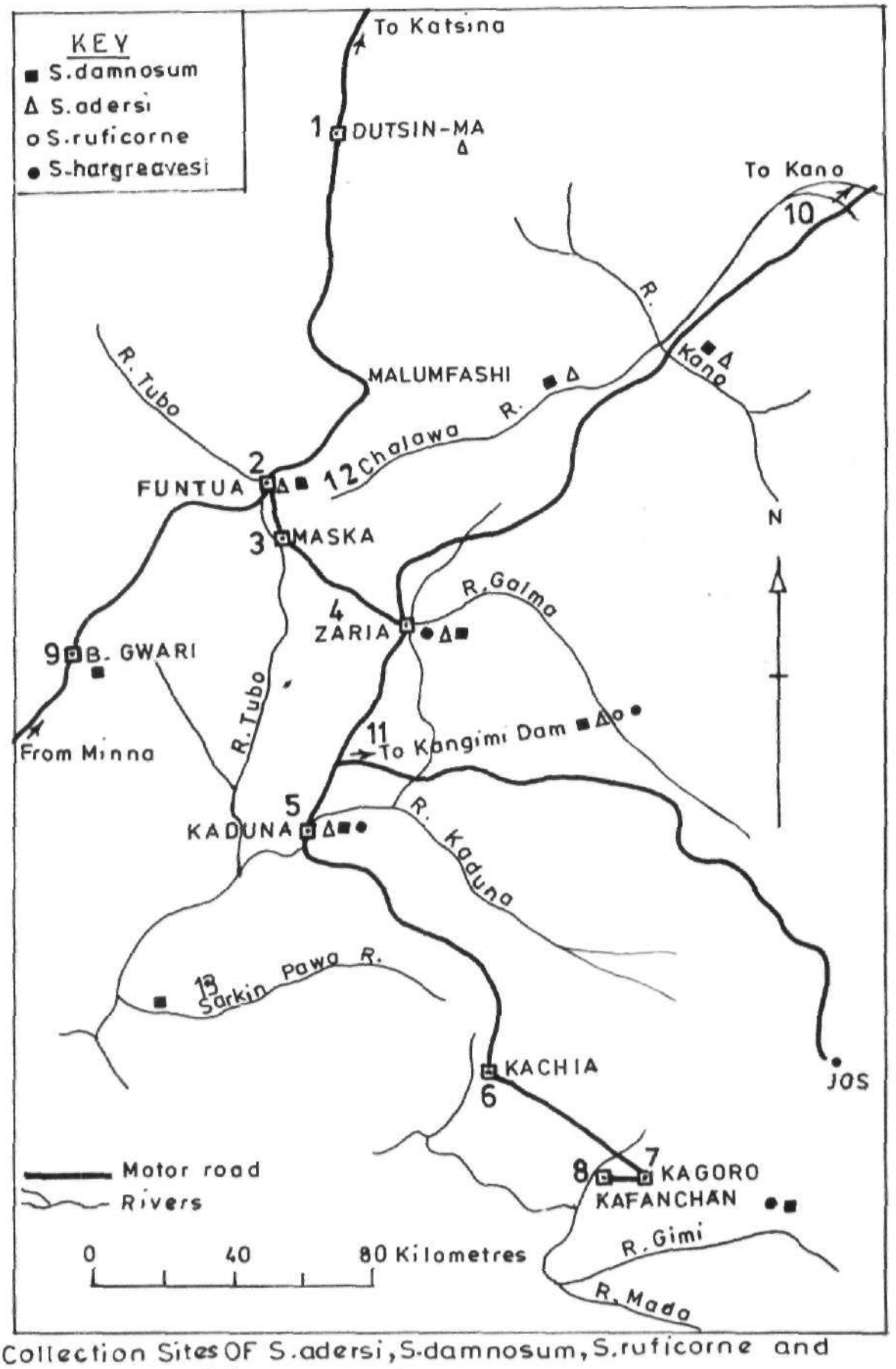
The purpose of the present study is to characterise four African Simuliids (S.sirbanum, S.hargreavesi, S.adersi, S.ruficorne) using the gross morphological landmarks of polytene chromosomes. A detail band to band analysis of the chromosomes of these species would reveal "evolutionary diversification and phylogenetic relationships" (Zimring, 1953) but this is beyond the scope of this study.

MATERIALS AND METHODS.

Larval samples of S.sirbanum, S.adersi, S.ruficorne, S.hargreavesi, S.alcocki, S.loutetense, S.unicornutum and S.cervicornutum were collected for a preliminary cytological study of larval salivary polytene chromosomes. Workable chromosomes were obtained from four of these species, namely S.sirbanum, S.adersi, S.ruficorne and S.hargreavesi. These four species were therefore selected for this study.

Materials were collected from River Kaduna, River Sarkin Pawa, Kangimi dam, Tiga dam, Mairuwa dam, Dutsin-Ma dam, Bagoma dam, River Kogum, University dam (at Ahmadu Bello University), and Balaw Creek. Details of collecting data are shown in the appendix while collection sites are shown on Map I next page.

For knowledge on the biology and taxonomy of the above mentioned species the reader is referred to Freeman and de Meillon (1953), Crosskey (1969) and Jamnback (1976). The larvae were identified with the aid of a larval and pupal key by Freeman and de Meillon (1953) and Crosskey (1969). The larvae were identified using the head pattern, ventral papillae, dorsal protruberances of the larvae and pupal respiratory histoblasts. Of the six instars, the fifth instar larvae are most preferable for cytological studies (Vajime, 1973).



ap.I. Collection Sites OF S.adersi, S.damnosum, S.ruficorne and S.hargreavesi

Simulium damnosum s.l. larvae were found in rapids and fast well aerated waters. They are usually found attached to rocks, vegetation or on dam spillways. In sandy bed rivers they were found attached to vegetation growing at the bottom. e.g. River Kaduna at Malali. S.damnosum s.l. larvae were collected throughout the year and it was easier to find them at the beginning of the rainy season.

S.adersi was usually found in slower flowing waters in which larvae were found attached to stones or vegetations. They were most abundant at the end of the rainy season and mid dry season; they were scarce during the late dry season. S.adersi were sometimes found in sympatry with S.sirbanum.

S.hargreavesi larvae were often found in sympatry with S.damnosum s.l. larvae. S.hargreavesi larvae were found attached to vegetation, rocks or basement of spillways in such abundance as to blacken the surface of their attachment. They were collected throughout the year but were abundant during the heights of the rainy season, that is, during July to October.

Simulium ruficorne was found in both fast flowing and still waters in Kangini dam. They were found attached to porous stones, rocks and vegetation. The morphology of the head pattern and the respiratory histoblast were the same in the majority of the

specimens; a few specimens collected from fast flowing water showed a slight variation in the head pattern. Method of collection and cytological techniques employed were those described by Rothfels and Dunbar (1953), Dunbar (1972). Larvae were collected in wet polythene bags placed in an ice chest containing ice blocks and brought to the laboratory for dissection and fixation. The majority of larvae collected as described above could be kept alive in the laboratory for about 15-20 hours. When long field trips were made to collect specimens, larvae were dissected and fixed in the field. Larvae were checked to ensure that they were alive before dissection. Immobilised larvae were observed to assume an 'S' shape and with a drop of the fixative were seen to wriggle. In the laboratory larvae were identified with the aid of a dissecting microscope before dissection. Thereafter, each larva was immobilised by placing on an ice cube and its abdomen was ruptured with a sharp needle.

The larval salivary glands are long transparent paired structures traversing about two thirds of the body length and then curving back.

The dissected larvae were immediately fixed in freshly prepared Carnoy's fixative (3 parts ethanol: 1 part glacial acetic acid); for S.adersi, S.hargreavesi and S.ruficorne, when a different concentration of Carnoy's (2 parts ethanol: 1 part glacial acetic

acid) was used for fixation better chromosome preparations were obtained. Fixed larvae were used immediately for squash preparations or were stored in tightly stoppered "Bijou" bottles in the refrigerator but not in the freezer compartment.

Larvae used for squash preparations were washed in distilled water for about ten minutes to remove excess fixative, thereafter they were hydrolysed in 0.1N HCl at 60°c for six minutes. The hydrolysed larvae were immediately placed in a tightly stoppered vial containing a small amount of Feulgen stain. Staining time varies from 5 minutes to 5 hours. Care was taken to ensure that larvae were removed from the stain as soon as the glands appeared red in colour; after this stage it was noticed that when the specimens were allowed to remain in the stain, the glands were destained appreciably. Well stained specimens were removed from the stain and were washed in three changes of tap water. Tap water has an effect on the stability of the stain and hardens the chromosomes. The fast green method of staining to identify the nucleolar organiser did not produce satisfactory results. However, using Feulgen stain Vajime and Dunbar (1975) located the position of the nucleolar organiser in S.damnosum s.l. the standard for the present study. Resemblance to the appearance of the nucleolar organiser in S.sirbanum was used to locate the

position of the nucleolar organiser in the other three species.

