Re-examination of Chromosomes of Three Species of Rice Planthoppers (Homoptera: Delphacidae)¹

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Chromosomes of homopterans are holocentric and do not show a localized centromere during cell division (HALKKA, 1959; WHITTEN, 1965). Cytogenetic studies of auchenorrhynchos species still have not advanced significantly, although the chromosome number and sex chromosomes have been determined in many homopteran species (KIRILLOVA, 1986). Also, there are discrepancies in the observations of some species of homopterans which are based on classical methods using testicular tissue.

In this paper, chromosome number and sex-determining systems of 3 rice planthopper species of economic importance, *Laodelphax striatellus*, *Nilaparvata lugens* and *Sogatella furcifera*, were re-examined using the air-drying technique, and different results from those reported by KIRILLOVA (1986) were obtained. The C-banding technique for the constitutive heterochromatin band was also applied to the air-dried specimens of planthopper embryos.

MATERIALS AND METHODS

Laodelphax striatellus (FALLÉN), *Nilaparvata lugens* (STÄL), and *Sogatella furcifera* (HORVÁTH) were collected in Izumo, Shimane and reared on rice seedlings at 25°C under a 16L-8D regime.

Testes of young adult males and embryos 3 to 4 days of age were used for the cytological study. We employed a modification of method from IMAI et al. (1977). Dissected testicular tissue or egg with cut with dissecting needles were incubated in a hypotonic solution, 0.075 M KCl, on a pre-cleaned slide for 15-20 min at room temperature. After the solution was removed, the materials were washed

with 60% ethanol-acetic acid (3:1 v/v) and crushed with needles. One drop of absolute ethanol-acetic acid fixative (3:1 v/v) was added on the slide. The dried materials were stained with a Giemsa solution at pH 6.8.

For the C-banding, the barium saline Giemsa method was employed. The slides prepared by the air-drying method with somatic embryonic cells were initially dipped in 0.2 N hydrochloric acid at room temperature for 60 min, followed by a rinse with distilled water, and then treated with 5% barium hydroxide at 40-50°C for 10-15 min. They were then incubated in 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate) at 55-60°C for 60 min, and rinsed with distilled water. The preparations were stained with the Giemsa solution.

RESULTS AND DISCUSSION

Laodelphax striatellus. The chromosome number of this species is reported to be $2n=33$ based on paraffin-sectioned preparations of testes (HIRAI, 1948). The present cytological preparations, however, showed the presence of 14 autosomal bivalents and one sex chromosomal univalent in meiotic division (Fig. 1A), and 29 or 30 chromosomes in somatic division (Fig. 1B). When the chromosome number of 158 cells from 38 preparations was counted, the ratio of cells which had 29 or 30 chromosomes was 39.9% in both cases (Fig. 2). Therefore, the male diploid number was 29 (28+XO) and the female one 30 (28+XX).

Nilaparvata lugens. Data of male chromosome number and sex chromosomes of this species are different among workers. SAITO et al. (1970) and PARIDA and DALUA (1979, 1981) reported that the male belonged to the heterogametic XO type and the male chromosome number was 29. LIQUIDO (1986) reported that the male had a diploid chromosome number of 29, and that the X chromosome showed anomalous segregation, i.e. meiotic divisions being sometimes reductional and sometimes equational. On the other hand, DEN HOLLANDER (1982) and SAXENA and BARRION (1986) reported that the sex-determining system was XX in the female and XY in the male, and that the diploid number was 30 in both sexes. The main difference among workers lies in the number of the male diploid chromosomes: 29 or 30. The

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Fig. 1. Chromosomes of rice planthoppers. A, C, E: meiotic chromosomes from testes of *L. striatellus*, *N. lugens* and *S. furcifera*, respectively; B, D, F: mitotic chromosomes from embryos of *L. striatellus*, *N. lugens* and *S. furcifera*, respectively. G, H: C-banding pattern of chromosomes in embryonic metaphase of *N. lugens* and *S. furcifera*, respectively. Arrows indicate sex chromosomes. Bar shows 20 μ m.

chromosome number of the somatic cells was, therefore, counted in 185 cells from 42 egg preparations. In *L. striatellus*, with a somatic chromosome number of 29 in the male and 30 in the female, the ratio of cells which had 29 or 30 chromosomes was 39.9% for both. On the other hand, in *N. lugens*, 66.5% of the cells had 30 chromosomes and

only 19.5% had 29 (Fig. 2). The results allow an interpretation that the chromosome number of *N. lugens* was 30 in both the male and female. Figure 1C illustrates the 14 autosomal univalents and the 2 sex chromosomes. Therefore, the male diploid number was 30 (28+XY) and the female one was also 30 (28+XX). This observation is consistent

Fig. 2. Frequency distribution patterns of chromosome number in somatic embryonic cells of *N. lugens* and *L. striatellus*.

with those of DEN HOLLANDER (1982) and SAXENA and BARRION (1986).

Sogatella furcifera. Male and female diploid chromosome numbers were first reported to be 27 and 28, respectively (PARIDA and DALUA, 1979; 1981) but later reported to be 29 and 30, respectively (DEN HOLLANDER, 1982; BARRION and SAXENA, 1984). This study showed that the male diploid number was 29 (28+XO) and the female one was 30 (28+XX) (Fig. 1E, 1F). The chromosome number and sex-determining system of *S. furcifera* were the same as those of *L. striatellus*.

C-banding. The C-banding technique was applied to the preparations for promoting further analysis of planthopper chromosomes. Figure 1G and 1H illustrate the C-banding patterns of chromosomes of somatic embryonic cells in *N. lugens* and *S. furcifera*, respectively. The C-banding pattern was different between the 2 species, the hetero-

chromatic region being on the edges of many chromosomes in *S. furcifera*. In homopterous insects, karyotype analysis is difficult because the metaphase chromosomes do not show restrictions. The air-drying method with banding techniques appears to be useful for further chromosomal studies of planthoppers and leafhoppers.

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