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Full Length Research Paper

Karyotypic studies of four species of the blackfly, *Simulium* (Diptera: Simuliidae)

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Karyotypic studies were carried out on the mitotic chromosomes from the supraoesophageal ganglia of four species of the blackfly, *Simulium*. The four species were *Simulium alcocki* Pomeroy, *Simulium damnosum* Theobald sensu lacto and two yet unidentified species of *Simulium* herein referred to as US 1 (unidentified species 1) and US 2 (unidentified species 2). A diploid number (2n) of 6 was obtained for all the species of blackfly studied. A modified method for preparing mitotic chromosomes from the supraoesophageal ganglia was developed. This involved placing the fixed paired supraoesophageal ganglia in 2.5% hydrochloric acid for 1 to 2 s and staining in FLP orcein for 10 to 15 min. Idiograms were constructed for all the four species studied. Standard methods were adopted in the classification and nomenclature used in describing the chromosomes of the four species of blackfly in the investigation. No structural aberrations were observed in any of the species studied.

Key words: *Simulium damnosum* s. l., *Simulium alcocki*, sensu lacto, karyotype, mitotic.

INTRODUCTION

The blackfly is the vector of the disease Onchocerciasis caused by the filarial nematode *Onchocerca volvulus* Leuckart (Nematoda: Filariodea), with about 1.8 million people affected worldwide (Wilson and Post, 1994). Onchocerciasis is a very debilitating disease with heavy infection resulting in blindness and intense pruritus. About 0.66 million people around the world are believed to be blind or with impaired vision, due to this disease. The rates of infection are as high as 15% (reaching 40% amongst human males of working age in areas of high infection) with the savannah region of West Africa having the highest rates (Waddy, 1969; Wilson and Post, 1994; Mafuyai et al., 1996). A skin condition known by the appellation “craw-craw” (pidgin English) recognised around the leg area and accompanied by intense itching is another manifestation of onchocerciasis in the forest region (Waddy, 1969). Others include restriction of economic and social activities such as farming and meetings/gatherings, respectively that coincide with the peak biting periods of the morning and evening of the blackflies.

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Simuliids are among a unique group of insects that have sibling species evolved in the genus with cytological evidence showing that they have an extraordinarily high rate of sibling speciation. A similar characteristic has been reported in other insects such as the mosquito genera *Anopheles* (Davidson, 1974), *Culex* and many of the fruitflies of the family Tephritidae (Berlocher, 1978). Sibling species are pairs or groups of closely related species that are morphologically indistinguishable but reproductively isolated and live sympatrically. Berlocher (1978) called them cryptic or isomorphic species. About 40 sibling species have been recognized within the *Simulium damnosum* complex alone.

Chromosome cytology is a useful tool in the identification of insect species and more so in the case of insect vectors of diseases, where it can assist in their control (Vajime, 1989). Mafuyai et al. (1996) reported six sibling species of the *S. damnosum* complex in Nigeria, further strengthening the view that chromosome cytology can be exploited to the advantage of the control of the disease as positive identification of anthropophilic species aids in the design of disease control measures.

Karyotype is the chromosomal characteristics (number, size and shape) of the body cells of an individual species. Certain parameters are used to describe the karyotype of

