

Karyo-Morpho-Evolutionary Studies of *Zonocerus variegatus* L., in South Eastern Nigeria

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Abstract: Currently, divergent views exist, among researchers working on the cytology, ecology, phenology and other aspects of *Zonocerus variegatus* L. This necessitates further studies aimed at reconciling these views. This present study was aimed at reconciling the diverse views on the cytology and karyotype of the ♂ and ♀ insects, using direct data from somatic chromosomes, supported by meiotic chromosome behaviour; and to relate the data obtained to their morphometric parameters, with a view to understanding their evolutionary status; exhibited sexual size dimorphism; with notes on their probable eco-phago-evolution. Two hundred ♂ male and 200 ♀ female insects of uniform age, first instar nymphs, were raised in 8 cages, each containing 25 ♂ s + 25 ♀ s. At maturity (able to copulate) 360 mature insects were sacrificed and used for the morphometric studies. Others were maintained for chromosome and karyotypic studies, adopting standard techniques. The results of the morphometric studies confirmed that the insect exhibited sex size dimorphism, with the ♀ being significantly larger in size than the ♂. The cytological studies confirmed that the ♂ had 19 acrocentric chromosomes, the ♀ 20 acrocentrics; with XO ♂ and XX ♀ sex determination mechanism. The karyotype indicated that the insect is advanced on the evolutionary ladder and this might be responsible for its ability to accumulate highly toxic substances, as a major survival factor and also develop the variegated coloration (for camouflage). Consequently, it lost its ability to fly, as a means of escaping from predators, as its relatives do.

Key words: Karyo-Morpho-Evolution • *Zonocerus variegatus* • Acrocentric Chromosomes • Sex Size • Eco-Phago-Evolution

INTRODUCTION

Zonocerus variegatus L. belongs to the Order Orthoptera; subfamily Pyromorphidae. It is found throughout West Africa to western Chad and the western border of the Central African Republic. It also occurs in a band from northeast Angola to central Kenya and Ethiopia (south of Eritrea) [1] (Fig. 1). It is common in Southern Nigeria and is known to be a polyphagous pest of food and cash crops [2-5]. The species is reported as having one population (the wet season) in the Northern part of it ranges [6, 7] but two populations (the wet and dry season) in the Southern part [8, 9]. Despite the fact that a lot of work has been done on the insect, especially on its ecology [9, 10], biology [7, 11, 12], economic

importance [13, 14] and phenology [15], not much has been done on its genetics [16] and several controversies still persist in many aspects of the insect studied, necessitating continued research work on this curious insect.

An overview of chromosome data from a cytogenetic viewpoint revealed that the Pyrgomorphidae have been studied sporadically [17-19]. According to Mestre and Chiffaud [20]; of the over 79 described species, less than 20% have been examined cytogenetically and the African species are therefore the most neglected. Apart from the meager research input into the African species, conflicting reports exist as to how many populations of the insect that exist in Southern Nigeria, as well as to the true classes of chromosomes found in *Z. variegatus*. Anya [9]

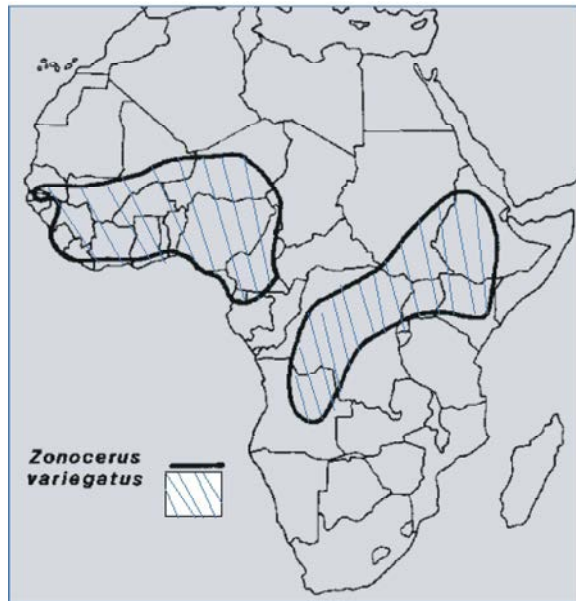


Fig. 1: *Z. variegatus* L., distribution in Africa [Adapted from Locust Handbook, NRI, Steedman (Editor)]

submitted that the dry and wet season populations, in South Eastern Nigeria do not mate with each other even when both are present in the same habitat. He stated that both populations were reproductively isolated and constitutes two different Mendelian populations. On the other hand, some authors [12, 21, 22] showed that the so-called wet and dry season Mendelian populations interbreed and constitute the same population. Writing on the cytology of the insect, some scientists [17, 23, 24] stated that the chromosomes of the male insect are interesting, being large and acrocentric, with only nine bivalents and an X-chromosome, but other [16] opined that all the chromosomes of the species are metacentric types.