Each washed specimen was placed on a slide containing a drop of 50% acetic acid on a clean slide, covered with a cover slip and squashed by tapping lightly on the cover slip with the index finger. The slide was then viewed under a compound microscope. Where the chromosomes needed further spread, the chromosomes were squashed again untill a satisfactory spread was obtained. Where the staining of the chromosomes was light, a drop of 1% Orcein in 50% acetic acid was introduced under the cover slip. Specimens stored too long produced very poor chromosome preparations.

The slides prepared as described above, that is, temporal mount, placed in acetic alcohol (1 part ethanol: 3 parts glacial acetic acid) overnight. The following day the slides were removed from the acetic alcohol, excess alcohol was blotted away, the coverslip was raised from the slide with the aid of a razor blade and both the coverslip and the slides were passed through a series of 80% and 100% alcohol saturated with barium hydroxide. Excess euparal was blotted away and the permanent slide was left to dry for at least one week.

To obtain the percent total compliment length (%TCL) (Table 1) of the salivary gland chromosomes flattened and evenly stretched specimens were traced using a camera lucida and the tracings

followed with fine cotton thread which was then measured on a centimetre scale overly stretched chromosomes were not included. The average length of each chromosome of each species were obtained. Each of this values was reduced to %TCL. Five nuclei and at least three individuals of each species were used for measurements.

Four types of films were used: Kodak Plus X-pan, K.B.17, Agfa Isopan and Ilford Pan F, of which Ilford Pan F gave the best photographs. Two types of photographic papers were used: Tura Report - Rapid. RRI (single weight) and Kodak Grade - 2 (single weight); both gave good photographic prints.

RESULTS.

S.sirbanum was used as the standard against which chromosomes of other species were compared with respect to the following chromosomal landmarks: chromosome number and size, arm ratios, degree of pairing, expanded region, nucleolar organiser, "double bubble", Balbiani Ring, Para Balbiani, "blister", homologous bands and chromosome ends.

Chromosome number and size:

Preliminary examination of the larval salivary polytene chromosomes of eight Simulium species (S.sirbanum, S.hargreavesi, S.adersi, S.ruficorne, S.elcocki, S.loutetense, S.cervicornutum

and S.unicornutum) showed that the major chromosomal landmarks of S.sirbanum, S.hargreavesi, S.adersi and S.ruficorne observed were reasonably discernable; the landmarks of the remaining species (S.alcocki, S.loutetense, S.cervicornutum and S.unicornutum) were not clear enough for even a comparative study because these species produced chromosomal preparations of inferior quality. In all the species studied the haploid number of each was three. S.hargreavesi produced the largest chromosomes: S.sirbanum, S.adersi and S.ruficorne produced workable chromosomes which were comparable in quality and size.

Arm ratios and idiograms:

The relative positions of the diagnostic landmarks are shown in the idiograms (Plate. I, Fig. 1, 2, 3, 4). The values on arm lengths and total chromosome lengths do not show desparity for similar findings throughout the Simuliidae. Chromosome II is identified by the presence of Balbiani Ring in IIS, Para Balbiani in IIL. Chromosome III is identified by the "blister" in IIIS. The longest chromosome is always identified as chromosome I, in these four species has the nucleolus (Table 1). In S.hargreavesi chromosome I is considerably longer than those of the other three species; but the arm ratios are almost the same as those of the other three species.

Data on arm lengths, arm ratios and percent total compliment lengths are given in Table 1.

Degree of pairing:

The degree of pairing varied from species to species. In S.sirbanum pairing of the homologues were so tight that the double nature of the chromosomes were difficult to recognise at a glance (Plate II, Fig. 5, 6, 7, Plate III, Fig. 19, Plate IX, Fig. 52). Similar observation were made on the majority of S.adersi individuals (Plate III, Fig. 22, Plate IV, Fig. 30). In S.hargreavesi (Plate II, Fig. 14, Plate III, Fig. 21, Plate IX, Fig. 53), S.ruficorne (Plate II, Fig. 12, 13, 15) and some specimens of S.adersi (Plate II, Fig. 10, 11) loose pairing was observed. The degree of loose pairing was more prominent in S.hargreavesi (Plate II, Fig. 14, Plate III, Fig. 21) and S.ruficorne (Plate II, Fig. 12, 13, 15) than in S.adersi (Plate II, Fig. 10, 11).

TABLE 1

ARM LENGTHS, RATIOS AND %TCL OF FIVE CHROMOSOME COMPLIMENTS OF EACH OF THE FOUR BLACKFLY SPECIES.

Chromosome I:

Species	IS	IL	Total	Arm Ratios	%TCL
S.sirbanum	28.16	30.08	58.24	1.1	43%
S.hargreavesi	30.52	35.64	66.16	1.16	46%
S.adersi	22.99	27.02	50.01	1.17	38%
S.ruficorne	28.60	32.52	61.12	1.13	46%

Chromosome II:

Species	IIS	IIL	Total	Arm Ratios	%TCL
S.sirbanum	14.20	23.70	37.90	1.66	28%
S.hargreavesi	15.28	23.37	38.65	1.53	27%
S.adersi	17.68	24.14	41.82	1.36	32%
S.ruficorne	14.28	20.68	34.96	1.44	27%

Chromosome III:

Species	IIIS	IIIL	Total	Arm Ratios	%TCL
S.sirbanum	13.58	24.53	38.11	1.8	29%
S.hargreavesi	12.71	25.55	38.26	2.01	27%
S.adersi	12.48	26.00	38.48	2.08	29%
S.ruficorne	12.50	22.65	35.15	1.81	27%

Expanded region:

The expanded region associated with the centromere is the main landmark of Chromosome I. In S.hargreavesi and especially S.sirbanum it has a distinctly greater diameter than the rest of the entire chromosome (Plate II, Fig. 5, 6, 8). In S.sirbanum the expanded region is large, more or less globular (Plate II, Fig. 5) or cylindrical (Plate II, Fig. 6) and is composed of a loose array of darkly stained granules (Plate II, Fig. 5, 6). The centromere is quite visible in some specimens (Plate II, Fig. 5, 6). Very light bands are seen on either side of the expanded region (Plate II, Fig. 5). S.hargreavesi is characterised by a large and long expanded region which is composed of a loose mixture of dark and lightly stained granules (Plate II, Fig. 8). The centromere is in the form of a thick dark band and is conspicuous in most cases (Plate II, Fig. 8). A few light bands were also observed in the expanded region (Plate II, Fig. 8, 9).

The expanded region was not observed in S.ruficorne and S.adersi (Plate II, Fig. 10, 12). S.ruficorne has a unique centromere which makes the species easy to identify. The centromere is conspicuously large and darkly stained in all the three chromosomes (Plate II, Fig. 12, Plate III, Fig. 26, Plate V, Fig. 39). In S.adersi the centromere is thick, prominent

but smaller than that of S.ruficorne (Plate II, Fig. 10). In both species there are very light band on either side of the centromere regions (Plate II, Fig. 10, 12). Another characteristic feature of S.ruficorne is ectopic pairing which was observed in this species to the exclusion of the remaining three species. Ectopic pairing was more common between Chromosome I and II (Plate II, Fig. 16, 17), however, in one specimen ectopic pairing was observed between Chromosome I and III (Plate II, Fig. 18).

Nucleolar Organiser:

A single nucleolar organiser was found on Chromosome I of each species. This feature was observed both homozygously (Plate II, Fig. 11, 13) and heterozygously (Plate II, Fig. 5, 9, 10, 12). The nucleolar organiser was found in the short arm of S.sirbanum and S.hargreavesi (Plate II, Fig. 5, 9) but in S.ruficorne and S.adersi the nucleolar organiser is located on the long arm of Chromosome I (Plate II, Fig. 10, 12). In S.sirbanum and S.hargreavesi two groups of characteristic dark bands marked 'a' were observed just before the nucleolar organiser (Plate II, Fig. 7, 9). In S.adersi these bands were located on the long arm immediately after the nucleolar organiser (Plate II, Fig. 10). In S.ruficorne these groups of bands are found on the short arm

(Plate II, Fig. 12). It must be stressed that in S.adersi, S.hargreavesi and S.ruficorne these groups of bands were read with difficulty because chromosomal preparations of these species were not sufficiently good. Also the characteristic group of dark bands marked 'b' after the centromere in the short arm of Chromosome I of S.adersi (Plate II, Fig. 10) were found in the IL arm in S.ruficorne (Plate II, Fig. 13). In S.sirbanum and S.hargreavesi these groups of bands were not observed.