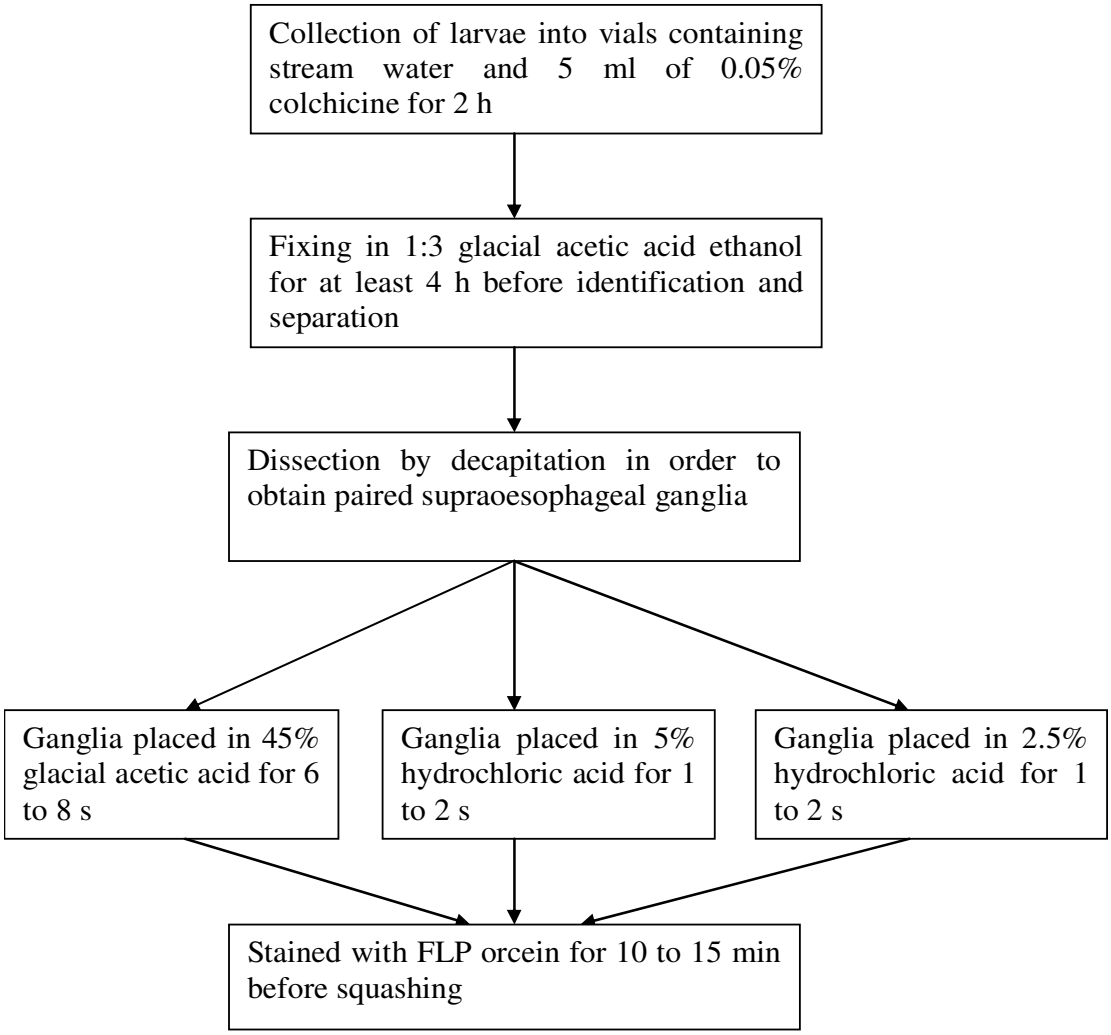

Figure 1. Flowchart showing the three methods of mitotic chromosome preparation employed.

an organism. These include the diploid number (2n), the position of the primary constriction (the centromere) in relation to the total length of the chromosome, presence or absence of structural aberrations and the fundamental number (NF), which is the number of chromosomal arms possessed in the diploid state.

Literature on the cytotaxonomy of blackflies in South-western Nigeria especially on their karyotype is extremely scanty. This study was therefore carried out in order to determine the karyotypic characteristics of blackfly species found within the study area.

MATERIALS AND METHODS

The study area was Obafemi Awolowo University Campus which is situated approximately between latitude 7°27′ and 7°35′ north and longitude 4° 31′ and 4° 39′ east, in the south western region of Nigeria. It is within the rainforest zone of Nigeria. Four sites on two perennial streams and two rivers were selected as the collection sites based on the following criteria: 1) Presence of sections on these water bodies which are fast flowing and 2) abundance of the larvae of the blackfly *Simulium* at these sites.

Blackfly larvae collection

One hundred (100) identified specimens (larvae) of each species were used for this study. Larvae were collected at the various sites, mainly from submerged leaves and sticks using a flexible forceps. The larvae were kept in vials containing stream water to which 5 ml of 0.05% colchicine was added (to increase the number of cells at the metaphase stage) and were left in the vials for 2 h.

