

## The Chromosomes of *Nilaparvata lugens* Stål. and Some Other Auchenorrhyncha

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The karyotypes of *Nilaparvata lugens* (Stål.) (Delphacidae), *Sogatella furcifera* (Horvath) (Delphacidae), *Nephotettix virescens* (Distant) (Cicadellidae), *Nephotettix nigropictus* (Motsch.) (Cicadellidae) and *Recilia dorsalis* (Motsch.) (Cicadellidae) have been studied with emphasis on *N. lugens*.

Saitoh, Kudoh and Mochida (1970) gave the male diploid number of *N. lugens* as  $2n=29$  ( $14_{11}+X$ ). The first meiotic division was reported as being reductional for the sex chromosomes giving two types of secondary spermatocytes, one with fourteen elements and the other with fifteen. Thus they concluded *N. lugens* had an XX, XO sex determining system.

Liquido (1978) supported the above but suggested that, with respect to the behaviour of the sex chromosome, sometimes the first meiotic division was reductional and the second meiotic division equational while at other times the situation was reversed. Both sequences were reported to occur within one individual. Liquido also reports that in some of his preparations the sex chromosome is composed of two elements of unequal length which he considers to be sister chromatids. Furthermore, since it has been reported that a biased sex ratio of 1 male: 3.64 females exists in field populations at IRRI (Kenmore, cited in Liquido 1978), it was postulated that the excess of females could result from a proportion of both meiotic divisions being equational.

A detailed study of meiosis in male *N. lugens* was undertaken to resolve the distribution of the sex chromosomes during meiotic divisions. The behaviour of the sex chromosomes in *N. lugens* was compared to that in the other mentioned species. Also, a survey of *N. lugens* was made to reveal if any geographic variation in the pattern of meiosis or in karyotype occurred within the species and, in view of the reported sex ratio bias, a number of single pair crosses were set up to establish if this might be due to meiotic drive.

### Methods

For chromosome preparations, gonads of newly emerged males and females were dissected out in insect Ringer, fixed in Carnoy's fixative (15 min), stained in a drop of 3% lacto-propionic orcein (15 min), rinsed with 50% acetic acid and squashed under a coverslip. The edges of the coverslip were sealed with nail varnish.

The crosses of individual pairs were conducted in melanex cages using 35–45 day old TN1 rice plants. The insects used in the crosses were from the “biotype 1” culture maintained at the International Rice Research Institute (IRRI) Los Baños, Philippines. All other insects were from colonies maintained at Cardiff, but originally obtained from Philippines, Australia, Bangladesh, Japan, Malaya, Solomon Islands and Sri Lanka.

### Results

The male chromosome number for *N. lugens* was found to be  $2n=30$  ( $14_{11}+XY$ ), for *N. virescens* and *N. nigropictus*  $2n=15$  ( $7_{11}+X$ ), for *R. dorsalis*  $2n=19$  ( $9_{11}+X$ ) and for *S. furcifera*  $2n=29$  ( $14_{11}+X$ ) (Fig. 1A, B, D, E, F, G+H).