"Double bubble":

In S.sirbanum the "double bubble" is seen behind the Balbiani Ring and is separated from the Balbiani Ring by three dark bands marked 'd' (Plate III, Fig. 19). In S.hargreavesi it is separated from the Balbiani Ring by four bands marked 'd' in the photographs (Plate III, Fig. 20, 21) all the four bands appear to be dark but a detail observation of the preparations shows that the four bands consist of two dark and two light bands. In S.sirbanum the "double bubble" is in the form of a puff with one central band marked 'e' (Plate III, Fig. 19). In S.hargreavesi the "double bubble" assumes the shape of a bulge with obvious dark bands, that is, the characteristic puff typical of the "double bubble" was not observed (Plate III, Fig. 21).

The "double bubble" was not observed in S.adersi and S.ruficorne (Plate III, Fig. 22, 23). In S.adersi and S.ruficorne there is a persistant structure in the form of two puffs separated by a dark double band and one light band (Plate III, Fig. 24, 25). These regions are labelled 'f'. In S.ruficorne these puffs are followed by five dark bands which lie directly behind the centromere (Plate III, Fig. 26), marked "g".

Ring of Balbiani:

The Ring of Balbiani is the major euchromatic structure found on the IIS arm. The expression of the Balbiani Ring varied from species to species and within species. For instance, in S.ruficorne in some specimens the Balbiani Ring is compact and in others it was blown up and glassy (Plate III, Fig. 25, 26). Similar observations were made in S.sirbanum and S.adersi (Plate III, Fig. 19, 24). In S.hargreavesi the Ring of Balbiani was found to be euchromatic but compact (Plate III, Fig. 21). Varied positions of the Balbiani Ring were observed among species. The position of the Balbiani Ring in S.sirbanum is found just before the centromere (Plate III, Fig. 19). In contrast the position of this marker, the Ring of Balbiani, in S.hargreavesi, S.adersi and S.ruficorne was situated near the end of the short arm (Plate III,

Fig. 21, 22, 23). In S.sirbanum the Balbiani Ring is followed by three dark bands marked 'h' which are located just before the centromere (Plate III, Fig. 19). These bands were not homologous to those observed below the Ring of Balbiani in S.hargreavesi, S.adersi and S.ruficorne (Plate III, Fig. 21, 22, 25). These observations suggest the presence of paracentric interspecific rearrangement in S.hargreavesi, S.adersi and S.ruficorne. To interpret the sequence of these rearrangements a detailed analysis of banding pattern of the species is required.

Para Balbiani:

The Para Balbiani is a euchromatic structure found on the long arm of Chromosome II and is the main land-mark of this arm. In S.sirbanum the Para Balbiani normally assumes the shape of an inverted 'u' like structure (Plate IV, Fig. 27), but in S.hargreavesi, S.adersi and S.ruficorne the Para Balbiani points upwards that is in these species it assumes the shape of a 'u' like structure and the relative positions of the marker were different in relation to standard. These observations indicate the presence of additional paracentric interspecific rearrangements (Plate IV, Fig. 28, 31, 33) in these species.

"Blister":

The "blister" is a lacerated puff found in the short arm of Chromosome III. It is the main marker of this chromosome. It occupies a similar position in all the four species (Plate V, Fig. 34, 35, 36, 37). It is easily detected in all the four species since it is always followed by two very thick bands marked 'j' (Plate V, Fig. 34, 35, 36, 37, 38). In one particular specimen of S.ruficorne the "blister" was involved in a heterozygous inverion (Plate V, Fig. 39).

Chromosome ends:

Apart from the main landmarks chromosome ends have proved to be good taxonomic characters since they carry groups of characteristic homologous bands. The shape of the chromosome ends aid to a certain extent in identifying the chromosomes. The shape varied from conical to flared.

The IS end was found to be conical in S.adersi (Plate VI, Fig. 42) and flared in the remaining three species (Plate VI, Fig. 40, 41, 43). Homologies were difficult to establish because the terminal bands of each chromosome were too compact to be read accurately (Plate VI, Fig. 40, 41, 42, 43).

The shape of the IL end was found to be flared in all the

four species (Plate VII, Fig. 44, 45, 46, 47). In addition a single darkly stained band marked 'k' near the IL end was homologous in all the four species (Plate VII, Fig. 44, 45, 46, 47). However, S.adersi can be clearly distinguished from the other three species by the presence of two characteristic euchromatic puffs (Plate VII, Fig. 46).

The IIS end was found to be flared and a prominent dark band marked 'l' at the base of the flared section is shared in common by all the four species (Plate III, Fig. 19, 20, 22, 23).

The IIL end was also flared but the flared section in S.sirbanum and S.ruficorne were unstained (Plate VIII, Fig. 48, 51). The corresponding section in S.hargreavesi and S.adersi were darkly stained (Plate VIII, Fig. 49, 50).

The degree of homology was found to be more extensive in the IIIS arm, that is from the IIIS end up to at least the "blister" marked 'm' (Plate V, Fig. 34, 35, 36, 37). The shape of the IIIS end was either conical, as in S.sirbanum and S.adersi (Plate V, Fig. 34, 36), flared as in S.hargreavesi (Plate V, Fig. 35) or pointed as in S.ruficorne (Plate V, Fig. 37). All four species, particularly S.sirbanum and S.hargreavesi were found to be remarkably homologous up to the "blister", thereafter the banding sequence of both species become incompatable and therefore

difficult to compare (Plate V, Fig. 34, 35).

The IIIL end was flared in all the four species (Plate IX, Fig. 55, 56, 57, 58). Homologies were difficult to ascertain because the bands near the end were not clear due to the unsatisfactory quality of the chromosomal preparations.

The section of Chromosome IIIL of S.sirbanum marked 'n' (Plate IX, Fig. 52) was found to be very clearly homologous to those of S.hargreavesi (Plate IX, Fig. 53) and S.edersi (Plate IX, Fig. 54) but not S.ruficorne. Homologies were ascertained to a certain extent in S.hargreavesi (Plate IX, Fig. 53), thereafter it was very difficult to compare since the bands are disturbed by a constriction. A complex paracentric intraspecific rearrangement was found in a specimen of S.ruficorne IIIL (Plate IX, Fig. 59).

DISCUSSION.

Data on the chromosome arm measurements and %TCL of the four species studied shows that Chromosome I of S.hargreavesi is longer than that of the remaining three species but the arm ratios of all the four species remain constant (Table II). Zimring (1953) has reported that Chromosome I and III of North American Simuliids can vary considerably in their %TCL than Chromosome II and that translocations, duplications or deletions are the causative agents. Considering S.hargreavesi deletion is ruled out since there is an increase in the length of the Chromosome I, and while translocation was not observed duplication cannot be implicated convinsingly because it was impossible to carry out the necessary detailed analysis of the banding pattern to reveal any duplications.

The failure to observe translocations, deficiencies and duplications in all the three species studied (S.sirbanum, S.adersi and S.ruficorne) was supported by the relative constancy of the arm ratios and the relative lengths (Table II) of their giant chromosomes. These observations indicate the conservative nature of evolution in the Simuliidae. This view is supported by Rothfels and Dunbar (1953) who observed that the arm ratios and relative lengths of the chromosomes of a long series of North American Simuliid species were remarkably constant.

The difference in the degree of pairing among the four species (Plate II, Fig. 5, 6, 10, 13, 14) brings out the fact that each of these four species has a characteristic pairing pattern as suggested by Rothfels and Dunbar (1953) who considered this feature to be a useful character in species identification. In species, where there was loose pairing, tight pairing was observed at the dark banded regions such as the centromere region in S.hargreavesi (Plate II, Fig. 8, Plate IV, Fig. 29, Plate IX, Fig. 54) and S.ruficorne (Plate II, Fig. 12, Plate III, Fig. 23). This observation is in agreement with the view held by Wallace (1966) that pairing attraction is a characteristic feature which lies in the darkly stained bands of some homologous chromosome sections but not in the chromosome as a whole. That is, bands of dark banded regions behave in this manner; and Rothfels and Dunbar (1953) have suggested that "difference in pairing signifies convergent development of pairing relations in several independent evolutionary lines or is caused by spurious association of unrelated forms in taxonomic artefacts".

Although the haploid number of chromosomes of the four species S.sirbanum, S.hargreavesi, S.adersi and S.ruficorne are the same, it was evident that S.hargreavesi possessed very large chromosomes compared to S.sirbanum, S.adersi and S.ruficorne.

This could be a reflection of high DNA content in the salivary gland chromosome of S.hargreavesi as reported by Rothfels and Dunbar (1953) in their work on the salivary gland chromosomes of S.vittatum.