In the words of Seino, Manjeli and Dongmo [25], the lack of karyotype information has hampered cytotaxonomic, phylogenetic and evolution studies on the African species of the family Pyrgomorphidae. If the species will play its envisaged principal role for teaching and research in basic animal cytogenetics in the region, there is great need for consensus of opinion as to its true chromosomal morphology and karyotype.

Most of the cytological studies carried out on the species were concerned essentially with meiosis in the male insect, with little or no attention on the somatic chromosomes of both the male and female insects. Nwankiti [16] studied meiosis in the male *Zonocerus* using

the testis, for both the wet and dry season populations. Iheagwam and Ene-Obong [22] reported on the reproductive, chromosomal and morphometric relationships of the so-called dry and wet season Mendelian populations of the insect.

Karyotype analysis and ideogram clarifies the taxonomic relationships among species of a genus and helps in the examination of the pattern of chromosome variation in relation to the taxonomic position and the life cycle of the taxa [26]. Studies of the karyotype and chromosome morphologies of a number of insects have contributed immensely towards their better understanding to man's benefit. Das [27] studied the mitotic complements of more than 30 species of Indian gryllids which showed many interesting chromosomal behaviour. Grinchuk and Mikhailova [28] studied the karyotype variation of salt-water individuals of *Glyptotendipes barbipes* inhabiting various localities and suggested that they had a common origin.

Zonocerus is variegated, which is of ecological significance. It has well developed wings and yet it hops rather than flying. Its ability to secrete a greenish fluid with very offensive odour (another survival adaptive feature), all combine to make *Zonocerus* a curious insect for researchers. The essence of this present work is to study the somatic chromosome morphology and elucidate the karyotype of the male and female insects; with a view to resolving the controversy among cytologists, as to the class of chromosomes possessed by the insects. This will be related to their morphological characteristics and evolutionary status as well sexual size dimorphism; with notes on the probable eco-phago-evolutionary advancement of the species.

MATERIALS AND METHODS

Culturing the Insects: Three hundred first instar nymphs, made up of 200 ♂s and 200 ♀s, of the same age were collected from the Department of Crop Science, University of Nigeria, Nsukka cassava experimental farm, as the eggs laid during the rainy season of 2015, hatched to produce the dry season population [8, 9]. They were placed in eight insect cages, with each cage containing 25 ♂ and 25 ♀ insects. The cages were similar to the one described by Youdeowei [4], with slight modifications. The insects were fed with cassava shoots, stuck into 750 ml bottles that were three quarter filled with tap water. The cages were kept on the benches in an open laboratory, near the window, with good ventilation. The cassava shoots were changed every three days.

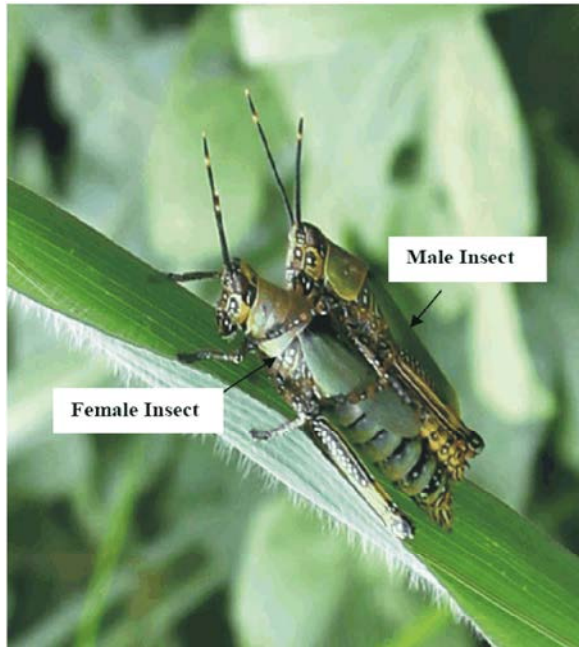


Fig. 2: Male and female *Zonocerus variegatus* (variegated grasshopper) about to copulate. Mag. x 1

Morphometric Measurements: With the first sign of the insects preparing to copulate (Fig. 2), 360 insects (180 ♂s and 180 ♀s) were sacrificed by placing them in a cellophane bag into which cotton wool saturated with chloroform was introduced. The insects died shortly after the application. They were used for the collection of the following morphometric data: Abdominal length, Outer wing length, Inner wing length, Length of thorax, Length of pronotum, Length of head, Length of hind leg, Body weight, Length of antenna, Number of body segments, Width of thorax and Width of head. The morphometric data were subjected to a t-test, to compare the features in the males with the females.