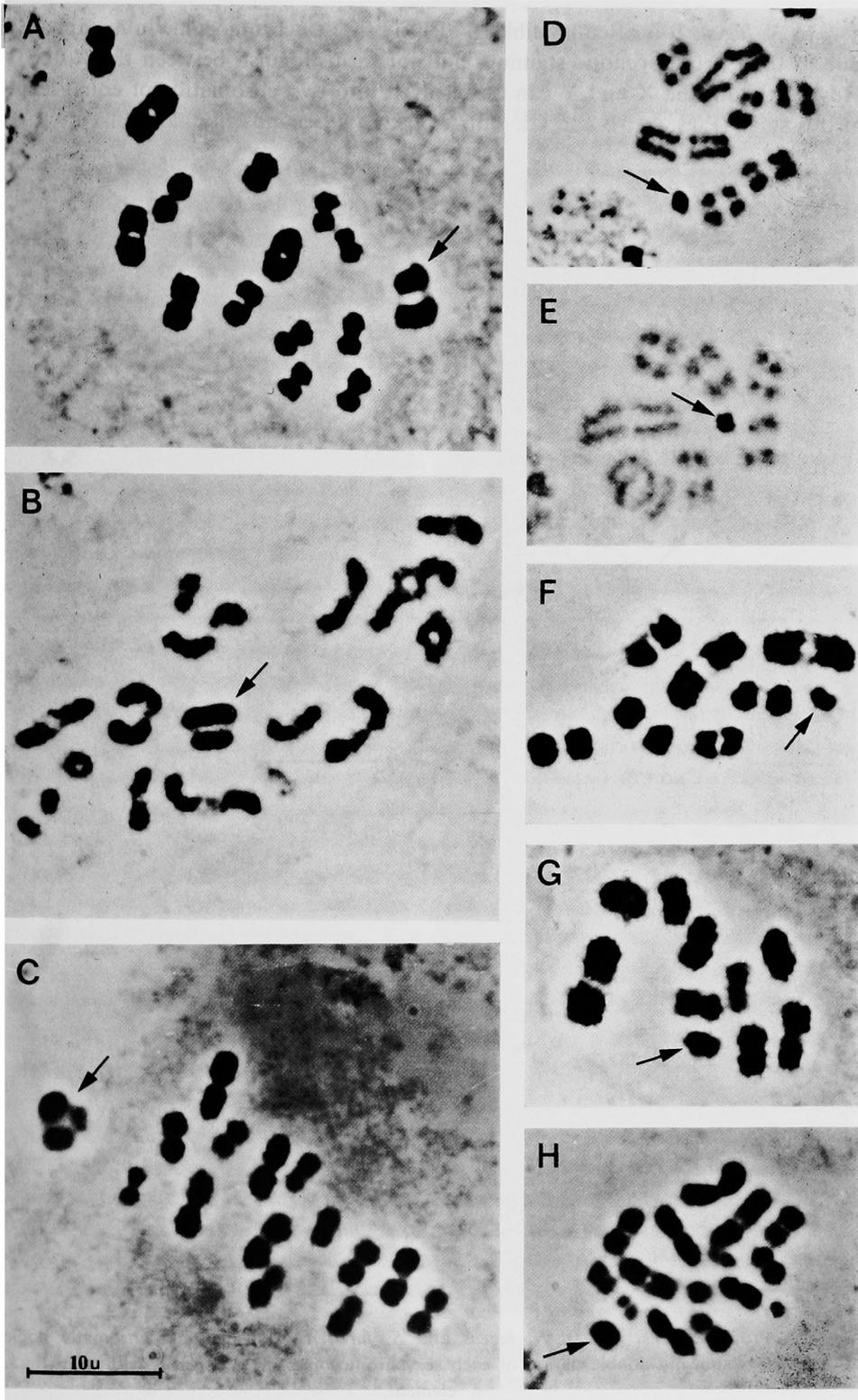
The sex chromosomes can be distinguished in the prophase nuclei of all the species as a heterochromatic body, the number of such bodies indicating the number of sex chromosomes present. In *N. virescens*, *N. nigropictus*, *R. dorsalis* and *S. furcifera* there is only one such body per nucleus, but in *N. lugens* many cells show two (Figs. 2A and B). The two heterochromatic bodies in *N. lugens* are of unequal size (Figs. 2A and C) and often fuse to form a single large body (Fig. 2A). Occasional individuals of *N. lugens* with an extra sex chromosome (Fig. 1C) displayed three such heterochromatic bodies (Fig. 2D).

In *N. lugens* during the diffuse diplotene stage only the sex chromosomes are visible (Fig. 2C), though often lying in close proximity to each other they do not seem to be intimately paired. In late diplotene each element of the sex chromosome pair can be seen to be double (Fig. 2C). Terminalisation of the chiasmata in the autosomes occurs during diakinesis and by metaphase the bivalents are terminally attached and oriented axially to the spindle (Fig. 3A). The sex chromosomes in contrast, do not appear to have any chiasmata between them, the bivalents orientate parallel to the equator, with the X and Y on different sides (Figs. 1A and 3A).

