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Brain Cells and Chromosomes of the Brown Planthopper *Nilaparvata lugens* (Stål)

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Cytological features during meiosis and at mitosis unravel the genetic system of any taxon. They furnish vital cues for observed genetic segregation and breeding behavior of the species. Mitosis governs a regular and an even distribution of essential cell constituents, specifically the hereditary components from a parental cell to two daughter cells through successive nuclear divisions. Also, the base line karyotype data are useful in understanding phylogenetic relationships among populations within a species.

Nuclear division or karyokinesis in the brown planthopper *Nilaparvata lugens* (Stål) consists of mitosis and meiosis. Mitosis is regularly associated with nuclear division of somatic cells; meiosis occurs in conjunction with the formation of reproductive cells or gametes. Most of the studies on *N. lugens*' cytology pertain to meiotic divisions at the primary and secondary spermatocytes (Saitoh *et al.* 1970, Liquido 1978, Den Hollander 1982, Saxena and Barrion 1982a, 1982b, 1983, 1986). Little is known about gonial mitosis of *N. lugens*. No cellular and chromosomal investigations have been made on somatic tissues such as the brain of *N. lugens*. We therefore studied the karyomorphology and morphometrics of the somatic chromosomes in brain cells of *N. lugens*.

Material and methods

A stock culture of *N. lugens* Biotype 1 was maintained on susceptible Taichung Native 1 rice plants in an insectary at IRRI. Fifty, first to fifth instar nymphs and newly emerged adults sampled from the culture were placed individually in vials and immobilized by freezing before subjecting them to air drying techniques (Crozier 1968).

The head of each individual was dissected in a 1% sodium citrate hypotonic saline solution. The cerebral ganglia were removed and transferred to fresh citrate solution for 10 to 20 min at room temperature. The ganglia were fixed in a glacial acetic acid-methanol mixture (1 : 3 by volume) and after 30 min transferred to a drop of 60% aqueous acetic acid on a glass microslide. To spread the brain cells, the cerebral tissue was macerated with a bent probe or the tips of a pair of forceps. When brain cells became transparent, they were covered with 3 to 4 drops of the fixative and the microslide was tilted back and forth repeatedly to spread the solution. The cells were fixed for 4 h and then stained with lacto-aceto-orcein solution (1 g orcein, 28 ml (85%) lactic acid, and 22 ml (28%) glacial acetic acid). A coverslip was applied to spread the stain. Mounted slides were placed in a covered petri dish and put on a hot plate (50°C) for 2 to 3 h.

For permanent mounting, the prepared slides were immersed in 70% ethanol in a coupling

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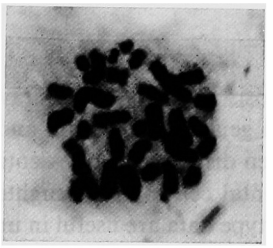
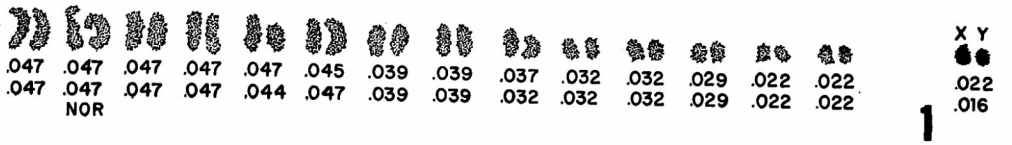
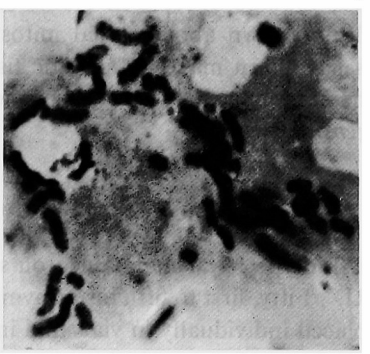
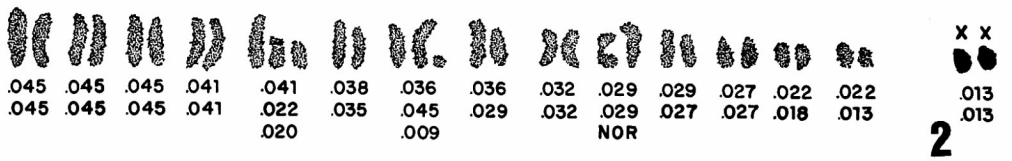
jar to loosen the coverslips. They were then dehydrated with 95% absolute ethanol and mounted in Canada balsam.