Cytological Studies: The remaining 20 ♂s and 20 ♀s left for cytological studies were randomly sampled intermittently from the cages and sacrificed like the ones used for morphometric studies. Dissecting the insects to collect the testis and hepatic caeca followed the protocol of Youdeowei [4]. This entailed mounting the insect, with its front view, facing the dissecting pan. All the wings were cut off before inserting a micro-scissors to cut open the insect dorsoventrally. Pouring water to submerge the cut open insect enables both the testes and the hepatic caeca to float for easy collection. The collected parts were

introduced into colchicine and kept in the refrigerator for 3 h at 8°C before transfer to Carnoy fluid for fixation. They were later transferred to 70% alcohol. Samples were taken from the stored specimen for slide preparation and study, following standard slide preparations techniques for meiotic and mitotic studies [29]. For meiotic studies, 2-3 testis follicles were mildly squashed on a clean slide with two drops of aceto-carmine stain. The squashed material was covered with a cover slip and allowed to stay for 3 minutes. The slide was placed in between a folded filter paper held with the left and on the lab bench while gentle pressure mounted on the squashed material with the right thumb to separate the cells. The cover slip was sealed unto the slide with a nail varnish and the preparation was viewed under the x 400 magnification for chromosome study. The preparation process for the mitotic studies was identical except that aceto-orcein stain was used and squashing was done with the hepatic caeca. Photomicrographs were taken of good mitotic and meiotic plates using Amscope Trinocular microscope SKU: T390B-3M fitted with 5MP Digital Camera, connected to an IBM laptop.

Karyotype Studies: Both arms of the somatic chromosomes were measured with a flexible tape after they were cut out from enlarged photomicrographs taken at x 400 magnification of the microscope but which were further enlarged four times during printing. Measurements were taken from ten cells of each sex and the homologues were easily identified from the cut photomicrographs. The pairs were arranged in ascending order of total length of chromosomes. In addition, homologues from clear meiosis I diplotene stages were identified and the bivalents were cut out from printed photographs to show the true position of the centromeres at such early stages. Measurement of the short arms (SA) as well as the long arms (LA) were made from the printed mitotic metaphase stages and these were used to compute the ratios of the SA to the LA as well as the Total Form percent (TF%) using the formula below;

$$TF\% = \frac{\text{Total sum of short arm lengths}}{\text{Total sum of chromosome lengths}} \times 100$$

following the methods of Huziwar and Costa and Forni-Martins Gunjan and Roy [30, 31, 32]. Arm ratio comparisons (SA/LA) was computed adopting the methods of Greilhuber, Speta and Greilhuber, Speta [33, 34] and was presented in graphical form using Microsoft Excel software.

Table 1: Comparison of Morphometric Characters in Males and Females of *Zonocerus variegatus* L.

Characters	Males	Females	t-stat	Probability
Abdominal Length	2.03±0.04	2.53±0.08	5.52	0.001***
Outer Wing Length	1.96±0.05	2.06±0.04	1.79	0.040 *
Inner Wing Length	1.74±0.05	1.74±0.03	-0.11	0.460 NS
Length of thorax	0.93±0.05	0.99±0.02	1.09	0.140 NS
Length of Pronotum	0.77±0.01	0.86±0.01	4.98	0.001***
Length of Head	0.78±0.01	1.13±0.31	1.16	0.130 NS
Length of Hind leg	3.93±0.05	4.11±0.04	2.88	0.001***
Body Weight	0.69±0.03	1.11±0.06	6.29	0.001***
Length of Antenna	1.59±0.04	1.50±0.02	-2.12	0.020 *
Number of body segments	11.00±0.00	11.00±0.00	65535.0	NS
Width of thorax	0.66±0.01	0.78±0.01	7.50	0.001***
Width of Head	0.27±0.01	0.31±0.01	5.55	0.001***

RESULTS AND DISCUSSION

Morphometric Studies: The results showed that *Zonocerus* culture could be easily maintained once adequate food is provided and the culture environment well ventilated with adequate natural lighting during the day. The mortality rate was less than 1%. This is not surprising since the ecological distribution of the insect spans vast areas of the tropical regions of Africa especially West Africa (Fig. 1). With the inability of the insect to fly most of the time sedentary life in the cage was not a serious handicap to the survival of the insects. Iheagwam [12, 21, 22] had shown that the insects could easily breed in captivity. This makes the insect an invaluable tool for diverse biological studies.