At metaphase I the sex chromosomes usually lie to one edge of the plate and comprise two unequal elements (Figs. 1A and B), each of which is presumed to consist of a pair of sister chromatids. The double nature of each element is supported by the fact that the thickness of each is similar to that of an autosomal dyad, while in the X O species the univalent X (Fig. 1E) is only half the thickness of an autosomal dyad.

In all species the first meiotic division is reductional for the sex chromosomes which usually lag behind the autosomes in anaphase (Figs. 3B and C). The presence of an X and Y in *N. lugens*, which segregate in the first metaphase, results in both second metaphase plates containing fifteen chromosomes, one with the X and the other with the Y (Figs. 4A, B+C). By contrast, the first meiotic division in the X O species gives two types of secondary spermatocytes one with and one without the sex chromosome (Figs. 4D, F and G). Those individuals of *N. lugens* with an extra sex chromosome displayed two types of second metaphase plates, one with fifteen and the other with sixteen chromosomes (Fig. 4E).

Fig. 1. Chromosome complements of 5 species with the sex chromosome arrowed. A+B, *N. lugens*. C, *N. lugens* showing the extra sex element. D+E, *N. virescens*. F, *N. nigropictus*. G, *R. dorsalis*. H, *S. furcifera*.



In *N. lugens* it is often possible to identify the sex chromosome in metaphase II due to their heteropycnotic staining, but not to distinguish between the X and Y. However, both the X and Y can be resolved into two chromatids of equal length at this stage (Figs. 4A, B and C). In *N. lugens* all second telophase cells appeared

