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Polytene Chromosomes of Zaprionus Vittiger Coquillett

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Abstract

The karyotype of *Zaprionus vittiger* consists of six chromosomes, five of which are banded polytene arms extending from a chromocentre in squashed preparations of the salivary glands. The sixth appears as a heterochromatic protrusion. The chromosomes are acrocentric and joined together at heterochromatin around the centromere to form a chromocenter which is easily broken. *Zaprionus vittiger* Coquillett were trapped in the university of Lags botanical Garden. Salivary glands chromosomes of *Zaprionus vittiger* Coquillett third instar larvae were prepared and examined under the microscope. The examination focused on the structure and characteristics of the terminal ends of different chromosomes which can be used to identify them in *Zaprionus vittiger* salivary glands chromosomes preparations.

Keywords: *Chromosome; Zaprionus; Drosophilidae*

Introduction

The family *Drosophilidae* in the order Diptera comprises more than 3,800 species [(1)] of small flies widespread in a variety of climates and environments throughout the world. The genus, *Drosophila* is paraphyletic, causing the genus Zaprionus to be included under it. There has been considerable debate as to what phylogenetic group *Zaprionus* belongs to, with the initial classification including it as a subset of the *immigrans* species group of the genus *Drosophila* because of its close relationship to that group, it is another invasive, human-commensal drosophilid that has been on the move globally [(2)]. Molecular studies of certain genes [(3)] tend to suggest that *Zaprionus* is closer to the *immigrans* group as first suggested by Throckmorton [(4)]. The flies are believed to have originated from the tropical region of Africa, the Middle East and South-East Asia and spread to other parts of the world [(5)]. Most of the species in Africa are endemic to the regions where they are found. Like other members of the family *Drosophilidae*, *Zaprionus vittiger* feeds on fermenting fruits[(6)(7)(8)]. They are small in size roughly 2.5-3.0mm in length, with a brown-coloured body, red eyes and a pair of conspicuous white silver-layered stripes with black margins, along the dorsal region of the head

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and thorax [5]. The males are slightly smaller than the females and possess the genital arch and sex comb typical of drosophila males. The average generation time of *Z. inermis* observed has been 15-16 days, with 9-10 days spent in the egg/larval stage and 6 days in the pupal stage, with extension recorded for drier media [9]. Temperature and humidity affect the generation time of drosophila generally, *Zaprionus* inclusive. However, no generation time has been found in the literature for *Zaprionus vittiger* Coquillett.

Polytene chromosomes are giant chromosomes found mainly in cells of dipteran larvae (salivary, midgut, rectal, malpighian and excretory tubules). The chromosomes appear to be present in the haploid number but each is formed by intimate side-by-side pairing of two homologous chromosomes. In some instances they appear to be actually fused while in other cases, they are in close contact but still distinguishable as separate entities [10]. The banding pattern is distinctive for each chromosome in any given species and corresponds to chromomeres seen at various stages of mitosis and meiosis [13]. It was H. J. Muller in 1940 that, on the strength of his proposition that the karyotype or particularly chromosomal arms in *Drosophila* have remained essentially intact throughout evolution, first designated the arms using letter A – F [14][15]. The recent invasion of *Z. indianus* in Brazil and other parts of South America has opened up opportunity for the study of a 'rare' ecological event [16].Quantitative traits of *Z. indianus* analyzed showed that American flies are larger than Africans, and demonstrate phenotypic variations along geographic lines [16][17][7].

The suggestion of that the genus *Zaprionus* provide species amenable to laboratory rearing and fruitful studies of ecology, evolutionary genetics and speciation, and even chromosomes behaviour in gene function is essentially true, throwing up a need for the preparation and identification of *Zaprionus vittiger* Coquillet salivary gland polytene chromosome[18]. The aim of this study is the examination of polytene chromosomes of salivary glands of *Zaprionus vittiger* Coquillett collected from the University of Lagos Botanical Garden. The examination focuses on the landmarks which can be used to identify each chromosome of *Zaprionus vittiger* Coquillett in squash preparations.