The larvae were then fixed in freshly prepared 1:3 glacial acetic acid ethanol (Clarke's fluid) for at least 4 to 5 h before identification and separation. A dissecting microscope was then used to: 1) positively identify *S. damnosum* and *Simulium alcocki* using the larval respiratory histoblast, head pigmentation, body shape and cuticular ornamentation (notably presence or absence of tubercular swellings), and then the larvae were separated into the different species found at a site and 2) the appropriate larvae were selected for chromosome work, that is, those with darkened larval respiratory filament or histoblast. Confirmation was done using the key produced by Freeman and de-Meillon (1953).

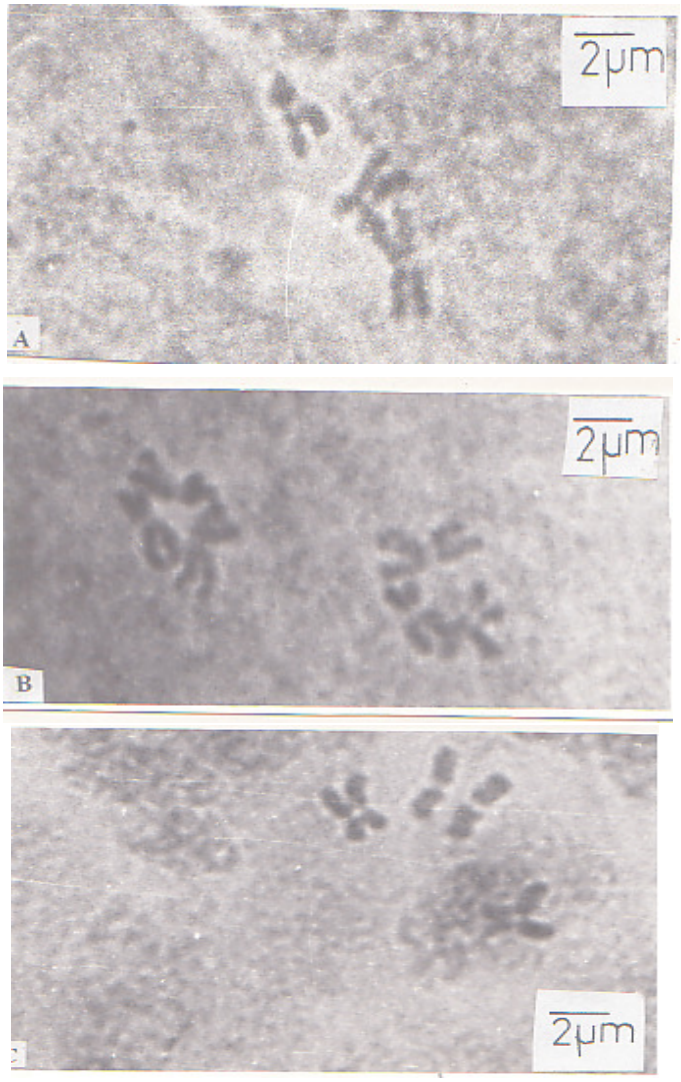

Plate 1. Photomicrograph of mitotic metaphase chromosomes of *S. alcocki* with 2n = 3 from the supraoesophageal ganglia using 2.5% hydrochloric acid. A: With 2n = 3 from the supraoesophageal ganglia using 45% glacial acetic acid showing two spreads; B: With 2n = 6 from the supraoesophageal ganglia using 2.5% hydrochloric acid.