With the exception of the male having a significantly longer antenna than the female and the body segments which were the same in both sexes, the female showed greater size in all the other morphometric characters studied (Table 1). Out of 12 characters measured, the female insect showed higher values in 9 characters. Of special interest were abdominal length, length of hind leg, length of pronotum, body weight, width of thorax and width of head; which showed very highly significant differences between the male and the female. The mean body weight of 0.69±0.03 g for the male insect and 1.11±0.06 g for the female reported in this study is in agreement with the range of body weights of 0.63 -0.87 g for the male and 1.11 – 1.40 g for the female, reported by Bamidele and Muse [35], from six locations in Southern Nigeria. This has shown consistency in the possession of larger body size by the females; a quality that may not be unconnected with their egg laying function for the continuity of the species.

The issue of sexual size dimorphism (SSD) has been a subject of great discourse not only in insects but for a large number of animals. Evolution of larger body size by

the female *Z. variegatus* has been attributed to the need for them to build up concentrations of metabolite substances preparatory to reproduction to enable them cope with their non-eating habit and the stress of carrying their partner for hours during copulation [36]. According to Kpindou *et al.* [37], *Zonocerus variegatus* L. females can oviposit up to five times and the interval between ovipositions varies from 14 to 32 days. They have an egg diapause and egg hatching is not synchronised. These observations, explain the almost perennial presence of the variegated grasshopper in southern Benin [37].

The oviposition process, which includes digging the soil, generally decreases the concentrations of tissue metabolites and weight is reduced [38]. On the other hand, during mating, the male releases a spermatophore, which contains nutrients and this contributes to the male's exhaustion. Mating has been described as an energy sapping exercise that affects the weight and fat-body nutrients of adult *Melontha melontha* (L.) [39]. Since grasshoppers are known to be polygamous, extraordinary female-male guarding periods suggest that long-duration guarding could be a phylogenetically inherited trait in the Pyrgomorphidae [40]. Wickler and Seibt [41] reported that *Z. elegans* males could remain mounted on the back of the females for up to 45 d. This is an enormous task. These considerations could possibly be part of the reasons for the smaller body size of the males not only for *Zonocerus variegatus* but also for many members of Pyrgomorphidae [17, 35, 40].

Cytological Studies: In the present chromosome investigation of the male and female *Z. variegatus*, the counts and the karyotype analysis confirmed the chromosome number of the male to be $2n=19$ (18A + XO) and that of the female to be $2n=20$ (18A+ XX). All the chromosomes were found to be acrocentric in nature and the chromosome formula for the male is depicted to be

Table 2: Genome characteristics for male and female *Zonocerus variegatus*

	♂ Genome Characteristics	♀ Genome Characteristics
Diploid Number	2n=19 (18A + XO)	2n = 20 (18A+ XX)
Chromosome formula	0 TC +19AC + 0SMC + 0 MC	0 TC + 20AC +0SMC + 0MC
Genome Length	80.10 μ	86.64 μ
TF%	0.118	0.109

A= Autosome, X= Sex chromosome, O = Absence of sex chromosome, TC= Telocentric chromosome, AC=Acrocentric chromosome, SMC= Sub Metacentric chromosome, MC= Metacentric chromosome. TF% = Total Form Percent.

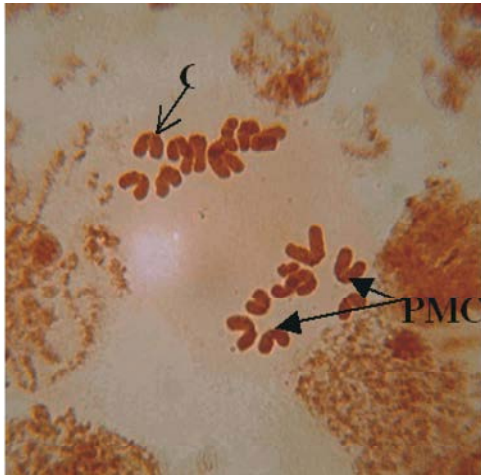


Fig. 3: Anaphase I of Male *Zonocerus variegatus* L. with clearly separated chromatids producing pseudo metacentrics (PMC).Mag. x 1200