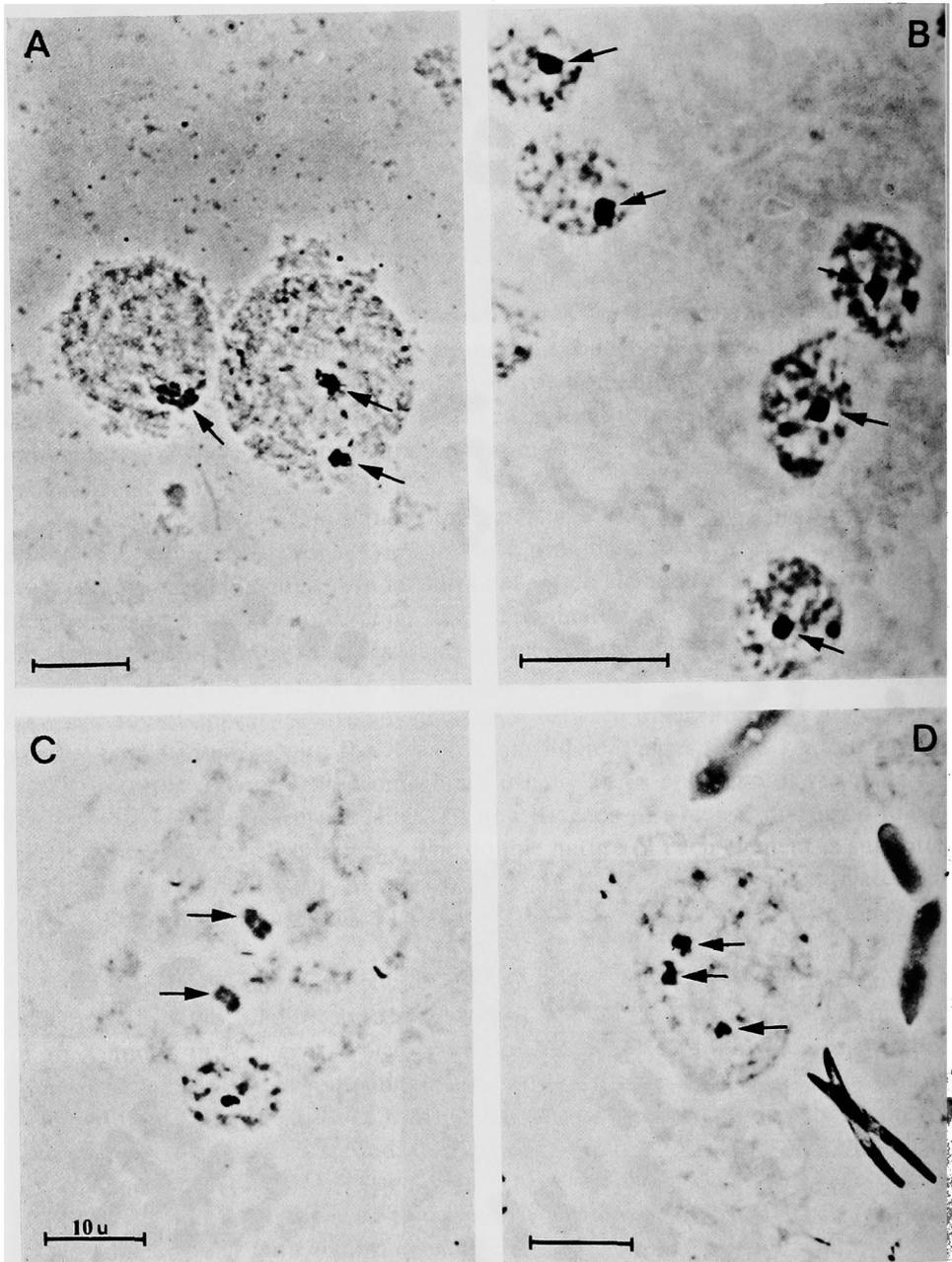


Fig. 2. Prophase cells. A+B, *N. lugens* and *S. furcifera* showing heterochromatic bodies. C, *N. lugens* showing double nature of each sex chromosome. D, *N. lugens* with an extra sex element.

to have a sex chromosome present as revealed by a heterochromatic body in each cell (Fig. 5B).