Materials and Methods

Zaprionus vittiger Coquillett used in this study were obtained by trapping flies from the University of Lagos Botanical Garden, Akoka. The traps were milk bottles with sliced oranges or pieces of ripe bananas in them to attract the flies. Traps were set under fruit trees and shady places where flies are expected to be, kept in the early evening and collected in mid morning, the best times for attracting the flies. The traps were collected after stopping the mouths of the bottles with cotton wool or foam stoppers. Different species of Drosophila and other insects were trapped, together with *Zaprionus vittiger* Coquillett. In the laboratory, *Zaprionus vittiger* Coquillett were separated, under a stereomicroscope, from the other flies that were trapped, after being etherized (anaesthsized with diethyl ether). The flies were transferred to, and raised in culture bottles containing a banana-garri medium [12] with the following composition: Distilled Water: 1000ml, Banana: 250g, Garri: 50g, Agar: 10g,Propionic acid: 5ml.

A pot containing the distilled water was heated on a hot plate to boil. Agar agar was added to it and stirred until it dissolved. The mashed banana was added to the boiling water and allowed to continue boiling for about 5 minutes. Garri was then added and mixed in thoroughly and after about 5 minutes the pot was removed from the hot plate. Propionic acid was added after the pot was removed from heat, to reduce its evaporation. The banana provides the sugar on which yeast could grow. The larvae feed on the growing yeast. The agar and garri bind the medium together, providing consistency. The propionic acid prevents the growth of mould that could contaminate the culture. The medium was poured immediately, while still warm, into vials (10cm x 2cm) and a few culture bottles, all sterilized. A funnel was used to avoid spilling it on the top or sides of the containers. The vials and culture bottles containing medium were arranged in a tray and covered with a cheese cloth to prevent contamination by flies, and left for about 24 hours to cool and solidify.

Five female flies and a male were transferred into each vial, and twelve females, three males into each bottle. The cultures were kept at room temperature until the females lay eggs and the larvae were

hatched. The cultures were then transferred to the incubator at 19 ºC. A drop of active yeast suspension was added to the medium to facilitate fermentation and provide more nutrients for the developing larvae. The reduced temperature increased the period of larval development leading to larger larvae. The third instar larvae crawl up the sides of the bottle at about the tenth day ready to pupate. At this stage, the salivary glands were at their maximum size and appeared as translucent structures with yellow fat bodies attached to them.

Chromosomes preparations were made using the salivary glands of the third instar larvae. Larvae that crawled to the sides of the bottle were picked and transferred to a glass slide with a drop of insect saline, under the stereomicroscope. Dissection was done using a pair of dissecting needles. One needle was used to hold the larva firmly; the other was placed directly behind the mouth parts to detach the head. The glands were isolated and the associated fat was removed as much as possible. The insect saline kept the glands from drying and prevents autolysis as the tissue degrades quickly. The salivary glands were then transferred to a drop of lactic acetic orcein to stain for 25-30 minutes, covered with a cover slip which is tapped lightly to disperse the chromosomes. A filter paper was placed over the cover slip and then pressed firmly with the ball of the thumb. The filter paper absorbed excess stain as it was forced from the space between the slides and the cover slip.

The salivary gland chromosomes preparations were examined with a compound microscope at different magnifications: low power (x10), and high power (x40). Slides that contained well spread chromosomes were preserved by sealing the edges. Photomicrographs of the preserved slides were taken under oil immersion (100x). The photomicroscope used was the Wild M20 microscope with a PS50 photoautomat and an MPS55 electronic control unit. Digital photographs of the slides were also taken under oil immersion using a Soc PC camera connected to a computer. Sections of overlapping images were merged together using Automated Photomerge on Adobe Photoshop CS3. Brightness and contrast of the images were adjusted for clarity where necessary.