From the mounted slides, the *N. lugens* karyotype, its mitotic index, chromosome counts, morphometrics at premetaphase, behavior during different mitotic stages, and other cellular features were determined. Camera lucida drawings (1250×) of brain chromosomes were made and photomicrographs (1000×, oil immersion) of mitotic stages were taken with a research microscope.









Figs. 1–2. 1, relative mean lengths of prometaphase brain chromosomes of male *N. lugens*. Magnification, 1000× (oil immersion). IRRI, 1986. 2, Relative mean lengths of prometaphase brain chromosomes of female *N. lugens*. Magnification, 1000× (oil immersion). IRRI, 1986.

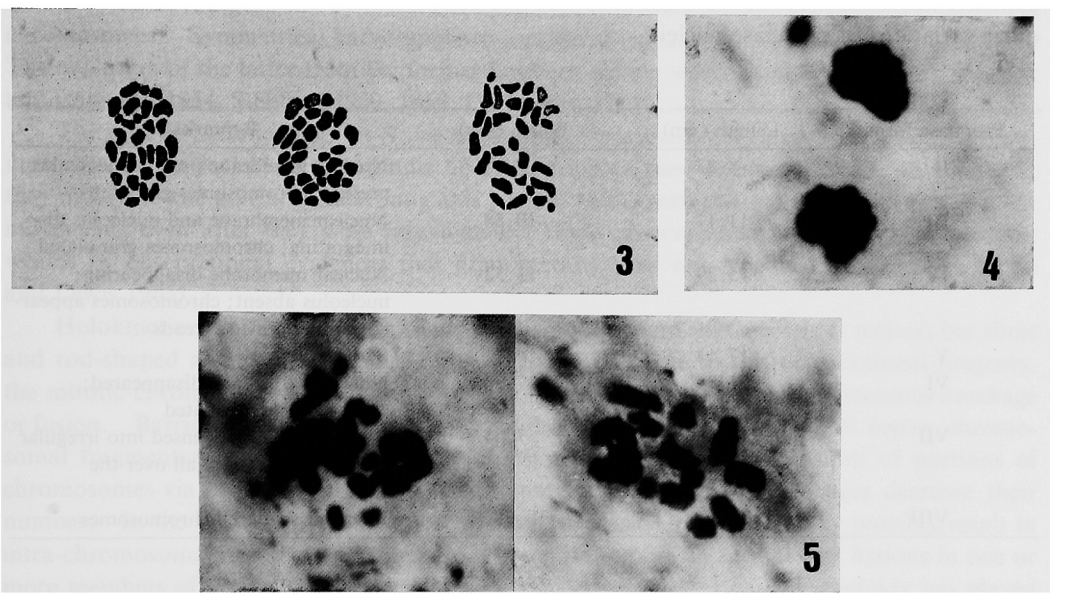
Results

Utilizing the standard air-drying techniques with lacto-aceto-orcein staining, the mitotic brain chromosomes of fourth-instar *N. lugens* nymphs were observed. From 100 individuals, the mean number of cells undergoing mitosis was 487 while that of non-dividing cells was 247. The mitotic index was therefore 67%.

Based on the late prophase or premetaphase chromosome counts, the normal diploid complement was 2n=30. The karyotype formula included 28 autosomes (A) and an XX(♀)

or XY(♂) sex chromosome system. The sex chromosomes were highly heterochromatic and maximally contracted. Furthermore, the XX- or XY-tandem consisted of chromosomes which were smaller than or equal to some autosomes. Usually, the X and the Y chromosomes had different shapes; the X chromosome was always bigger than the Y chromosome.

The early metakinetic chromosomes (Figs. 1, 2) were short and rod-shaped, each having two chromatids. They were ideal mitotic elements for karyotyping. The sister chromatids comprising the chromosomes were barely observed due to the lack of a distinct centromere. They appeared to have diffuse or scattered spindle attachments implying that *N. lugens* chromosomes are holokinetic. At metaphase the chromosomes occupied the spindle body with the chromosomal long axis at right angles to the polar axis.