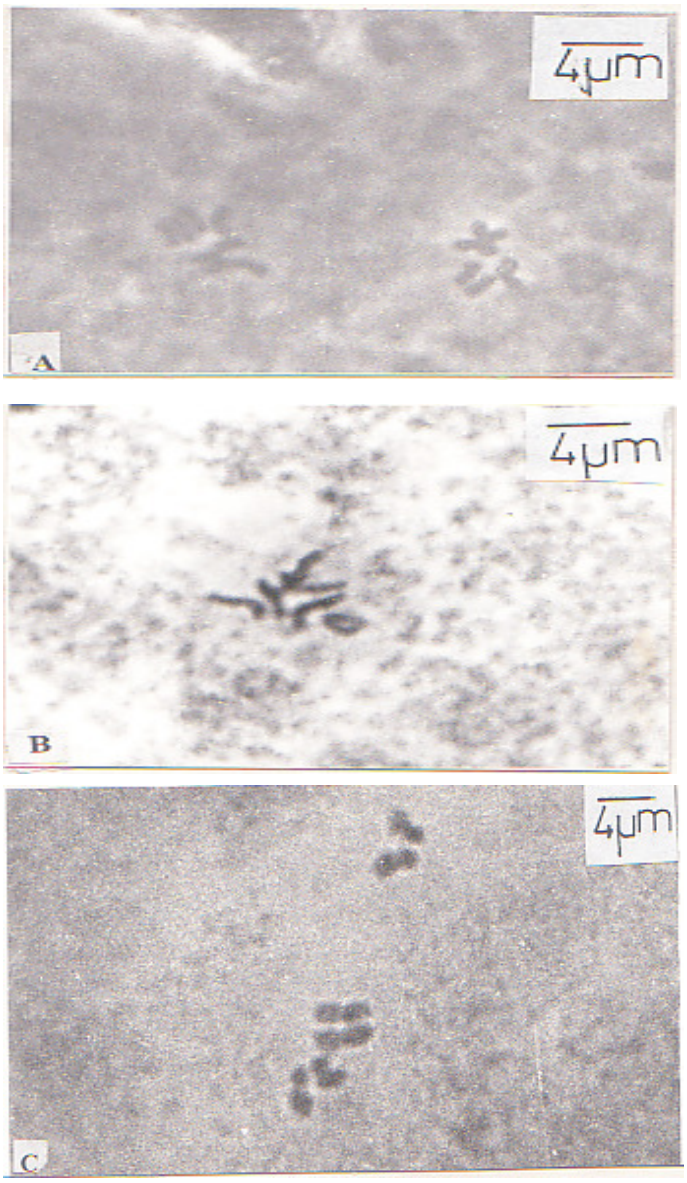

Plate 2. Photomicrograph of mitotic metaphase chromosomes of (A) *S. damnosum* Theobald s.s. with 2n = 6 from the supraoesophageal ganglia using 2.5% hydrochloric acid; (B) *Simulium* species (US 1) with 2n = 3 from the supraoesophageal ganglia using 2.5% hydrochloric acid; (C) *Simulium* species (US 2) with 2n = 6 from the supraoesophageal ganglia using 2.5% hydrochloric acid.

Pupa stages were also collected simultaneously and used for positive identification of the larvae. In fact, species-specific characters, such as gill form and branching, cocoon shape, etc., which are used for positively identifying blackfly species exist both in the larval and pupa stages (Freeman and De-Meillon, 1953). Some of the pupae of the various species were reared to adulthood and preserved as demanded by standard simulid taxonomic procedures. All larvae were kept in the fridge until needed.

Chromosomal preparation

The supraoesophageal ganglia were obtained by decapitating the larva. The head came off with the paired supraoesophageal ganglia attached to it. Three different protocols were adopted for preparing mitotic chromosomes slides. The first, which is the standard protocol used for *Drosophila* (Gatti et al., 1994), involved placing the ganglia in 45% glacial acetic acid for 6 to 8 s and staining in FLP orcein (Olorode, 1974) for 10 to 15 min before squashing. The second involved placing the ganglia in 5% hydrochloric acid for 1 to 2 s before staining in FLP orcein for 10 to 15 min, while the third involved placing the ganglia in 2.5% hydrochloric acid for 1 to 2 s before staining in FLP orcein for 10 to 15 min and squashing (Sorungbe, 2000).