Results

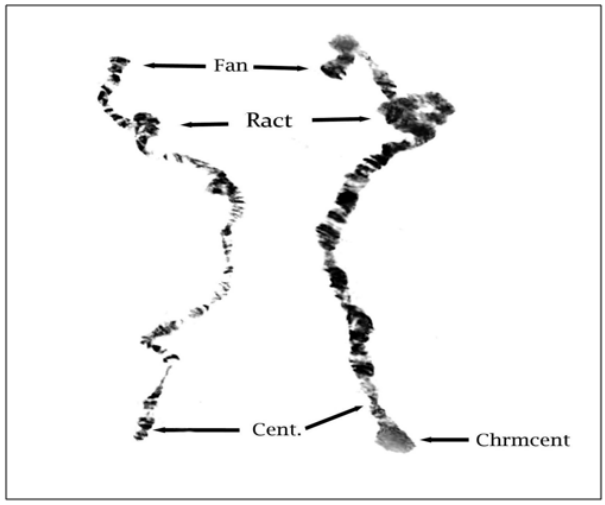

Fig 1: Chromosome A with the centromere, chromocenter, the Fan terminal region and the region of constant activity (Ract). Note the difference in the Ract between the two examples.

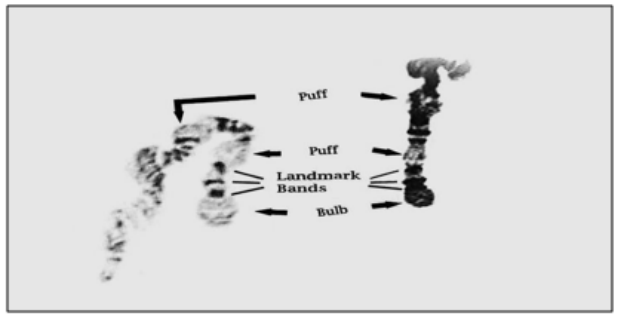

Fig.2a Bulb-shaped free terminal end of chromosome B. Some landmark puffs are shown

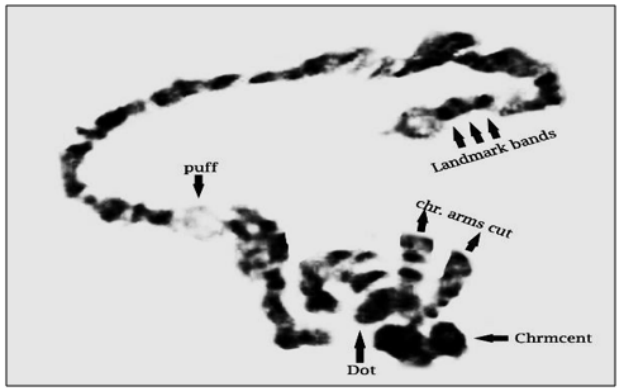

Figure 2b: A full length chromosome B traced from the chromocenter.

The dot chromosome is also seen here.