Fig. 5: Oogonial Mitosis of Female *Z. variegatus* showing true acrocentric (TA) chromosomes with sister chromatids visible only on the very long arms (SCOLA) of the chromosomes Mag. x 1200

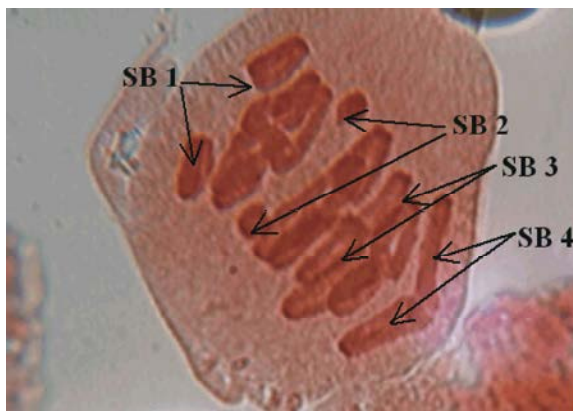


Fig. 4: Early Anaphase I of ♂ *Z. variegatus* L. showing mild separation of chromatids (true acrocentrics) as the bivalents separate (SB1- 4) to the opposite poles Mag. x 1200

0 TC +19 AC + 0 SMC + 0 MC, for the female it is 0 TC + 20 AC + 0 SMC + 0 MC. The genome length for the male was found to be 80.10 μ while for the female it was found to be 86.64 μ (Table 2). The chromosome number, type and sex determination mechanism were all found to be in agreement with earlier reports [17, 24, 42]. This however

differs from the report of Nwankiti [16], who asserted that all the chromosomes were metacentric in nature. Apparently this conclusion was arrived at, after looking at the chromosome configuration at meiotic anaphase 1 where the sister chromatids of the very long arms, repel each other. The centromeres and the very short arms are hardly visible at lower magnifications (Figs. 3 and 4); consequently the separating homologues assume a pseudo-metacentric configuration, which could be misleading.

Incidentally the true karyotype of an organism may not be fully worked out without studying both the mitotic and meiotic chromosome behavior. Fig. 5 shows the mitotic chromosomes of the female insect, with the sister chromatids of the long arms slightly visible while the centromeres are obscured. At meiotic metaphase II (Fig. 6), some of the centromeres attached to the very short arms could be seen. Convincing evidences that the chromosomes are truly acrocentric are shown by the mitotic chromosome plates (Figs. 7 and 8). The centromeres are visible, appearing as if they are the end of the chromosomes. This is in agreement with the

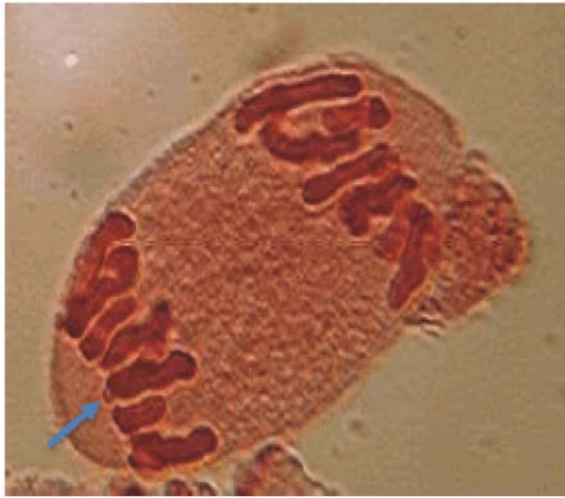


Fig. 6: Metaphase 11 of ♂ *Z. variegatus* L. showing I- shaped chromosomes lined up at the equator. Blue arrow shows a very distinct short arm and the position of the centromere on one chromosome. Mag. x 1200

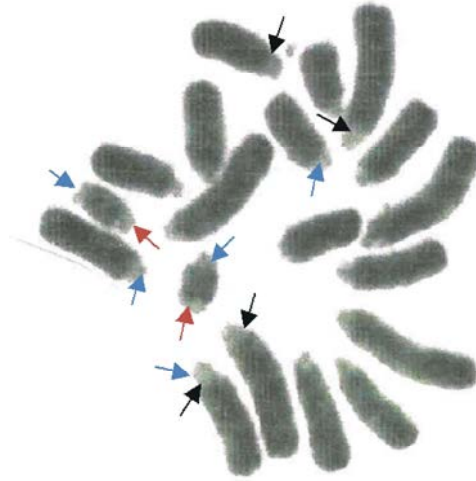


Fig. 7: Mitotic metaphase cell of *Z. variegatus* L. ♂ showing chromosome number ($2n=19$) and morphology. Red arrow shows satellite chromosomes. Black arrows show position of centromere. Blue arrows show the short arms. Mag. x 1400