Squashing was carried out after applying a size 0 coverslip and application of pressure with the thumb. Excess stain was removed by placing the slide in between a fold of filter paper and applying pressure over the entire area of the coverslip. Several cells in the metaphase stage of mitosis from the supraoesophagel ganglia were

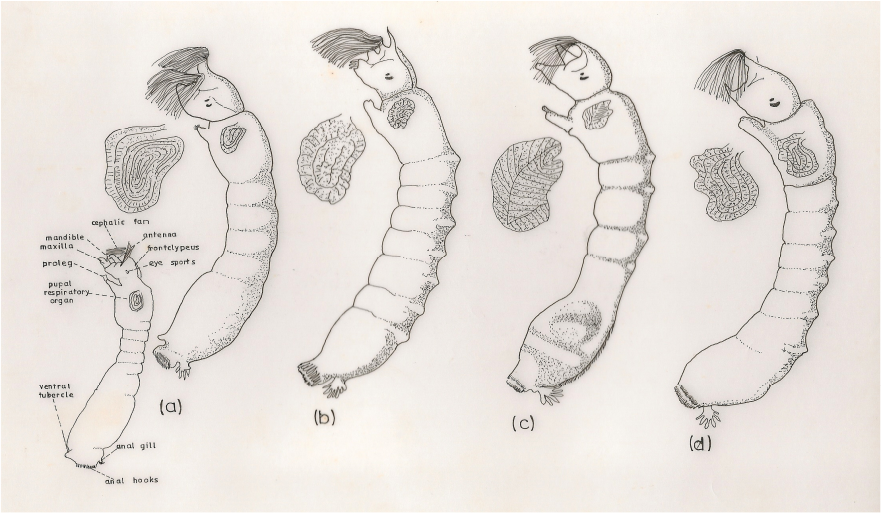

Figure 2: Diagram of the four different species of blackfly larvae and their larval respiratory histoblast. **(a)** S. alcocki Pomeroy **(b)** S. damnosum Theobald **(c)** Simulium species (US 1) **(d)** Simulium species (US 2)

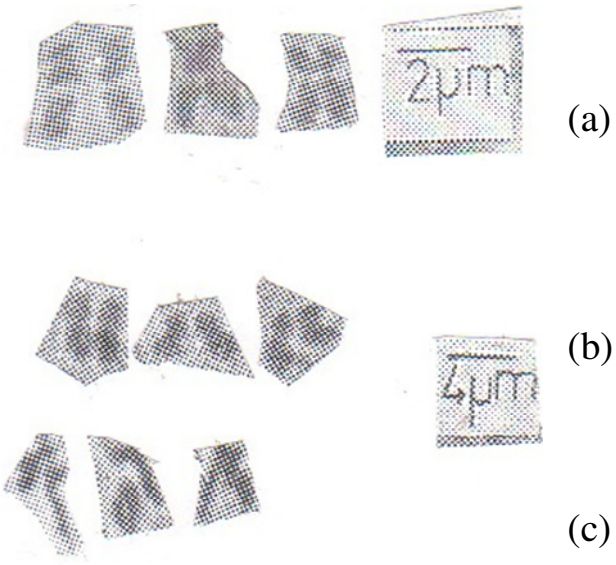

Plate 3. Photomicrographs of karyotypes of *S. alcoki* (a); *Simulium* sp. (US 1) (b); *Simulium* sp. (US 2) (c).

observed with an average of five good spreads from over 30 larvae for each species.

Measurement of chromosomes

Prepared slides were scanned at 4, 10 and 100x oil immersion of a compound light microscope. Measurements of total length and arms of mitotic chromosomes were carried out using eye graticule. Short arm (s) and long arm (l) of well spread mitotic chromosomes were measured and the location of the centromere was noted. Chromosome nomenclature and classification was according to Abraham and Prasad (1983). Photomicrographs of good preparations were taken.

RESULTS

In all, four species of the blackfly *Simulium* were found in the campus of the Obafemi Awolowo University, Ile-Ife. These were *S. alocki* Pomeroy, *S. damnosum* Theobald and two unidentified species of *Simulium* referred to as US1 and US2 herein (Figure. 2).