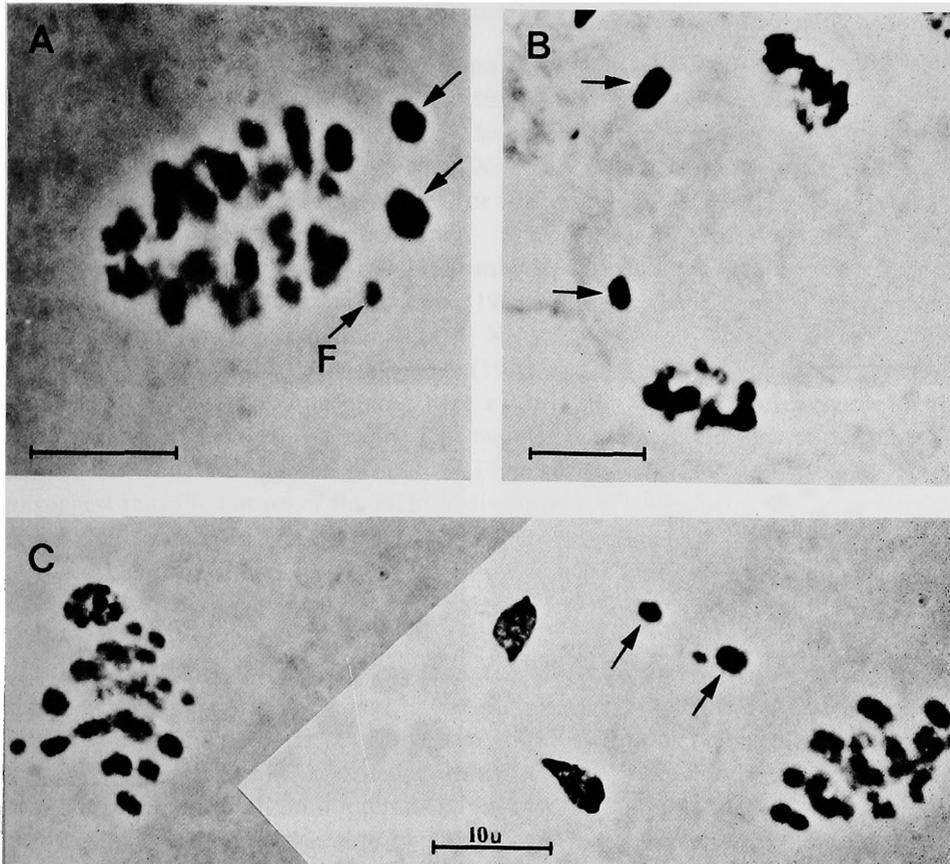
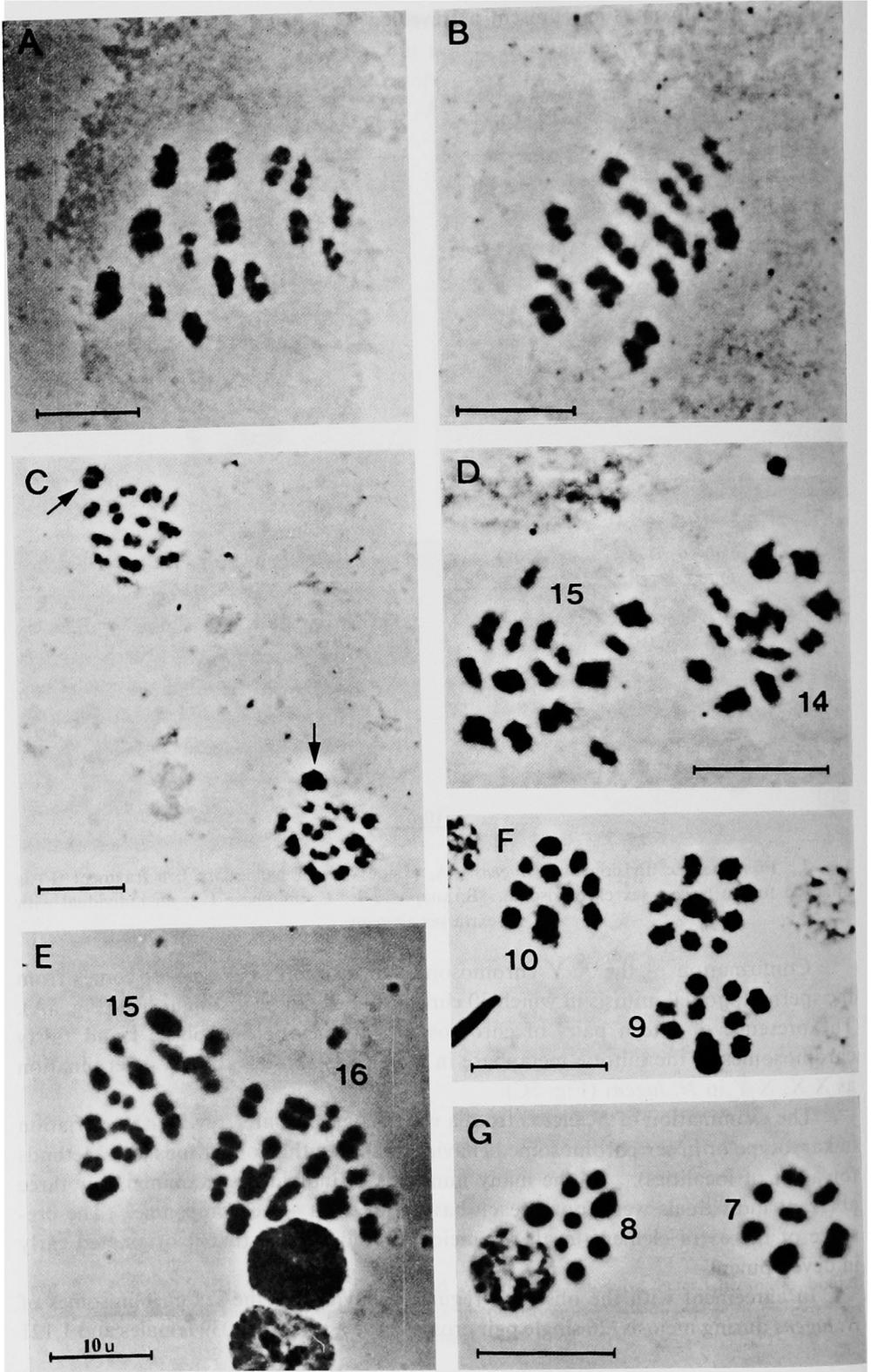


Fig. 3. First meiotic division in *N. lugens*. A, a side view of metaphase I, a fragment (F) is attached to the largest sex chromosome. B, anaphase I. C, anaphase I in an individual with an extra sex element.