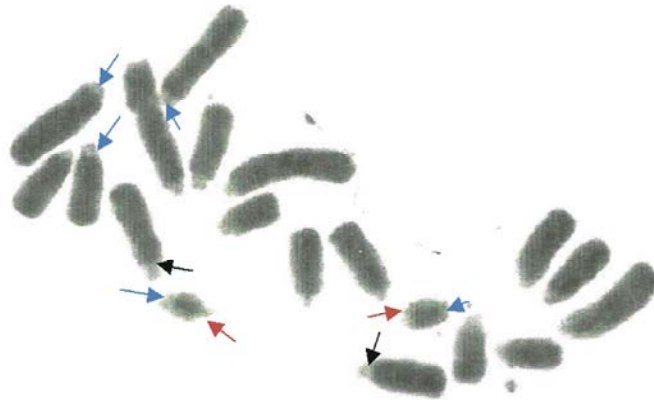


Fig. 8: Mitotic metaphase cell of *Z. variegatus* L. ♀ showing chromosome number ($2n=20$) and morphology. Red arrow shows satellite chromosomes. Black arrows point to the position of centromere. Blue arrows show the short arms. Mag. x 1400

reports of Seino *et al.* and Faluyi and Olorode and Hewitt [17, 24, 42]. that all the chromosomes of *Z. variegatus* are acrocentric. Furthermore, looking at the meiotic I (Figs. 9 a and b), the position of the centromere on the very long chromosomes that are still condensing and coiling show they are acrocentric in nature. This is in line with reports of chromosome morphologies among the Pyrgomorphidae from other parts of the world [43, 44, 45].

Karyotype Studies: Based on the theories of Levitsky and Stebbins [46, 47] which are supported by evidences by Liang and Chen [48] that chromosomes do indeed evolve

in a direction from symmetry to asymmetry as a new evolutionary feature; *Z. variegatus* L. is viewed as advanced on the evolutionary ladder. The phenotypic manifestation of these advanced features include the ability of the species to feed on plants containing toxic substances to acquire defensive chemicals that repel, if not kill predators; keep its variegated quality which offers it natural camouflage against predators in its environment. Being adequately protected, the species does not spend energy flying around to escape from predators, hence the species conserves its hopping habit, despite the fact that it has well developed wings.

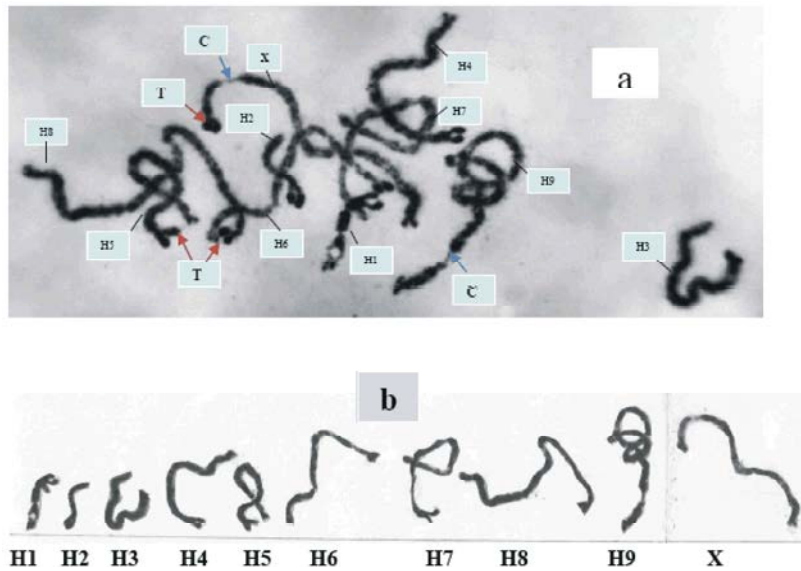


Fig. 9a: Diplotene stage of Meiotic Prophase I of the ♂ *Zonocerus variegatus* L. showing 9 Bivalents and the X sex chromosome. The prominent centromeres (C) on two bivalents are shown with blue arrows indicate the telocentric nature of the chromosomes. The prominent Telomeres (T) on the short arms of some chromosomes are shown in red arrows. Mag. x 1600

