Chromosome Morphology and Meiosis in Zonocerus Variegatus L. (Orthoptera, Pyrgomorphidae)

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The grasshopper, Zonocerus variegatus of the family Pyrgomorphidae has a male diploid chromosome number of 19, with an XO constitution (Oyidi 1968, Lasebikan and Olorode 1972). Although these investigators described the chromosomes as acrocentric, Nwankiti (1983b) described them as metacentric. The meiotic aberrations detected by Lasebikan and Olorode (1972) and Olorode and Akingbohungbe (1975) were not reported by Nwankiti, (1983a, b). Since the chromosomes of Z. variegatus are relatively large and not too many, it is not expected that there would be any disagreement on the morphology of the chromosomes. It should also be possible to detect consistent meiotic aberrations. This paper re-examines these issues.

Materials and methods

The fifty grasshoppers used for this study were adult males collected between January and May on the University of Lagos campus. They were killed with chloroform either on the day they were captured or on the next day. The grasshoppers were then dissected in 0.67% NaCl solution.

Five fresh testicular follicles were placed on each glass slide, cut into three or four pieces and covered with a drop of 2% lactic-propionic orcein stain. After about 15 min, a cover glass was placed on the material. The cover glass was held in place while it was tapped gently with a dissecting needle, to disperse the cells and force out the excess stain. The preparation was squashed further between folds of bibulous paper to absorb the excess stain. The edges of the cover glass were sealed with nail varnish. Photomicrographs were made under the 100x oil immersion objective of a Wild M20 microscope with MPS 55 photoautomat attachment.

Results and discussion

Chromosome Morphology:

In spite of the fact that the grasshoppers were not treated with colchicine the individual chromosomes could be identified and counted in a polar view of the mitotic metaphase (Fig. 1a). The chromosomes appeared to be in C-metaphase because there was no relational coiling between sister chromatids. This appearance and the fact that what should be the short arms of the chromosomes were not clearly visible as paired structures (chromatids) could lead to misinterpretation, as by Nwankiti (1983b), that a pair of sister chromatids are instead the two arms of a folded metacentric chromosome. Close examination however, shows that there is a constriction separating the long arm from the short arm which also has some of the lampbrush appearance of the long arm. In addition, in some cases, the two chromatids of the short arm can be detected (Fig. 1a). The interpretation of folded metacentric chromosomes would be plausible only if the figure which Nwankiti (1983b) called mitotic telophase were so, but that is in fact a polar view of metaphase as in the present case. Furthermore, the mitotic anaphase/
Fig. 1. (x1,200). a. Mitotic metaphase in the testes (polar view). Sister chromatids are not closely associated, similar to C-metaphase. Primary constrictions (centromeres) are almost terminal (arrows). s = chromatids of the short arm. b. Pachytene: The heteropycnotic X-chromosome (x) is double-stranded but the unpaired terminal portions of the bivalents are 4-stranded (arrows). c. Diplotene: The heteropycnotic X-chromosome is double-stranded. In all bivalents only a maximum of 4 strands (2 pairs of sister chromatids) can be detected at the chiasmata (arrows). Terminalization in some bivalents (r) would result in some apparently ring-shaped bivalents in metaphase-I. d. Metaphase-I (Polar view): The negatively heteropycnotic X-chromosome is 2-stranded. Each bivalent is 4-stranded at the chiasmata. r = "ring"-shaped bivalents. arrows = chiasmata. e. Metaphase-I (side view) = Centromeres (short arrows) with the two attached chromatids in the bivalents are oriented toward opposite poles and metaphase stretch has changed apparently ring-shaped bivalents into a-shape (r). Long arrow = the short arms of the X-chromosome.

telophase and anaphase-/telophase-II stages differ normally only with regard to the number of chromosomes and not the number of strands per chromosome, because each stage is preceded by division of the centromeres. The chromosomes in anaphase-II in Fig. 2e are clearly single-stranded rod-shaped structures which are not folded, confirming that they are acrocentric.
Fig. 2. (×1,000). a and b. Anaphase-I: Double stranded V-shaped dyads indicate acrocentric chromosomes. Entanglement of surface material between homologues may cause elongation of some chromatids (Long arrow) or fibrous connections between chromatids (short arrows). c. Anaphase-I (polar view): The dyads are acrocentric. The tips of the shorter chromatids (arrows) are out of focus, not broken. d. Metaphase-II (polar view): Each dyad has only two arms and is acrocentric. Centromere (arrow). e. Anaphase-II: All the chromosomes are single-stranded.

The other meiotic stages also do not support the contention that *Z. variegatus* has metacentric chromosomes. In pachytene (Fig. 1b) the positively heteropycnotic X-chromosome and the bivalents appear double-stranded but the unpaired ends of some bivalents are evidently double-stranded, indicating that each bivalent contains four chromatids. Although centromeres are not visible in such portions, the repulsion between homologous chromosomes is probably an indication of their location.

In diplotene (Fig. 1c) the four-strandedness of the bivalents is evident in some parts, especially at the chiasmata but it is obscured in other parts by the lampbrush appearance of the
chromosomes. The positively heteropycnotic X-chromosome is visibly double-stranded and there is not indication of folding at the centromere. In both diplotene and diakinesis the shapes of some bivalents are indicative of the position of the centromere. In Fig. 1c the simplest and most plausible interpretation of the cross-shaped bivalents is that the chromosomes are either acrocentric or telocentric (John and Lewis 1975). These bivalents and the fact that chiasmata and the four strands are clearly visible in some bivalents confirm that the strands are the four chromatids expected, contrary to Nwankiti's (1983b) interpretation.