The presence of heavily stained granules forming the expanded region, as observed in S.sirbanum (Plate II, Fig. 5), suggest that it is formed by the coalescence of the heterochromatic section of the homologues forming the giant chromosome (Suzuki, Griffiths and Lewantin, 1981). The presence of very light bands on and adjoining the expanded region as in S.hargreavesi (Plate II, Fig. 8) suggests that it is formed by the disorganisation of bands. Similar observations have been made by Rothfels and Dunbar (1953) who have reported that "regions of chromosomes not typically banded but either deeply or amorphously stained or disaggregated and flaring apart are termed heterochromatic". The morphology of the expanded region is an important feature in characterising segregates. As may be seen in Plate II, Fig. 5 and 8, the shapes of the expanded region in S.sirbanum and S.hargreavesi are unique; and this feature is absent in S.adersi and S.ruficorne.

It is important to note that the position of the nucleolar organiser in all the four species studied was intraspecifically constant and interspecifically variable (Plate II, Fig. 7, 9, 10, 12).

These observations emphasize the importance of the nucleolar organiser as a diagnostic taxonomic character. Throughout the simuliidae the nucleolar organiser is more often situated near the centromere in Chromosome I. The nucleolar organiser was found in three species in the same relative position but in S.adersi it was in the apparent long arm. (Plate II, Fig. 12). In S.ruficorne the nucleolus was found in the other arm, away from associated bands (Plate II, Fig. 10). This finding suggests a heterobrachial rearrangement as reported by Zimring (1953) in six North American species. In all the four species studied there was a single nucleolar organiser located on Chromosome I only. This observation is in contrast to that reported for Chironomids which are characterised by a de novo appearance of the nucleolar organisers (Zimring 1953). The long arm bearing the nucleolar organiser in S.adersi is possibly the short arm since the arms of the chromosomes used could have been unequally stretched. The occurence of a pericentric inversion could have also caused the shift of the nucleolar organiser to the long arm. The former explanation is more acceptable since pericentric inversions more often cause the production of inviable gametes.

There was considerable variation in the expression of the morphological features discussed above. This pattern of

variation was present within and among species. The expression of the Balbiani Ring varied from a lacerated puff with a defined contour, as was observed in S.hargreavesi (Plate III, Fig. 20, 21) to an exploded structure as was observed in S.sirbanum (Plate III, Fig. 19), S.adersi (Plate III, Fig. 24) and S.ruficorne (Plate III, Fig. 26). The expression of the "blister" was quite conspicuous; this took the form of a locerated distended structure in all the four species (Plate V, Fig. 34, 35, 36, 37).

In contrast the "double bubble" was not observed in S.adersi and S.ruficorne (Plate III, Fig. 22, 23) while the Para Balbiani was inconspicuous in some members of S.adersi. These contrasting observations remind one of the work of Pelling (cited by Beerman and Clever, 1964) on giant chromosome puffs. Using autoradiograph techniques Pelling is reported to have shown that puffs are produced as a result of gene activity and Beerman (1952) postulated that "a particular sequence of puffs represents a corresponding pattern of gene activity" and that the bands involved simply loose their sharp contour and assume a diffuse appearance as was observed in S.ruficorne (Plate III, Fig. 25) or the bands may look as though they had exploded into large cottony loops as was observed in S.adersi (Plate III, Fig. 24). It may therefore be informed that the obscure nature of the "double bubble" among

species and the Para Balbiani within the species S.adersi, suggests lesser or no gene activity at the loci occupied by the two features.

According to Michelke (1953 cited by Beerman and Clever 1964) pre-existing puffs can regress completely and Pelling (cited by Beerman and Clever 1964) has shown that the major product of puffs is RNA which is the active ingredient in the synthesis of protein required for larval development. These observations led to the suggestion that "the pattern of puffing along a chromosome is a quantitative reflection of the pattern of synthetic activities from gene to gene". In addition to the above findings experiments by Hans-Joachim Becker (cited by Beerman and Clever 1964) have also shown that ecdysone induces puffing. Thus any of the three factors, the complete regression of pre-existing puffs, lack of protein synthesis or ecdysone could be responsible for the lack of the formation of a puff such as the "double bubble". Even when the "double bubble" was absent or the Para Balbiani obscure the bands adjoining the expected position of these features remain unchanged. (Plate III, Fig. 22, 23). This suggests that although the shape of bands change during development, the banding pattern remains unchanged. This is why studies involving detailed analysis of banding sequences are among the most reliable means of identifying segregates.

Inspite of the variation in the expression of morphological landmarks discussed above the features that were found to be most useful in species diagnosis were the expanded region, the nucleolar organiser, the Balbiani Ring, the Para Balbiani the "blister" and chromosome ends. In all the species studied the expression of these diagnostic landmarks was variable but the features themselve were constant. In contrast, although the expression of the remaining morphological features, that is, the "double bubble" and the Para Balbiani were also variable, these features were not constant; that is apart from S.sirbanum and S.hargreavesi both features were observed in some but not in all the individuals of all the species studied. It is not surprising that the two "unstable" morphological features mentioned here could not be observed in some specimens. According to Beerman and Clever (1964) the absence of either features could be attributed to the elimination of the hormone (ecdysone) or to lesser or no gene activity. Therefore, the constant presence of the "unstable" morphological features in S.sirbanum and S.hargreavesi suggests that observations on the two species involved, only individuals that reached a developmental stage at which the "unstable" features could be expressed.

It has already been stated that chromosome ends, expanded

regions and chromosomal sections around them are among the principal interspecific homologies observed in this study; the expanded regions or centric regions in species that lack expanded regions have been more reliable in detecting homologies than the other chromosomal morphological features (Plate II, Fig. 5, 6, 7, 9, 10, 12, 13). These observations are in accordance with Zimring's findings that chromosome ends and the regions adjoining the expanded regions are rarely involved in inversions. This finding is expected since radiation experiments and cytological observations have shown that no inversions involve the fusion of unbroken chromosomal ends; that is inversions usually involve the reunion of broken chromosomal ends. (Zimring 1953).

The preservation of the homologous bands adjoining the expanded region, that is, the absence of breaks near the centromere region, has been explained by Zimring (1953) as selection against the deletrious effects of pericentric inversions in reproduction. This view has been supported by Philip's work (1942 cited by Zimring 1953) on chiasma preference in the study of meiosis in Chironomos dorsalis.

Homologies of key sections in the IIIS and IIIL arms were quite conspicuous and curious. In the IIIS arm all the four species share in common the same banding sequence up to the

"blister" (Plate V, Fig. 34, 35, 36, 37). In the IIIL arm, however, only S.sirbanum, S.hargreavesi and S.adersi show homology of key bands marked 'n' (Plate IX, Fig. 52, 53, 54). These key bands were not observed in S.ruficorne. The homology between S.sirbanum and S.hargreavesi (Plate V, Fig. 34, 35) is understandable since they belong to the same group (group VII) on morphological grounds as reported by Crosskey (1960), while that between S.adersi and S.sirbanum is not surprising since they belong to the same genus. What is surprising is the homology between S.ruficorne of Eusimulium and the other three species (S.sirbanum, S.hargreavesi and S.adersi) which belong to a closely related subgenus Simulium. These observations are indicative of the conservative nature of evolution in the Simuliidae. (Rothfels and Dunbar, 1953, Rothfels, 1981).

The position of the nucleolar organiser in S.ruficorne reveals a shift of the nucleolar organiser which should have a lesser lethal effect on fertility than that of a pericentric inversion since the centromere would not be involved in such a shift. The occurrence of a shift could be explained in two ways: the de novo appearance of the nucleolus or as a result of pericentric inversions.

The frequency of pericentric inversions is extremely low among

the simuliids because of the fact that pericentric inversions are known to cause the formation of inviable gametes; it is therefore presumed that selection operates against these kinds of rearrangements (Zinring 1953, Rothfels and Dunbar 1953, Dunbar 1965, Vajime 1973).

On the other hand the frequency of paracentric inversions are common in the Simuliidae. (Rothfels and Dunbar 1953, Zinring 1953, Pasternak 1963). In the present study there were several clues indicating paracentric rearrangements. The most conspicuous of these clues were the positions of the Balbiani Ring in S.hargreavesi, S.adersi and S.ruficorne. In contrast to the S.sirbanun position (Plate III, Fig. 19) the terminal position of the Balbiani Ring in S.hargreavesi, S.adersi and S.ruficorne (Plate III, Fig. 21, 22, 23) suggests the occurrence of one or more interspecific paracentric inversions. Similarly, the difference in the position of the Para Balbiani in S.hargreavesi (Plate IV, Fig. 28) and S.adersi (Plate IV, Fig. 31) suggests the presence of at least one interspecific paracentric inversion. A single heterozygous inversion was observed in the short arm of Chromosome III in S.ruficorne. (Plate V, Fig. 39).