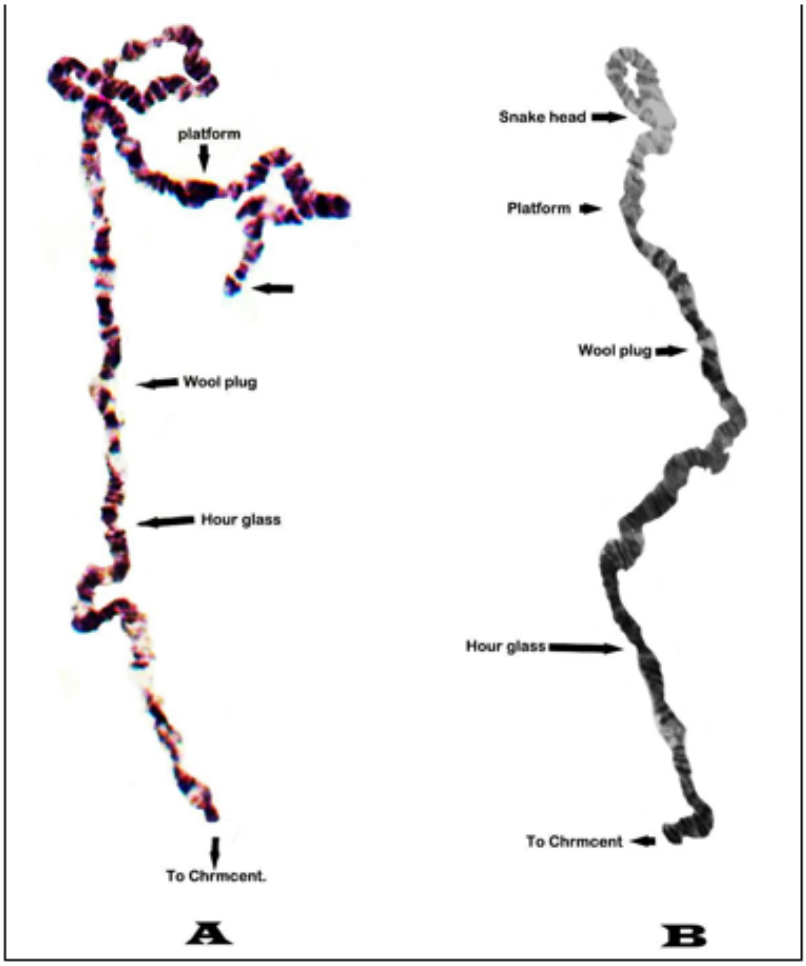

Figure 3: Chromosome C showing various landmarks

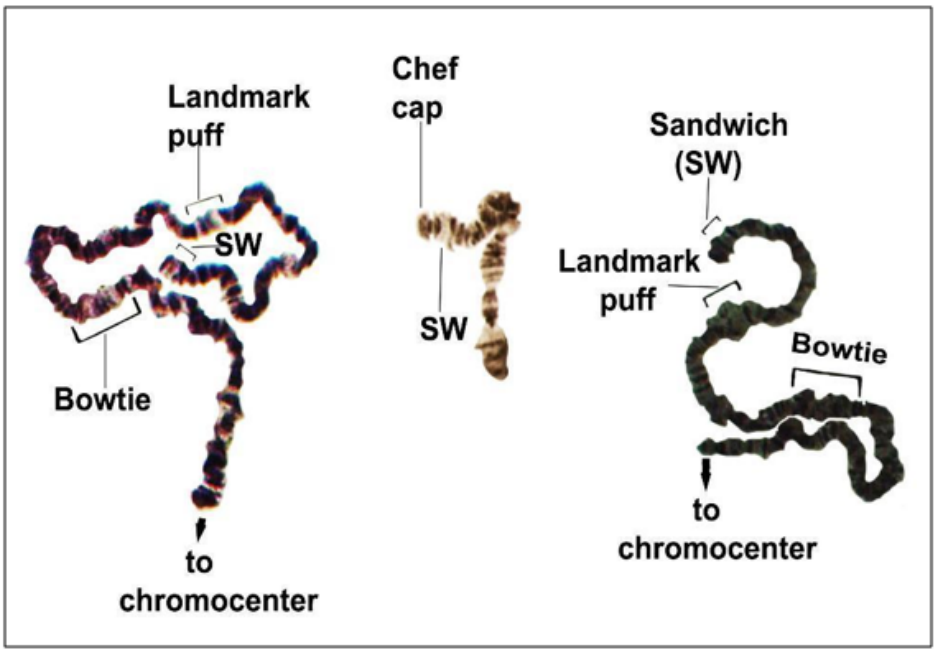

Fig. 4: Chromosome D

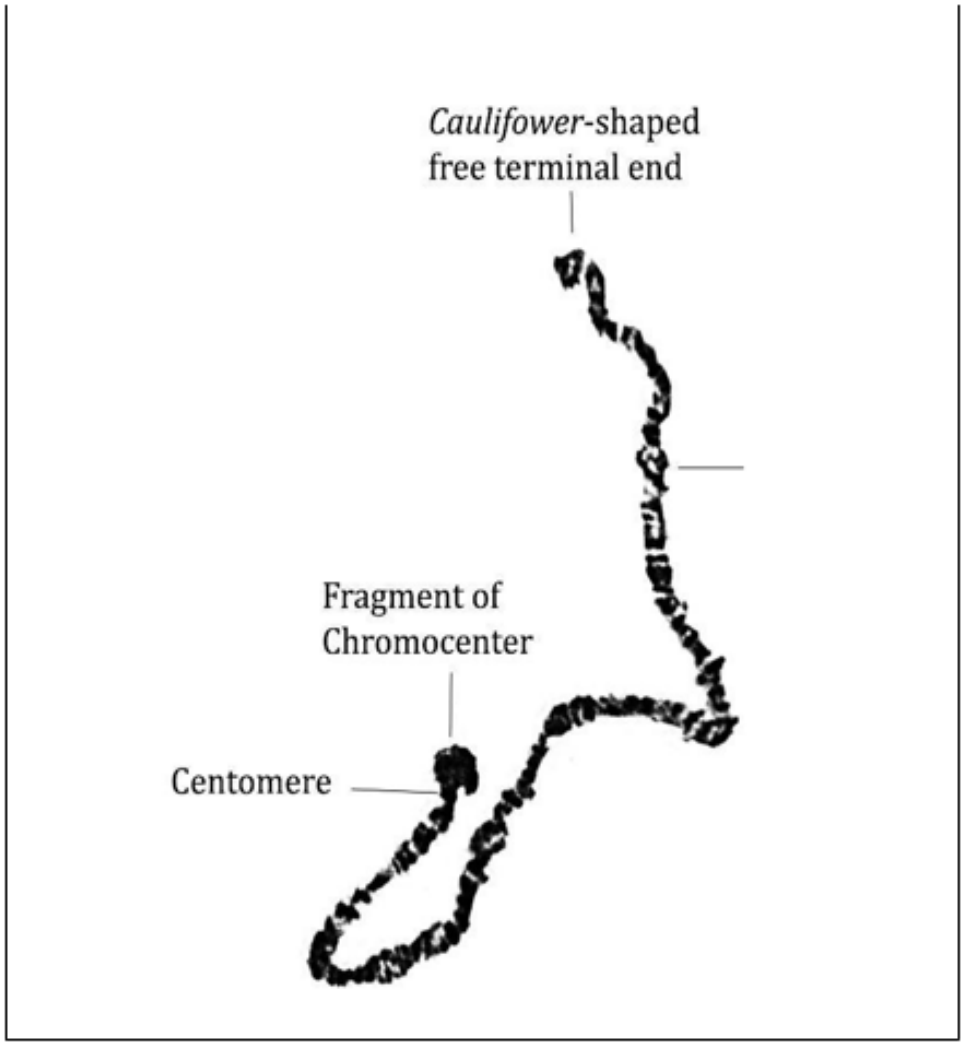

Fig. 5: Chromosome E

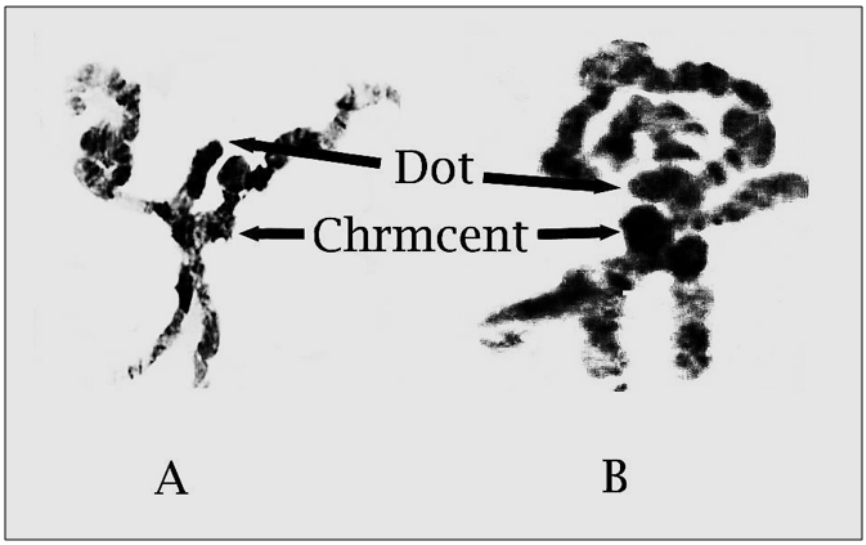

Figure 6: Dot chromosome protruding from the chromocenter

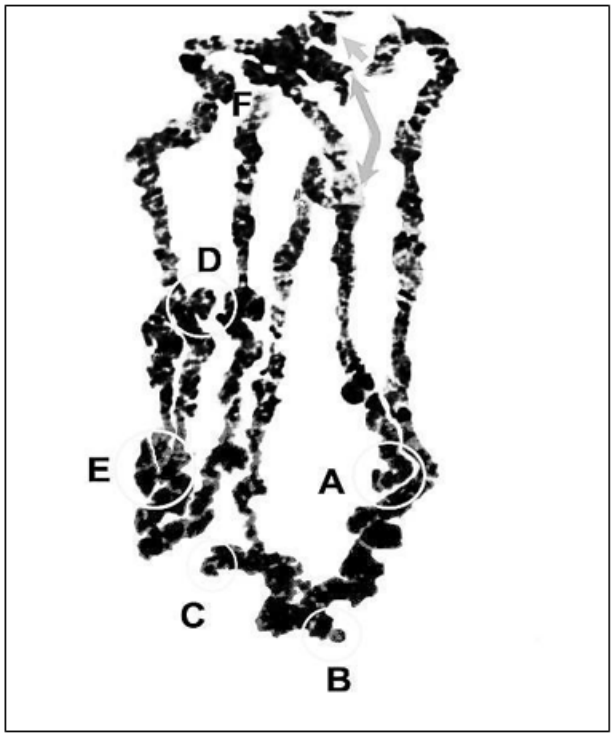

Figure 7: A completecomplement of the *Zaprionus vittiger* Coquillett salivary gland chromosomes. Grey arrows indicate likely breaks of the arms as the radiate from the chromocenter. White circles enclose the free terminal ends of the chromosome arms.

Discussion

The salivary gland chromosomes of *Z. vittiger* Coquillett observed in our preparations are polytene and consist of five chromosomes and one dot chromosomes joined at a chromocenter. The long acrocentric chromosomes twist and coil around itself and one another. Bands and puffs are evident on all the chromosomes except the dot chromosome whose bands are not readily visible. The dot chromosome is seen as a heterochromatic protrusion in those plates that contain the chromocenter. The chromosomes do not spread easily and are relatively fragile, fragmenting easily into multiple pieces. They also frequently undergo ectopic pairing both in the intercalary region and at the telomeres. The ectopic pairing may occur between bands located in close proximity or relatively far apart and even between chromosomes. The chromosomes frequently overstretch and become thin and the bands far apart. Small inversions were observed. Chromosome loops were also observed. Each polytene chromosome can be identified through the differences in banding pattern and puffs. The chromosomes frequently break off from the chromocenter but can still be identified by the pattern at the tip of each chromosome which is unique to the chromosome.