Figs. 3–5. 3, camera lucida drawings of segregating anaphase chromosomes of *N. lugens*. Magnification, 625×. IRRI, 1986. 4, Late telophase chromosomes of *N. lugens*. Magnification, 1000× (oil immersion). IRRI, 1986. 5, Somatic fusions of chromosomes of *N. lugens* resulting to hypoploids. Magnification, 1000× (oil immersion). IRRI, 1986.

In some brain cells of fourth-instar nymphs, the holocentric complement consisted of X-Y bodies and 28 autosomes, two chromosomes of which had distinct acrocentric constriction and were interpreted as the nucleolar organizing region (NOR). The segment distal to the constriction was the satellite. This type of genomic complement was associated with heterogametic male *N. lugens*. In terms of shapes and relative sizes of chromosomes, the male autosomes were symmetrical whereas the female autosomes were somewhat asymmetrical. Normally, males had 16 linkage groups while females had 15. In some cases, the female complement included some supernumerary or m-chromosomes.

The total chromatin in female *N. lugens* was 11.90 μm, and in male, 5.74 μm. Females possessed longer chromosomes than did males. In karyotype analysis of premetaphase chromosomes of males, the relative lengths of autosomes ranged from 0.022 to 0.047. The NOR measured 0.047, whereas the X and the Y chromosomes measured 0.022 and 0.016, respectively (Fig. 1). In contrast, the relative length of autosomes in females ranged from 0.013 to 0.045. The relative length of NOR was 0.029, while that of the X-chromosome was 0.013 (Fig. 2).

Very few cells, (3 cells per individual), immediately after metaphase were detected undergoing anaphase. Anaphase occurred so fast that it was least trapped in the fixed brain tissue. During anaphase, the "broadside-on" segregation of chromosomes to the pole commenced at the central core directing towards the periphery of the genomic pool. The disjuncted group at each polar ends possessed 15 chromosomes or 30 chromatids (Fig. 3). There was no gradient or localized repulsion in chromosome. Supernumerary fragments also moved to the poles. In contrast, the frequency of telophase cells was high (30 cells per individual). At early telophase, the 30 chromatids at each polar ends formed clumps measuring 5.88 μm long and 4.70 μm wide. However, at late telophase (Fig. 4), the clumps became more compact and were 2.98 μm long and 2.34 μm wide. Later, cytokinesis ensued and formed two daughter nuclei. The first prophase nucleus measured 7.05 μm by 5.88 μm. Each nucleus had a circular 1.76 μm diameter nucleolus. Aside from the prophase nucleus previously mentioned, there were seven other variations of prophase nuclei:

Prophase form	Length (μm)	Width (μm)	Remarks
II	8.23	7.64	Nuclear membrane present; nucleolus present; chromosomes granulated
III	11.17	10.58	Nuclear membrane and nucleolus disintegrating; chromosomes granulated
IV	15.29	13.52	Nuclear membrane disappearing; nucleolus absent; chromosomes appeared as intertwined threads
V	17.64	14.11	Same as above
VI	11.76	7.06	Nuclear membrane disappeared; chromosomes elongated
VII	8.23	7.06	Chromosomes condensed into irregular forms and scattered all over the nucleus
VIII	17.64	10.58	Irregular scattered chromosomes

Thus during the regular mitotic cell cycle in *N. lugens* Biotype 1, prophase was the longest stage, followed by telophase and metaphase; anaphase was the shortest.

Some brain cell nuclei displayed either an increase (*agmatoploidy*) or a decrease (*hypoploidy*) in chromosome numbers. Hypoploidy resulted from somatic chromosomal fusions (Fig. 3). On the other hand, agmatoploidy resulted from chromosomal fragmentations leading to the appearance of extra chromosomes, such as m-chromosomes (Fig. 5). On the whole, hypoploidy was more frequent than agmatoploidy in *N. lugens* Biotype 1.

Discussion

The regular mitotic division in brain cells of *N. lugens* Biotype 1 occurred only in fourth-instar nymphs. Other cellular metabolic events, such as the first growth period (G_1), the synthetic phase (*S-phase*) and the second growth period (G_2) during interphase, possibly occurred in other instars and adults. In contrast, newly-emerged males and females were ideal for the study of gonial cells and chromosomes (Saitoh *et al.* 1970, Liquido 1978, Den Hollander 1982, Saxena and Barrion 1982a, 1982b, 1983, 1986). Mitosis of brain cells and gonial meiosis in *N. lugens* were observed using different procedures. The squash technique and lacto-aceto-orcein staining were suitable for studying testicular meiocytes (Saxena and Barrion 1982a). However, mitosis was better resolved by air-drying techniques and lacto-aceto-orcein staining

(Crozier 1968, 1970).