Fig. 9b: Meiotic Karyotype of Diplotene of Prophase I of the ♂ *Zonocerus variegatus* L. showing 9 Homologues and the X Sex Chromosome. Mag. x 1400

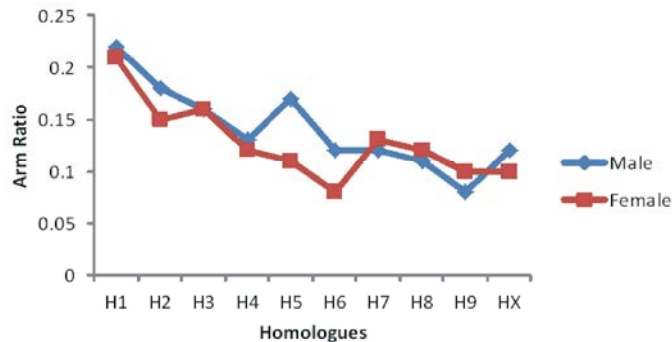


Fig. 10: Arm Ratio Comparison of Male and Female *Z. variegatus* Homologous Chromosomes.

Based on the system of chromosome classification and nomenclature described by Abraham and Prasad [49], the range of arm ratios for both the male and female insects which was between 0.08 -0.22 signifies that all the chromosomes were all nearly sub-terminal (Fig. 10). The close identity of the chromosomes as seen from the metaphase plates and karyotypes (Figs. 7, 8, 11) and the arm ratios comparison (Fig. 10) is as expected. The difference in genome length between the two sexes ($\geq 80.10\mu$, + = 86.64μ) may be mostly due to genetic factors without ruling out environmental influences. The longer genome length of the female could involve possession of more genes responsible for larger size of the females as can be seen from the forms of the two

sexes (Fig. 2) and the measured morphometric characters (Table 1). Also the shifts in centromeric positions as seen on homologues 2, 5 and 6 may also contribute to the observed differences in sizes.

In the same line, Kiauta [50] working on the dragonfly chromosome also reported variation in size with considerations to its significance in the taxonomy of the order Odonata. He found that the relative size of metaphase chromosomes varied in dragonfly; and this difference in chromosome size may be used for the classification of certain intra-specific forms. He noted intraspecific variation in chromosome size with the female being longer in size ($6.03 - 12.84\mu$) than the male ($5.7-8.6\mu$).

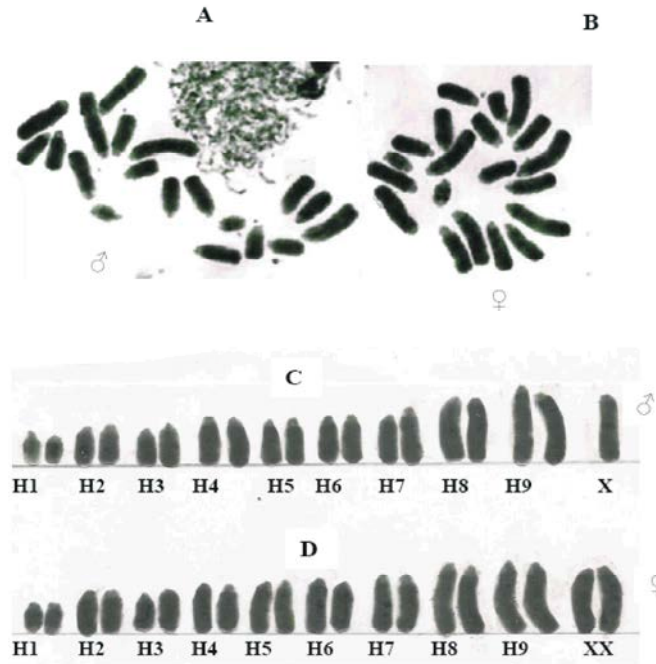


Fig. 11: A. Mitotic Metaphase Plate of male ♂ *Zonocerus variegatus* L. Mag. x 1200
 B. Mitotic Metaphase Plate of female ♀ *Zonocerus variegatus* L. Mag. x 1200
 C. Ideogram of chromosome complement of male ♂ *Z. variegatus* L. Mag. x 1200
 D. Ideogram of chromosome complement of female ♀ *Z. variegatus* L. Mag. x 1200

Also the work done by Grinchuk and Mikhailova [28] on the comparative karyological analysis of species of *Glyptotondipes baripes*, which revealed both similarities and variations in their karyotypes; which they attributed to the differences to intraspecies divergence, is in agreement with this report. Yadav [51] also found differences in size variation in the chromosomes of *Crocothemis erythraea* at metaphase in both males and females in his karyotype variability study of the species *Crocothemis erythraea*.