Though not many full lengths of the polytene elements were observed in the preparations, many free ends were seen: some from chromosomes broken in the middle, some broken off the chromocentre, and others free ends. The possible free terminal ends were observed in various plates as free and away from the chromocenter. One free telomere tip observed in the preparations is shaped like a fan. The chromosomes with the fan shaped terminal end is designated chromosome A. chromosome A also has a region located on the first quarter from the *Fan,* with frequent twist, loop or ectopic pairing and a constriction on the distal part of the chromosome before the chromocenter.

Another type of terminal end observed is shaped like a bulb. The tips of this terminal end possesses a bulb or jar appearance, with a base and a narrow neck wearing a large dark 'collar' which is actually the first in a series of three bands around the neck of the bulb . The chromosome named chromosome B in this study have a characteristic puff after the three bands described above. Though not many full lengths of the polytene elements were observed in the preparations, many free ends were seen: some from chromosomes broken in the middle, some broken off the chromocenter, and others free ends. The possible terminal ends were observed in various plates as free and away from the chromocenter. One free telomere tip observed in the preparations is shaped like a fan. The chromosomes with the fan shaped terminal end is designated chromosome A. chromosome A also has a region located on the first quarter from the *Fan,* with frequent twist, loop or ectopic pairing and a constriction on the distal part of the chromosome before the chromocenter.

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Chromosome C has a free end shaped like a snakehead or arrowhead a somewhat triangular shape with a constriction on the base, from which the chromosome continues. The base is actually on a band making it look like the 'corona' around the neck. Other distinguishing features on the chromosomes C include an interband region somewhere around the first quarter from the terminal end of the chromosome that appears to be plugged with wool. A region with a deep constriction, which nearly segments the chromosomes, with swellings on both sides of the constriction is also found along the length of this chromosome. The constriction and swellings confers on the chromosome the shape of an 'hourglass', the word with which it is described in the plates The hourglass appears in the last quarter of the chromosome as it approaches the centromere.

Chromosome D had a unique fluffy crescent shaped free terminal end. The free end has a region of banding patterns that looks like a 'sandwich' (a region of three bands, the middle band just barely visible, giving the middle portion the appearance of a sandwich stuffing) just after the fluffy tip.. The base of the crescent is constricted. This free terminal end has been called chefs cap because it bears semblance a cook's headwear. In plate 16A, the 'chefs' cap is lost but the *sandwich* is evident. Also evident is a region of two lighter bands, a dark middle constricting band and another darker band all enclosed in constrictions on both sides. On the whole, the region has the appearance of a bow tie.

The fifth chromosome, chromosome E, has unique free terminal end probably shaped like a Cauliflower, like a half-moon and slightly corrugated. A puff may be present about six bands after the *cauliflower*, but just about the end of the first quarter from the free end, constant puff activity is present, Various broken ends were observed.

The tightly twisted and coiled polytene chromosomes were fragile and broke easily. In most preparations observed, more often than not the chromocenter was broken, leaving the chromosomes to attach in incomplete numbers to the broken chromocenter the fragility of *Zaprionus indianus* chromosomes is due to low polyteny [11].

The Karyotype of *Z. vittiger* Coquillett has a complement of five polytene chromosomes and a dot chromosome, a karyotype observed in all species of *Zaprionus* and some *drosophillids*[11] [9] [15]. The acrocentric chromosomes are joined at the centromere to form the chromocenter. The dot chromosome is not readily observed in most chromocenters either because the chromocenters are broken along with it or in cases where they were intact; the chromosome was entangled in it. Though often entangled, it was still observed as heterochromatic dark protrusion from the chromocenter in some preparations.

The terminal ends can be differentiated from the centromeres which are usually relatively more rounded or just slightly pointed when broken off the chromocenter. The terminal free ends in contrast exhibit various shapes and textures. This appears to be a general centromere and terminal end structural appearance respectively in *Zaprionus* species as it can also be seen in the maps of *inermis, tuberculatus* and *indianus* [9] [4]. In keeping with some works on *Zaprionus* chromosomes [9] [14]descriptive terms are used to describe the terminal ends and other prominent structures. Some of the terms are borrowed from available literature if they describe similar structures, the rest of the descriptions used are objects in popular use. The use of descriptive words is to facilitate easy understanding and visualization of the shape (and in a few instances, the texture) of structures and regions of the chromosomes.