From the results it is apparent that Chromosome III has undergone less change than Chromosomes I and II. A complete

analysis of the banding pattern should reveal more homologies and structural rearrangements, however, this is beyond the scope of this study. Other limitations of the present study include the quality of chromosomal preparations. S.hargreavesi, S.adersi and S.ruficorne yielded poor quality preparations in comparison to S.sirbanum which can be identified even with the naked eye. The larvae of the former had to be identified with the aid of a dissecting microscope. Larvae exposed in this manner often yield poor chromosome preparations (Zimring 1953). This was experienced in the present study and confirmed by Vajime (1982, per. comm.). Since these preparations were not good enough for detailed analysis, it was not possible for example to establish the exact limits of the homologies among species using the chromosomal segments that adjoin the expanded or centromere regions. Information on phylogenetic relationships among species would require detailed studies of both interspecific and intraspecific rearrangements. Studies along these lines are more likely to expose the variability in African Simuliids, and reveal the presence of sibling species to throw more light on the taxonomy of the Simuliidae in Africa.

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APPENDIX I

COLLECTION DATA.

Sample No.	Location	Site On Map	Date of Collection	Total Collected	Species	Collector
1.	Kangimi Dam	11	9-4-81	45	S.r., S.a.	C.G.V., P.K.
2.	Tiga Dam	10	15-4-81	1	S.d.	P.K.
3.	River Kaduna(Malali)	5	18-4-81	145	S.d., S.a.	P.K.
4.	River Kaduna(Malali)	5	31-4-81	180	S.a.	P.K.
5.	River Kaduna(Malali)	5	20-5-81	4	S.a.	P.K.
6.	Kangimi Dam	11	27-5-81	none	S.r.	P.K.
7.	Tiga Dam	10	4-6-81	200	S.d.	P.K.
8.	River Sarkin Pawa	13	18-6-81	none	S.d.	P.K.
9.	Kangimi Dam	11	25-6-81	250	S.r.	P.K.
10.	Tiga Dam	10	23-6-81	15	S.d.	P.K.
11.	R. Kogum (Kafanchan)	8	1-7-81	none	-	P.K.
12.	Kangimi Dam	11	20-7-81	50	S.r.	P.K.
13.	Tiga Dam	10	23-7-81	70	S.d.	P.K.
14.	University Dam	4	23-7-81	100	S.h.	C.G.V., P.K.

Sanple No.	Location	Site On Map	Date of Collection	Total Collected	Species	Collector
15.	University Dam	4	27-7-81	120	S.h.	P.K.
16.	University Dam	4	5-8-81	125	S.h.	P.K.
17.	University Dam	4	7-9-81	50	S.d.	P.K.
18.	Mairuwa Dam Dutsinma Dam	2 1	9-9-81	225	S.a.	P.K.
19.	Bagona Dam	9	13-9-81	200	S.d.	P.K., A.U.E., M.E.D..
20.	University Dam	4	14-9-81	160	S.d., S.h.	P.K.
21.	University Dam	4	22-9-81	140	S.d, S.h.	P.K.
22.	University Dam	4	29-9-81	80	S.d, S.h.	P.K.
23.	University Dam	4	2-10-81	85	S.d, S.h.	P.K.
24.	University Dam	4	5-10-81	110	S.d, S.h.	P.K.
25.	University Dam	4	7-10-81	90	S.h.	P.K.
26.	Kangimi Dam	11	14-10-81	110	S.d, S.h.	P.K.
27.	Kangimi Dam	11	22-10-81	60	S.d, S.h.	P.K.
28.	River Kaduna (G.G.)	5	25-1-82	150	S.d, S.a.	S.P.
29.	Balaw Creek(Kagoro)	7	30-1-82	30	S.l, S.c., S.al.	P.K.

Sample No.	Location	Site On Map	Date of Collection	Total Collected	Species	Collector
30.	Kafanchan W. Falls	8	31-1-82	100	S.h.	P.K.
31.	River Kaduna(G.G.)	5	3-2-82	150	S.d, S.h.	S.P.
32.	River Kaduna(G.G.)	5	25-3-82	120	S.d, S.a.	S.P.
33.	River Kaduna(G.G.)	5	1-5-82	30	S.d, S.a.	S.P.
34.	Kangini Dam	11	20-4-82	100	S.r.	S.P.
35.	River Kaduna(G.G.)	5	27-4-82	15	S.a.	S.P., P.K.

G.G. = Government Gardens

S.d. = S.dermosum s.l.

S.h. = S.hargreavesi

S.a. = S.adersi

S.r. = S.ruficorne

C.G.V. = C.G. Vajime

P.K. = P. Kunar

A.U.E. = A.U. Ezealor

M.E.D. = M.E. Dyer

S.P. = S. Presertphon

PLATE 1

Idiograms

Fig. 1 *S.hargreavesi*

Fig. 2 *S.sirbanum*

Fig. 3 *S.aderai*

Fig. 4 *S.ruficorne*

Ex - expanded region

C - centromere

NO - nucleolar organiser

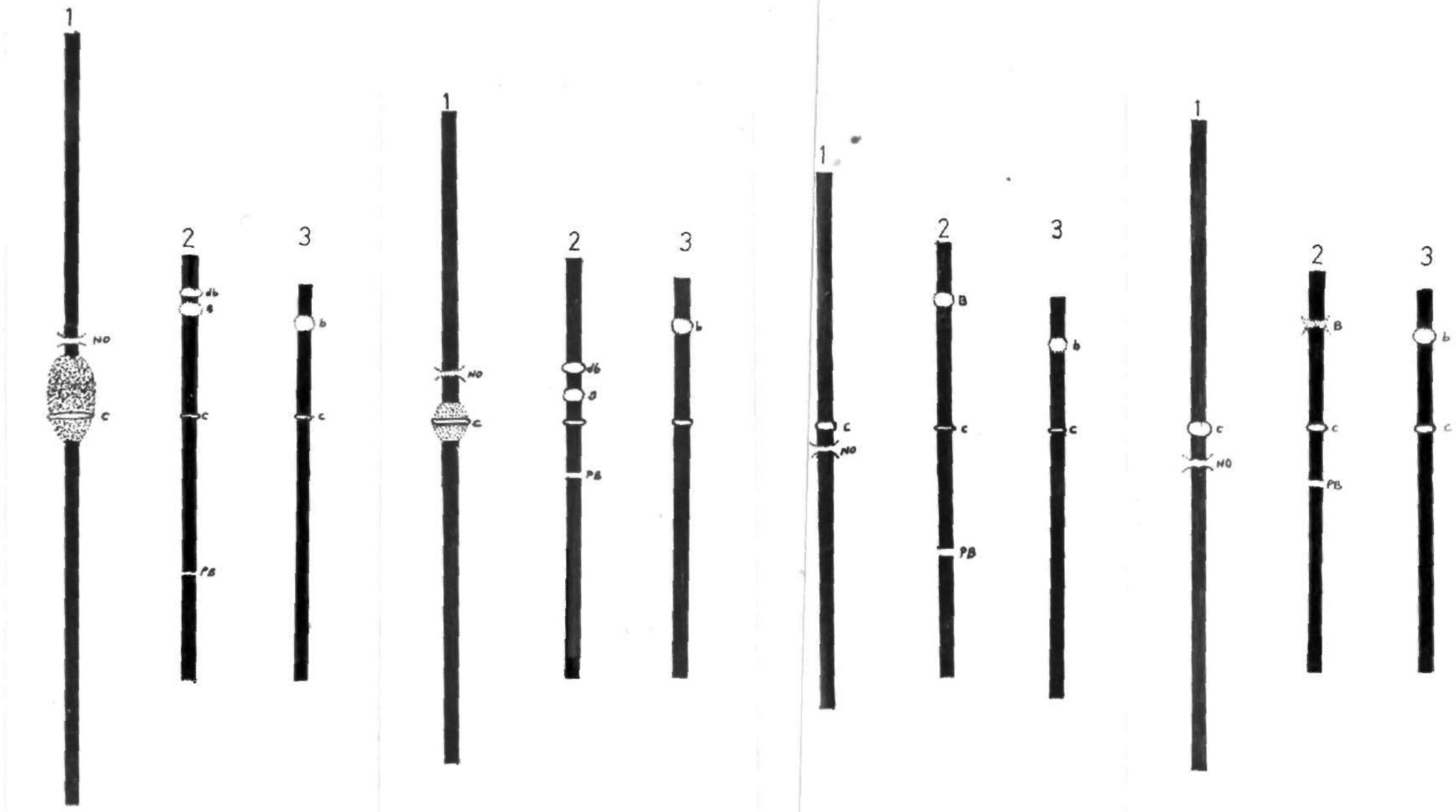
EC - ectopic pairing

db - "double bubble"