In metaphase-I the orientation of the bivalents at the equator of the spindle is normally syntelic, with the centromeres on opposite sides of the metaphase figure. Therefore, the shape of each bivalent at metaphase-I is determined by both the position of the centromere and the number of chiasmata (John and Lewis 1975). Since there is always repulsion between centromeres, there would be no ring-shaped bivalents if the chromosomes were telocentric. In the case of acrocentric chromosomes, ring-shaped bivalents would be very rare because chiasmata would be rare in the short arm. Although some apparently ring-shaped bivalents were observed in our slides, careful examination of polar views of such bivalents even at metaphase-I. (Fig. 1d) showed that in some only one of the two chiasmata was terminal. Such bivalents were found to be similar to some of the more easily interpreted α-shaped forms found in diplotene (Fig. 1c). Put differently, some of the α-shaped diplotene bivalents would appear ring-shaped in polar view of metaphase-I if the arms of the α-shape were very short and out of focus (Fig. 1d). The bifurcated dark-staining end of the otherwise negatively heteropycnotic X-chromosome probably corresponds to the two chromatids of the short arm of an acrocentric X-chromosome (Fig. 1e).

In anaphase-I all the chromosomes moving to each pole are V-shaped and each arm is single-stranded (Figs. 2a, b, c). This configuration is what is expected as a result of the repulsion between sister chromatids characteristic of anaphase-I. Thus metacentric chromosomes would appear as four armed "V-shaped" structures while acrocentric and telocentric chromosomes would be two armed, with the centromere as the point of convergence in both cases (John and Lewis 1973, White 1973).

The repulsion between sister chromatids is less in metaphase-II, but it is also obvious that all the chromosomes are acrocentric (Fig. 2d). Since centromeres normally do not divide until anaphase-II, the mitotic metaphase and metaphase-II chromosomes are similar in that each is made up of a pair of sister chromatids and a centromere, except that there is greater contraction and divergence of sister chromatids in metaphase-II. Thus in the light of the expected and observed similarity between the chromosomes of the two stages, it would be wrong to describe the double-stranded acrocentric chromosomes as folded metacentric chromosomes.

In anaphase-II and telophase-II all the chromosomes are single-stranded rod-shaped structures as would be expected of acrocentric chromosomes (Fig. 2e). Mitotic anaphase and telophase chromosomes are usually similar to those in the corresponding stages in meiosis-II owing to the fact that the centromeres divide and lead the consequent single-stranded chromosomes to the poles. There are however, no such similarities between Figs. 2e and 1a, although Nwankiti (1983b) had interpreted the latter as mitotic telophase showing folded chromosome arms. Since it is unlikely that two stages which normally have similar chromosome morphology would be so clearly different in the same organism, the correct interpretation of Fig. 1a should be mitotic metaphase as stated earlier. The chromosomes of Z. variegatus are therefore acrocentric, a conclusion which is consistent with the appearances of the chromosomes in the mitotic and meiotic stages observed and the karyotype in the family Pyrogomorphidae (White 1973).
Meiotic Aberrations:

The observations in this study do not support the conclusions by Lasebikan and Olorode (1972) and Olorode and Akingbohungbe (1975) that chromosome aberrations are a regular feature of meiosis in Z. variegatus. No acentric fragments were detected nor were there any dicentric bridges or even laggards which should have resulted in the formation of micronuclei. In virtually all cases disjunction in anaphase-I occurred first in the smallest bivalents (Fig. 2b). However, in the large bivalents there were some cases of either late disjunction or difficulties in disjunction probably due to stickiness between telomeric portions of non-sister chromatids, sometimes resulting in stretching of the affected chromatids (Fig. 2a). The stickiness is probably due to tangled uncondensed material from the lambrush-like chromosomes of prophase. This material is detectable in anaphase-I, as thin connections, especially between sister chromatids of the X-chromosome or between separated homologues, in some cases making one chromatid appear longer than the other (Fig. 2b). In extreme cases the failure of the telomeres to slip through the tangled material quickly could lead to stretching of the chromatids with enlarged telomeres (Fig. 2a). However, it is questionable whether such entanglements lead to breakages because no acentric fragments were seen. Other cases of unequal chromatids as in Fig. 2c could have been due to the fact that the whole chromosome was not lying in one plane.

The apparent absence of meiotic aberrations in the population studied strongly suggests that the smaller wet season population is not a result of meiotic aberrations, as suggested by Olorode and Akingbohungbe (1975). It is more likely that the aberrations detected by the investigators cited were artefacts of fixation and not a regular occurrence in natural populations of Zonocerus variegatus.

Summary

The morphology of the chromosomes of the grasshopper, Zonocerus variegatus was studied from mitotic metaphase and meiotic stages in squashed preparations of fresh testicular material. The chromosomes (2n = 19) were found to be acrocentric as in most of the Pyrgomorphidae and not metacentric as reported in one study. No meiotic aberrations were detected.

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References

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