The free terminal ends of the chromosomes were analyzed and used to identify each chromosome along with a few other noticeable landmarks. The landmarks were mainly puffs of certain characteristic or a set of noticeable and recognizable band patterns. Regions with any constant activity like twisting and coiling also served as points of reference.

Chromosome A has a tip shaped like a hand fan. In some cases, the *fan* appeared totally 'open', in others, the angle of spread appeared to be less but generally the angle is midway between obtuse and acute. The arc is composed of two bands with a narrow

interband region, and the inner band is a bit wider than the outer one. However, in some chromosomes, the interbands region was not observed, causing the arc of the *fan* to appear as having a large band. The chromosomes also had other distinguishing features.

In the first quarter of the chromosomes from the free end, a region of constant looping and twisting is observed. The looping activity may be progressive: mild or obvious and protruding. The chromosome does not fragment as much as other members of the complement. An observation of the full length chromosome displays a constriction just before the centromere. The karyotypes of *inermis* and *tuberculatus* also both presented chromosomes with Fan and Chinese fan tips respectively and the Chromosome 4 of *Z. inermis* which possess this tip also has the 'S' constriction observed in this study in subregion 80C just before the centromere [14][9].

The *bulb* of chromosome B is the most important feature of this chromosome. The puffing activity present after the three bands from the tip of the free end is the first of regular puffing activity observed along the length of the chromosome. Full lengths of chromosome B are often difficult to obtain because it frequently twists and coils, and pairs with other chromosomes.

A 'snakehead' or 'arrowhead' tip of Chromosome C sometimes may not be clearly triangular but somewhat more round. However, the base and constriction on the "neck" was still distinguishable. The chromosome is relatively longer and sections of it can always be identified in chromosome coils through its other distinguishing features. The wool plug is not just like any other interband region, but is whitish and narrower on one side. This causes a slight kink on this spot on the chromosome. A few bands after this (five to six bands) is a spot, the *platform,* where no band is distinct (though it should actually have spanned three to four bands), but rather may be undergoing an undefined chromatin activity. The constriction on the *hourglass* should actually be expected to be a site of breakage; but surprisingly the chromosome is often seen full length with the *hourglass* intact.

The tip did not however resemble any of the tips of other *Zaprionus* spp. Chromosomes when compared with the maps of *tuberculatus*[14] and *inermis*[9]. The morphological characteristics described as 'bloody snake head', and 'snakehead' in the *inermis* map are somewhat different in both shape and location from this tip.

The sandwich of chromosome D free terminal end is usually easily recognized though the 'Chef cap' tip may be lost, possibly due to the narrow region that exists where the chromosome continues from it. The *bowtie*, often located in the middle region, provides an additional feature to distinguish this chromosome.

The free terminal end of Chromosome E has a somewhat *cauliflower* shape. It frequently breaks and attaches itself to other chromosomes. The region leading to the free terminal end showed indistinct narrow dark bands interspersed with large light bands in our preparations However a diagonal band across one of such light bands about one-fifth of the chromosome length from the free end appeared to be associated with the *cauliflower* free end.

Conclusion

A high number of ectopic pairings were observed in the study. These are random pairings between non-homologous regions of the chromosomes (usually the regions are heterochromatic) [10]. The abundant ectopic pairings may actually be the reason for the fragile nature of *Zaprionus vittiger* chromosomes, since the chromosomes are likely to break at these points of pairing during preparations [19] have described these regions of intercalary heterochromatin as 'weakpoints' due to underreplication of DNA at these regions. Chromosome A may likely be the sex chromosome because it stains lightly due to is unpaired status in males, a feature also observed by [20]on *Zaprionus inermis* chromosome.

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