B - Balbiani Ring

PB - Para Balbiani

b - "blister"



S.m.hargreavesi
1

S.sirbanum
2

S.adersi
3

S.ruficorne
4

PLATE 2

Expanded Region and Nucleolar Organiser

Fig. 5, 6, 7 - S.sirbamun

Fig. 8, 9, 14 - S.hargreavesi

Fig. 10, 11 - S.adersi

Fig. 12, 13, 15, 16, 17, 18 - S.ruficorne

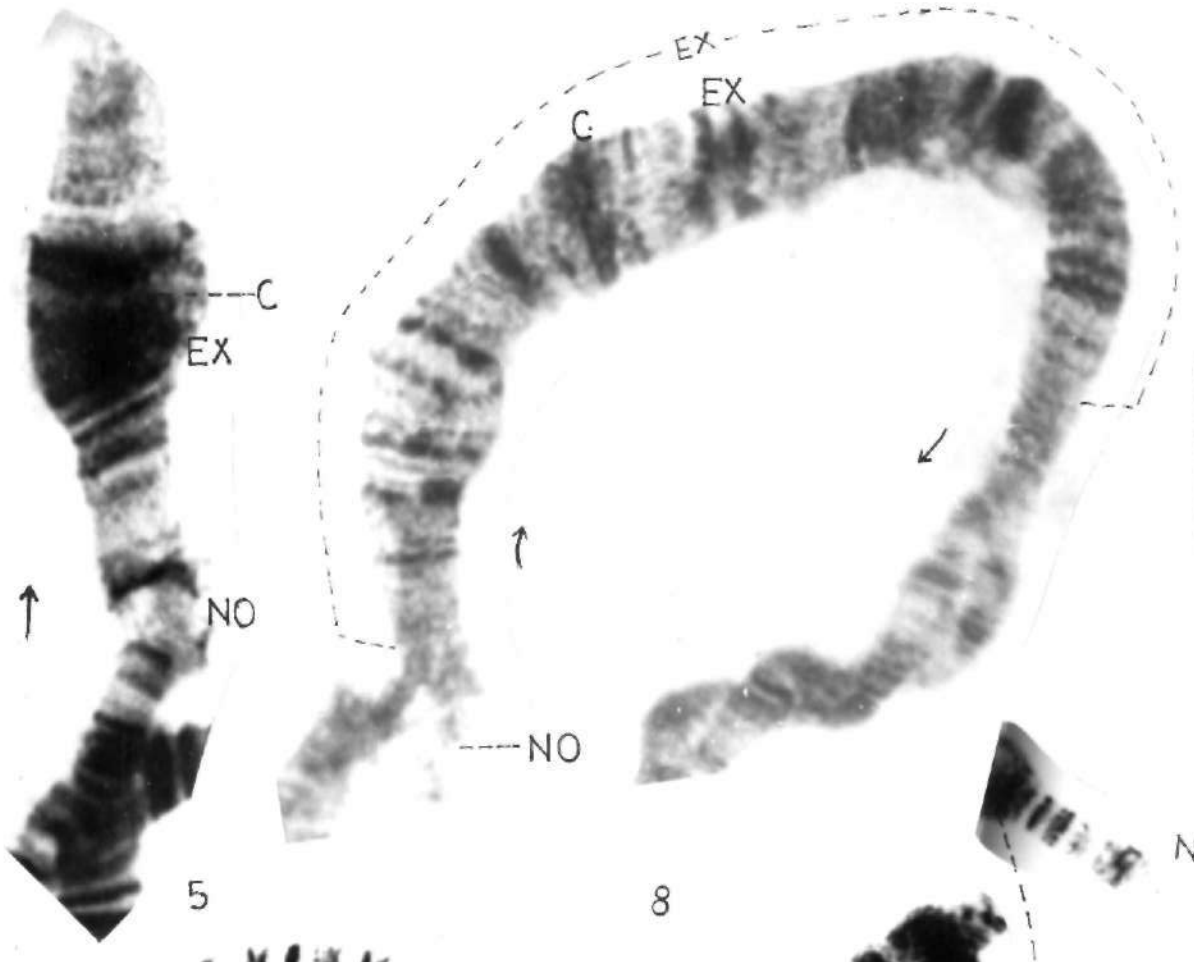
EX - expanded region

C - centromere

NO - nucleolar organiser

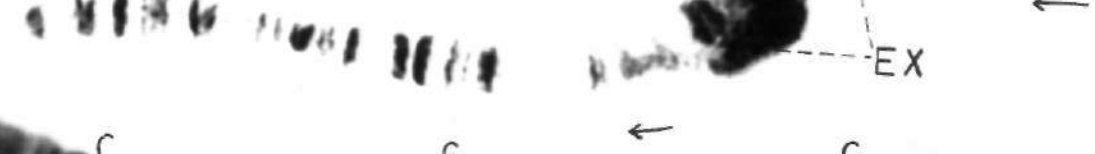
EC - ectopic pairing

↓ - Direction of arrow indicates short to long arm.



5

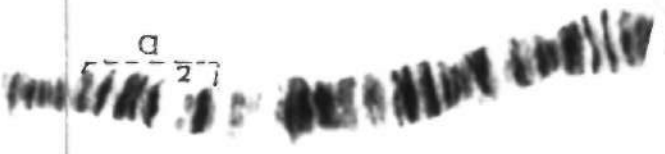
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6



9



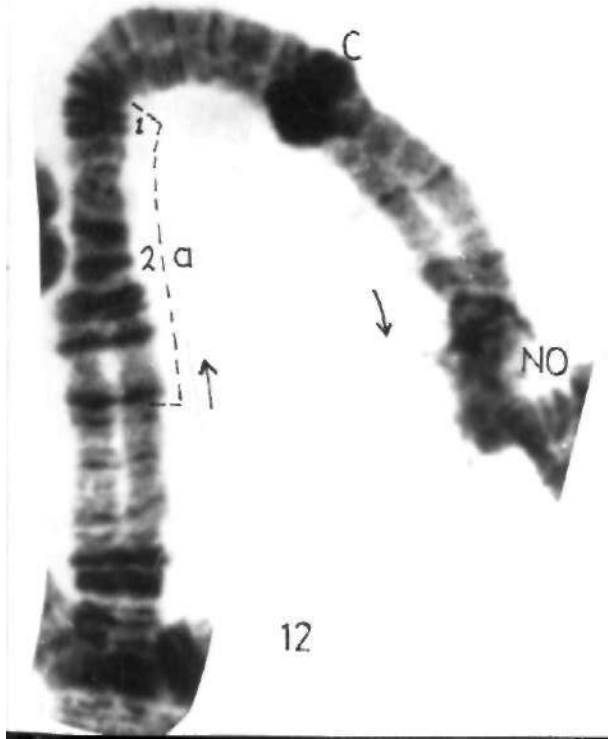
7



10



11



12



13



14



15



16

PLATE 3

Short arm end of Chromosome II

Balbiani Ring and "double bubble"

Fig. 19 - S.sirbanum

Fig. 20, 21 - S.hargreavesi

Fig. 22, 24 - S.adersi

Fig. 23, 25, 26 - S.ruficorne

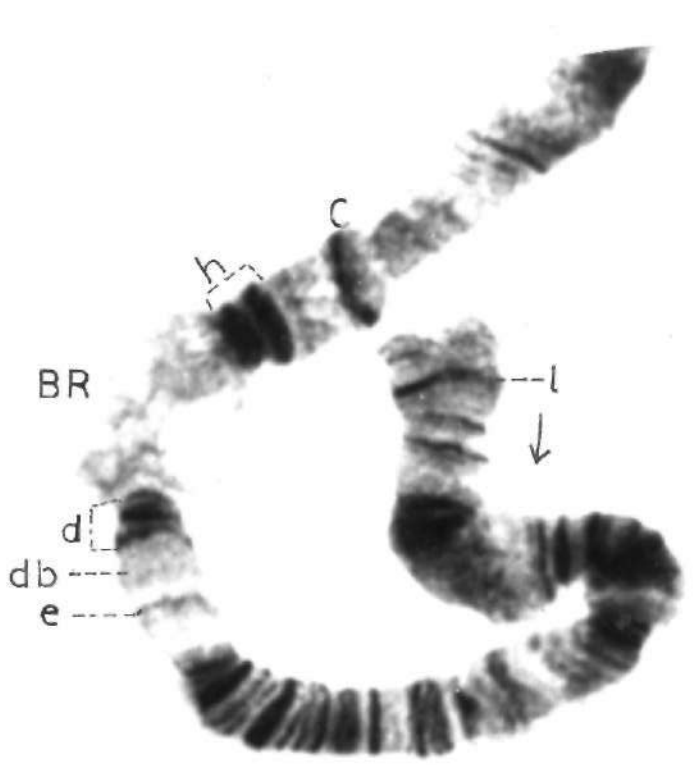
BR - Balbiani Ring

db - "double bubble"