Both meiosis and mitosis showed that the diploid genome and sex chromosome systems of *N. lugens* are identical 2n=30 (28 autosomes plus XX(♀) or XY(♂). However, the genomic configuration at premetaphase differed. The mitotic premetaphase karyotype showed 28 autosomes and positively heteropycnotic XX or XY sex chromosomes. On the other hand, the meiotic premetaphase I comprised 14 bivalent (II) autosomes and XX(♀) or XY(♂) which were synapsed homologs. The number of chromosomes in *N. lugens* (30) is within the reported range (24 to 37) in delphacids, 29 being the modal number of chromosomes (Bhattacharya and Manna 1973, Halkka 1959, 1962, Halkka and Heinonen 1966). The heterogametic XY system exists in several insect orders, but only in Hemiptera-Heteroptera has the mechanism of segregation been explained (Darlington 1939).

The male *N. lugens* has symmetrical karyotype while the female possesses asymmetrical chromosomes. Symmetrical karyotypes are considered more primitive than asymmetical ones. The evolution of the latter from the former has been clearly worked out (White 1945, Babcock and Cameron 1934, Stebbins 1950, 1958, Darlington 1965).

The mitotic chromosomes of *N. lugens* Biotype 1 possessed diffuse centromeres. The chromosomes were attached to the spindle fibers throughout their entrire lengths. At anaphase, they moved to the pole with their long axis parallel to the metaphase plate. They had similar movements even after they were fragmented. These observations further supplemented the wealth of observational evidences that homopterans have non-localized kinetochores (John and Lewis 1965).

Holokinetic chromosomes are usually small, globular and isodiametric at meiosis but short and rod-shaped at mitosis (Thomas and Yonke 1981). Due to this constrictional property, the mitotic chromosomes of *N. lugens* are highly unstable and prone to spontaneous breakage or fusion. Barrion and Saxena (1985) reported that during gonial meiosis in *N. lugens*, chromosomal fragmentation increases the chromosome number. Meanwhile, fusions of portions of chromosomes via reciprocal translocation at subterminal points of breakages decrease their number. In the brain cells of *N. lugens*, the primary mechanisms that may possibly result in intra-chromosomal numerical repatterning are the Robertsonian fusions and fissions in one or more members of the genome. Increase in chromosome number of agmatoploidy has played a role in karyotypic evolution of holokinetic chromosomes in many families of Hemiptera (Thomas and Yonke 1981). Brain cells of female *N. lugens* exhibited agmatoploidy through the production of one or more m-chromosomes, but on the whole hypoploidy was more frequent than agmatoploidy. According to several reports (Whitten 1968, Whitten and Taylor 1969, John and Claridge 1974) agmatoploidy and hypoploidy do not result in unbalanced products and may often lead to establish chromosomal polymorphism. Stebbins (1950, 1971) has even suggested that a decrease in chromosome number will have a selective advantage in specialized or pioneer habitats because the rate of gene combination will be lower and hence tend to preserve adapted genotypes. If the new genome is to completely replace the original, the new homozygote must have a selective advantage over the heterozygote as well as the original homozygote (White 1968).

Summary

The karyomorphology and morphometrics of chromosomes in brain cells of fourth-instar *N. lugens* Biotype 1 nymphs were studied. The following features were noted: mitotic index 67%, normal diploid complement 2n=30; karyotype formula, 28 autosomes, plus XX(♀) or XY(♂). Males had symmetrical chromosomes with higher relative mean lengths than the females' asymmetrical chromosomes.

Mitotic chromosomes of brain cells possessed diffuse centromeres or were holocentric. During regular mitosis, prophase had the longest duration, followed by telophase. Metaphase and anaphase were short duration stages. At prophase, 8 differentiating forms of nuclei were observed.

The karyological changes in the basic diploid number were either increases (*agmatoploidy*) or decreases (*hypoploidy*) in chromosome numbers. Hypoploidy was more frequent than agmatoploidy.

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