According to Stebbins [47] in nearly all animals, the karyotypes of the two sexes differ from each other with respect to both the, degree of heterochromatination and the gross morphology of a particular pair of the chromosomes. Morphological differences in the relative size of the female (larger) to the male (smaller) were also observed. This is in accordance with a similar study done by Singh and Gupta [52] on some Indian species of Drosophilidae and they recorded differences with respect to the morphology of both sexes.

ECO-Phago-evolutionary Considerations: Current observations in the field indicate that despite the fact that this insect does not fly, it is demonstrating greater and better survival abilities. This ranges from longer stay in

the field, with a tendency for an overlap between the rainy season and dry season populations, in the Southern Eastern part of the country, to concentration on feeding on plants like *Manihot esculenta* (cassava) known for its high cynide content, Croton, *Vernonia amygdalina*, apart from other well-known plants like *Chromolaena odorata*, known for their content of toxic substances. Compounds extracted from Croton plants are known to be effective molluscicides [53], powerful piscicides [54]. Oils extracted from *Vernonia amygdalina* are known to have very strong insect repellent properties [55]. Boppre, Boppre and Billeret *al.* and Timbilla and Lawson [56, 57, 58]; have written extensively about the pharmacophagus relationship in which *Z. variegatus* L. consumes the flowers of *C. odorata* as a drug for pyrrolizidine alkaloids (PAs) used for defense against antagonists.

Kubitza *et al.* [59] reported that N-Oxygenation of pyrrolizidine alkaloids (PAs) and the accumulation of PA N-oxides within the haemolymph of *Zonocerus variegatus* L. result in two evolutionary advantages for the insect: (i) they circumvent the defence mechanism of their food plants and (ii) they can use PA N-oxides to protect themselves against predators, which cannot cope with the toxic PAs.

It is suspected that with this feeding tendency, the insect could over time accumulate more concentrated clastogenic compounds for protection against predators. This however could have effect on its chromosomes. Chromosomes are known to be prone to damage by clastogenic compounds. More detailed studies of the chromosomes of the insect, especially at the molecular level, are needed to investigate a possible tendency of evolution from monocentric to holocentric chromosomes which are known to be better adapted to clastogenic environments. According to Zedek and Bureš [60], because chemicals, including clastogens, are part of plant defence against pests, holocentric herbivorous insects could display a broader host range and/or feed more often on plants displaying higher concentrations of clastogenic compound. Several studies provide clues suggesting that the possession of holocentric chromosomes may indeed be advantageous in clastogenic environments. Holocentric chromosomes should therefore provide a selective advantage by directly protecting DNA in times of exposure to agents causing chromosomal fragmentation, i.e. clastogens [60].

On the other hand, even if accumulation of clastogenic compounds result to chromosomal fragmentation, [60] had observed that due to their extended kinetochore and the attachment of spindle microtubules along their entire length, holocentric chromosomes tolerate fragmentation; hence, they may be advantageous in times of exposure to factors that cause chromosomal fragmentation (clastogens). It is stated that holocentric organisms may, indeed, thrive better than monocentric organisms under clastogenic conditions and that such conditions of various duration and intensity have occurred many times throughout the history of Earth's biota. This accumulation of clastogens and yet to be determined toxic substances has additional significance in the role *Zonocerus variegatus* may be playing in the global food chain.

Despite the fact that Ladeji *et al.* and Solomon *et al.* and Omotoso and Adesola [61, 62, 63] had reported about the high nutritional quality of *Zonocerus variegatus*, with the suggestion that it could be used in dietary supplementation in developing countries like Nigeria as well as included in animal feed formulation; caution must be exercised in the consumption of this insect, which (to the authors best of knowledge), appear to be currently consumed only by humans. There may be yet to be unraveled reasons for its avoidance by other creatures in the food chain. Not long ago, Ehigie, Okonji and Ehigie [64] reported the presence of Thiaminase, a toxic enzyme,

in the gut of *Zonocerus variegatus* and cautioned that in areas where *Zonocerus* is consumed, it should be prepared using extensive and prolong cooking to avoid suffering from thiamine deficiency. Despite the extensive publications that currently exist on this grasshopper, so much is yet to be learnt, hence the need for more collaborative research on the insect to unravel its great scientific potentialities.

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