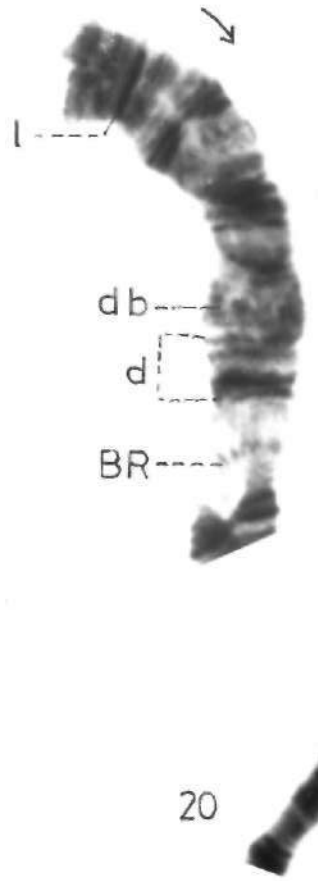
C - centromere

P - puff

↓ - Direction of arrow indicates short to long arm.



19



20



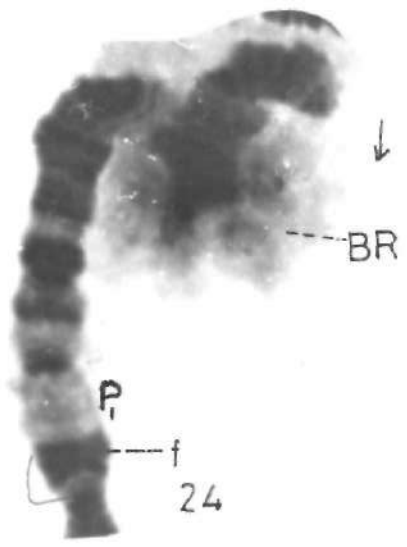
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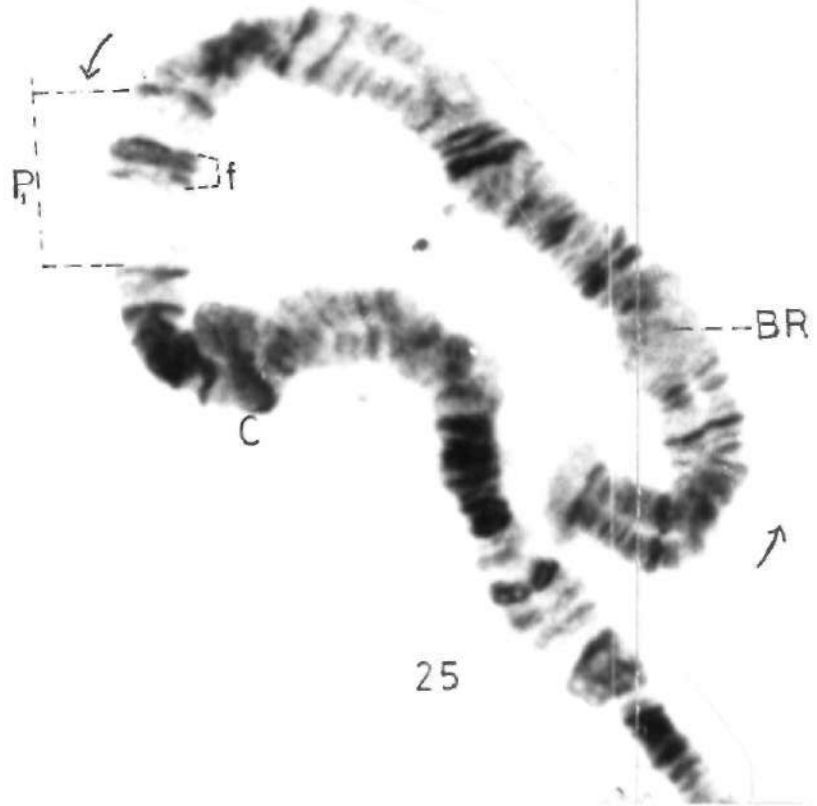
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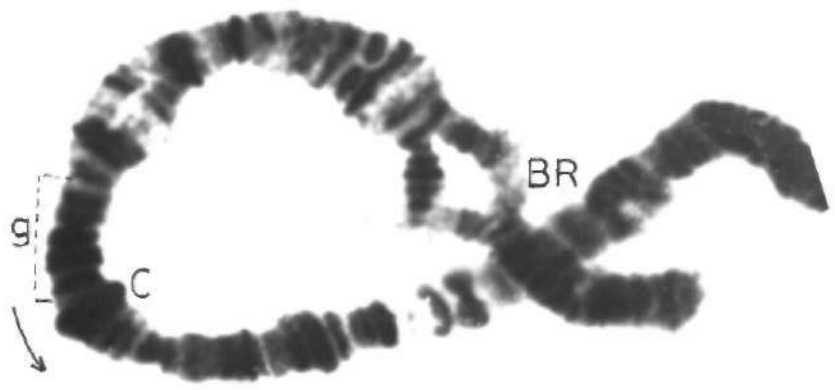
23