The diagnostic characters used for the identification of *S. alcocki* and *S. damnosum* were shapes of the larvae of each species, head pigmentation, shape of histoblast (larval respiratory organ) and presence of seta, shape of pupal cocoon, structure and number of pupal respiratory filament of each species (Freeman and De-Meillon, 1953). US1 and US2 had similar species-unique diagnostic characteristics. Every specimen analysed for mitotic chromosomes was carefully checked for these traits.

In all the four species investigated, a chromosome complement of 2n = 6 was observed (Plate 1, Figures. A, B and C; Plate 2, Figures A, B and C). The karyotype of all species was described. The longest pair of chromosomes is considered as 'marker' chromosomes for the purposes of description. The centromeric indices together with the chromosome nomenclature are shown in Table 1. For *S. alcocki* as with all species analysed, chromosome 1 was the longest and was considered the marker chromosome. Along with chromosome 2, both had the same percen-tage length of short arms (14.37%). Chromosome 3 was the shortest in all the species considered. For *S. damnosum*, centromeric position was median, with chromosomes 2 and 3 been nearly median and nearly submedian, respectively.

US1 and US2 were both unique in terms of having the same centromeric position. While US1 was nearly submedian for all the chromosome pairs, US2 was nearly median for its chromosome pairs.

None of the species in the study possess secondary constrictions on any of the chromosome arms.

DISCUSSION

The normal blackfly complement of three pairs of chromosomes (2n = 6) obtained in this study agrees with those of previous workers such as Bedo (1977), Crosskey (1990) and White (1979) who also reported that all the three pairs were metacentric, while the other two pairs were submetacentric.

In this study, the three pairs of chromosomes observed for all the species were all nearly submedian (nsm) for US 1 and all nearly median (nm) for US 2, while *S. alcocki* and *S. damnosum* showed variations in chromosome morphology. Structural aberrations of any type were not observed in any of the species investigated.

Crosskey (1990) also reported that within the *Simulium aureum* group, two pairs of chromosomes (2n = 4), the lowest number found in any insect, occurs in *S. manese* and *Cnephia laponica*. These chromosomes numbers would strongly suggest that the blackflies constitute an evolutionary conserved species.

It was observed that the use of 2.5% hydrochloric acid as the hydrolyzing agent had better results than 45% glacial acetic acid because it greatly reduced, and in some cases eliminated the undesirable effect of parallax. Quite often, the surrounding tissue in the 45% glacial acetic acid treated preparations was very thick. As a result, the surrounding tissue took up the stain so deeply that there was little contrast between the chromosomes and the surrounding tissue.

The extremely small number of chromosomes reported for the genus *Simulium* seemed to indicate evolutionary conservatism since number and structure seem to vary little within the species. This argument again is supported by the assertion of White (1979) that an organism with a low diploid value has automatically reduced the chances of recombination within its genome, hence the less likelihood of generating variation within the genus.

The Diptera is an outstanding example of a large taxonomic group characterized by low chromosome number, and the suborder Nematocera to which the family Simuliidae belongs are considered as the lower diptera. White (1979) believes that it is legitimate to conclude that there are some kinds of special barrier, which operates against increase of chromosome in the diptera.

This is not the case within the genus *Drosophila* where a reasonable amount of variation in chromosome morphology and number exist as exemplified in the *Drosophila virilis* group (Mettler and Gregg, 1969). Lamizana et al. (2001) used sex chromosomes variation from the polytene chromosomes as a basis for identifying three cytotypes of *S. squamosum* from Cameroon and Nigeria, while Henry et al. (2009) described the salivary gland polytene chromosome characteristics of *Simulium dentatum* from the Himalayan region. The use of polytene chromosomes is ideal for cases where morphological characters are insufficient in discriminating species as in the case of sibling species, while mitotic chromosomes are sufficient enough to be used for identification to the species level.

In conclusion, the karyotypes of the four species of simuliids described in this study were basically similar to those reported for other blackfly species. Characteristically, there were three chromosome pairs for each species. Very little interspecific variation appeared to occur in terms of number, size and the absence of structural aberrations.

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