24



25



26

PLATE 4

Para Balbiani

Fig. 27 - S.sirbanum

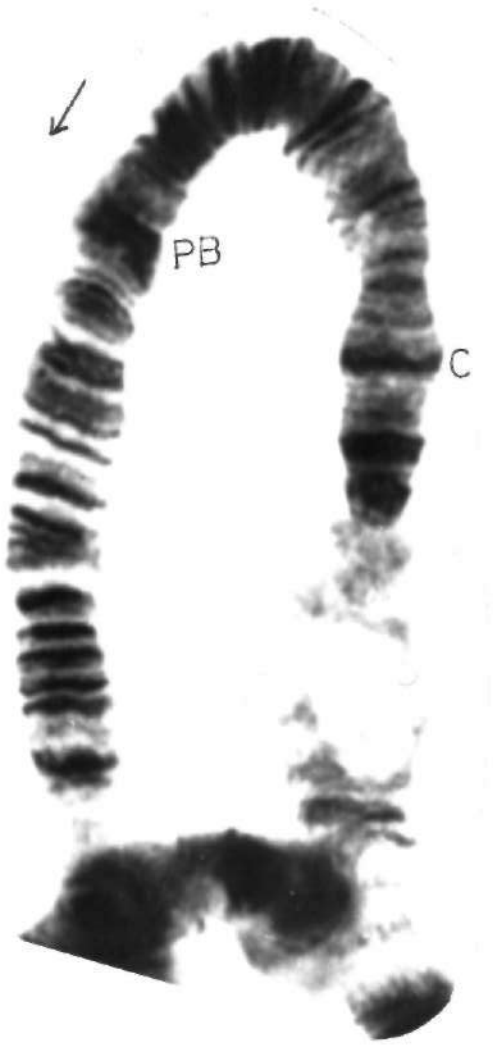
Fig. 28, 29 - S.hargreavesi

Fig. 30, 31 - S.adersi

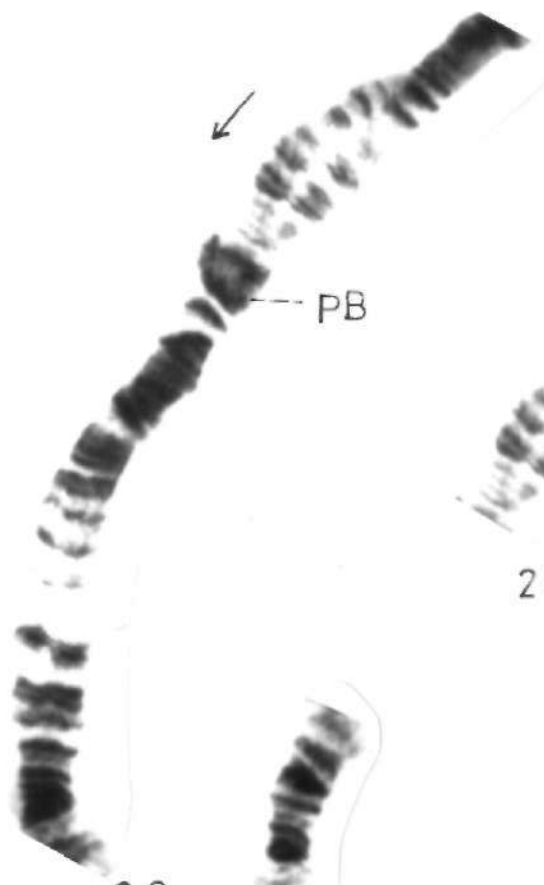
Fig. 32, 33 - S.ruficorne

PB - Para Balbiani

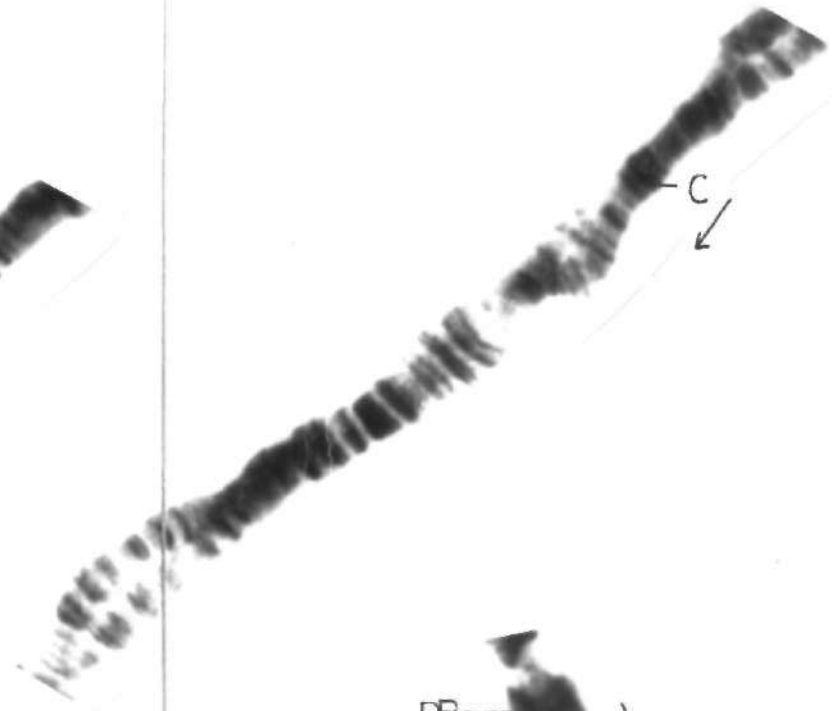
↓ - Direction of arrow indicates short to long arm.



27



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PLATE 5

Short arm end of Chromosome III

Blister

Fig. 34 - S.sirbanum

Fig. 35 - S.hargreavesi

Fig. 36, 38 - S.adersi

Fig. 37 - S.ruficorne

'bl' - blister

Fig. 39 - S.ruficorne shows an inversion involving the "blister" - 'bl' for to 'i'.

↓ - Direction of arrow indicates short to long arm.



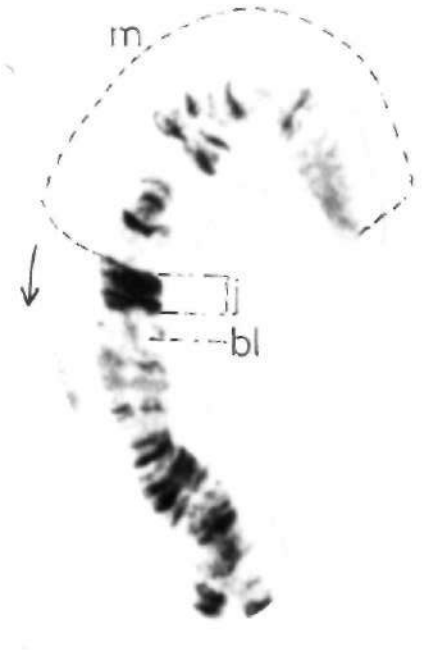
34



35



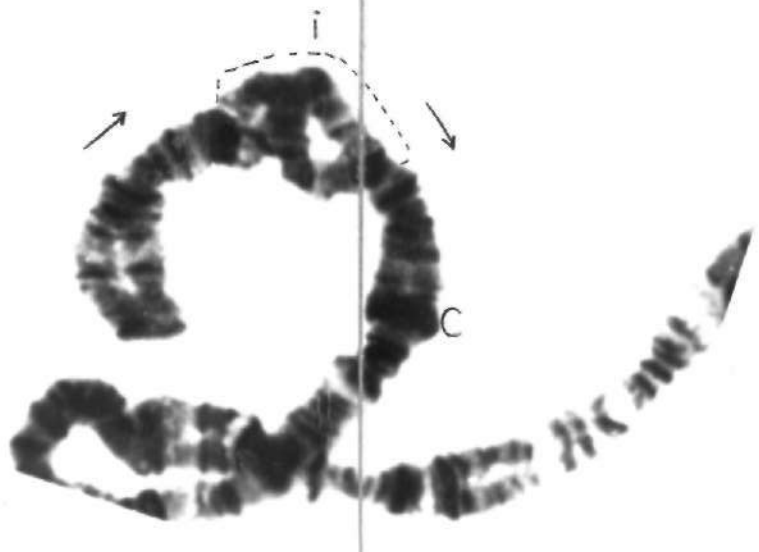
36



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PLATE 7

Long arm end of Chromosome I

Fig. 44 - S.sirbanum

Fig. 45 - S.hargreavesi

Fig. 46 - S.edersi

Fig. 47 - S.ruficorne

I_2 , I_3 - puffs

↓ - Direction of arrow indicates short to long arm.



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PLATE 8

Long arm end of Chromosome II

Fig. 48 - S.sirbanum

Fig. 49 - S.hargreavesi

Fig. 50 - S.adersi

Fig. 51 - S.reticorne

↓ - Direction of arrow indicates short to long arm.



48



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51



52

Middle part of the long arm of Chromosome III

Fig. 52 - S.sirbanus

Fig. 53 - S.hargreavesi

Fig. 54 - S.adersi

Long arm end of Chromosome III

Fig. 55 - S.sirbanus

Fig. 56 - S.hargreavesi

Fig. 57 - S.adersi

Fig. 58 - S.ruficorne

Fig. 59 - S.ruficorne shows an inversion involving the long arm of Chromosome III - refer to 'i'.

↓ - Direction of arrow indicates short to long arm.



52

55



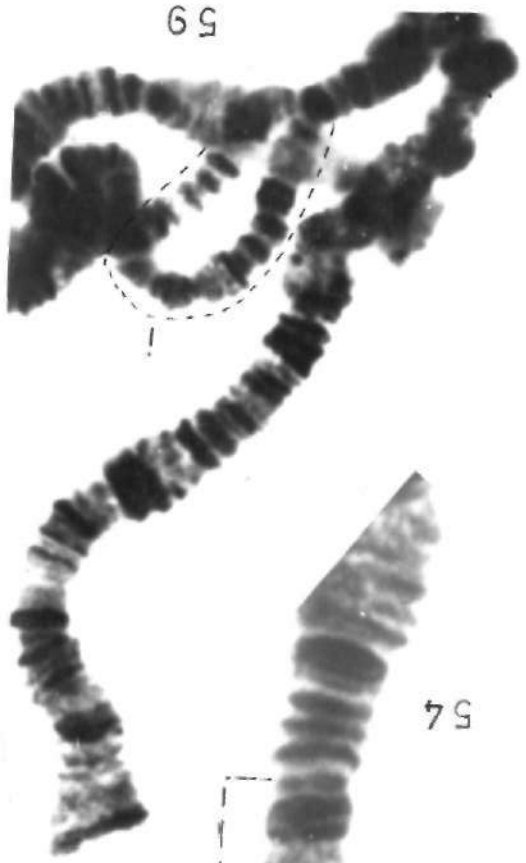
56



57



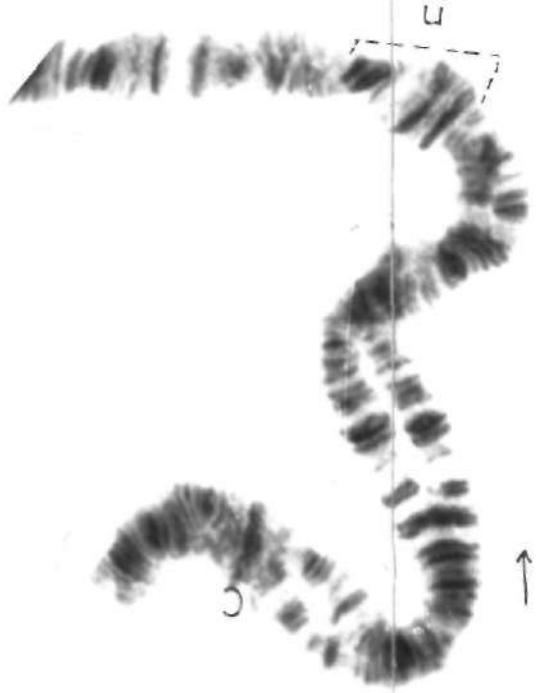
58



59



54



53