Confirmation of the X Y chromosome constitution of *N. lugens* comes from the spermatogonial mitosis in which 30 chromosomes can be distinguished (Fig. 5A). The presence of fifteen pairs of chromosomes in meiotic metaphase I and thirty chromosomes in the mitotic metaphase in females establishes the sex determination as X X, X Y in *N. lugens* (Fig. 5C).

The examination of *N. lugens* from a wide geographic area revealed no variation in karyotype or in sex chromosome behaviour between the populations (see Methods for a list of localities). Of the many hundreds of individuals examined only three aberrant individuals were found, each having an extra sex chromosome. The presence of the extra element in all the meiocytes did suggest that it originated early in development.

In agreement with the observed regular behaviour of the sex chromosomes of *N. lugens* during meiosis, 26 single pair crosses gave a total of 1,039 females and 1,121



males which was not significantly different from a 1:1 sex ratio.

### Discussion

Previous workers give chromosome numbers of  $2n=15 (7_{11}+X)$  for *N. cincticeps* (Uhler) (Mitsuhashi 1966, Saitoh *et al.* 1970) and *N. apicalis* (Motsch.) (= *nigropictus*, Ghauri 1971) (Kurokawa 1953), but Bhattacharya and Manna (1967) give a chromosome number of  $2n=13 (6_{11}+X)$  for *N. bipunctatus* (= *virescens*, Ghauri 1971). No explanation can be offered for this discrepancy in chromosome number from *N. virescens* reported in this paper. *R. dorsalis* (= *Inazuma dorsalis*, Nielson 1979 in Maramorosch and Harris 1979) may be identical to *Deltocephalus dorsalis* examined by Bhattacharya and Manna (1967) (Halkka, pers. comm.), the chromosome numbers are the same  $2n=19 (9_{11}+X)$ .

Unlike Saitoh, Kudoh and Mochida (1970) and Liquido (1978), the chromosome number of *N. lugens* is interpreted here as  $2n=30$ . The sex chromosome in the males instead of being single at the first meiotic metaphase consists of two unequal elements considered to be an X and Y (Figs. 1A and B). No evidence was found to suggest that the nature of the meiotic divisions was labile, being sometimes reductional and sometimes equational. All five species showed a reductional division of the sex chromosomes in the first meiotic division and an equational division in the second, following the standard pattern for Auchenorrhyncha (White 1973, Halkka 1959, Whitten and Taylor 1969).

The nature of the pairing between the autosomes and that between the sex chromosomes differs in *N. lugens*. The pairing between the autosomes is initially side by side with chiasmata between them. The chiasmata undergo terminalization and the bivalents remain attached terminally. This terminal association of autosomes is common to all homopterans so far studied (John and Claridge 1974). Sex chromosome pairing is also side by side, but is looser than that of the autosomes and achiasmate. This is considered to be non-specific heterochromatic pairing. They remain paired in this fashion until they segregate in anaphase I. Such pairing of the sex chromosomes is typically found in heteropterans and homopterans (White 1973). The side by side pairing and orientation parallel to the equator during metaphase I facilitates the reductional division of the sex chromosomes.

As homopterans have non localised centromeres (John and Lewis 1965, White 1973) fragmentations and fusions of chromosomes do not result in unbalanced meiotic products and often may be perpetuated to establish polymorphisms, variations in chromosome number or karyotype (John and Claridge 1974, Whitten 1968, Whitten and Taylor 1969). Also polymorphisms within a species for sex chromosome composition have been reported in *Dicranotropis hamata*, *Rhopalopyx preysleri*, (Halkka 1959), *Parabolocratius albomaculatus* (Battacharya and Manna 1969) and in *Oncopsis flavicollis* (John and Claridge 1974). However, Vilbaste (1962)

Fig. 4. Metaphase II. A, B+C, *N. lugens*, all plates have 15 chromosomes. D, *S. fucifera* showing 14 and 15 chromosome plates. E, *N. lugens* with an extra sex element showing 15 and 16 chromosome plates. F, *R. dorsalis* with 9 and 10 chromosomes. G, *N. virescens* with 7 and 8 chromosomes.

has shown that the alleged 7 (X Y) "race" of *R. preysleri* in fact is a valid species *R. adumbrata*.

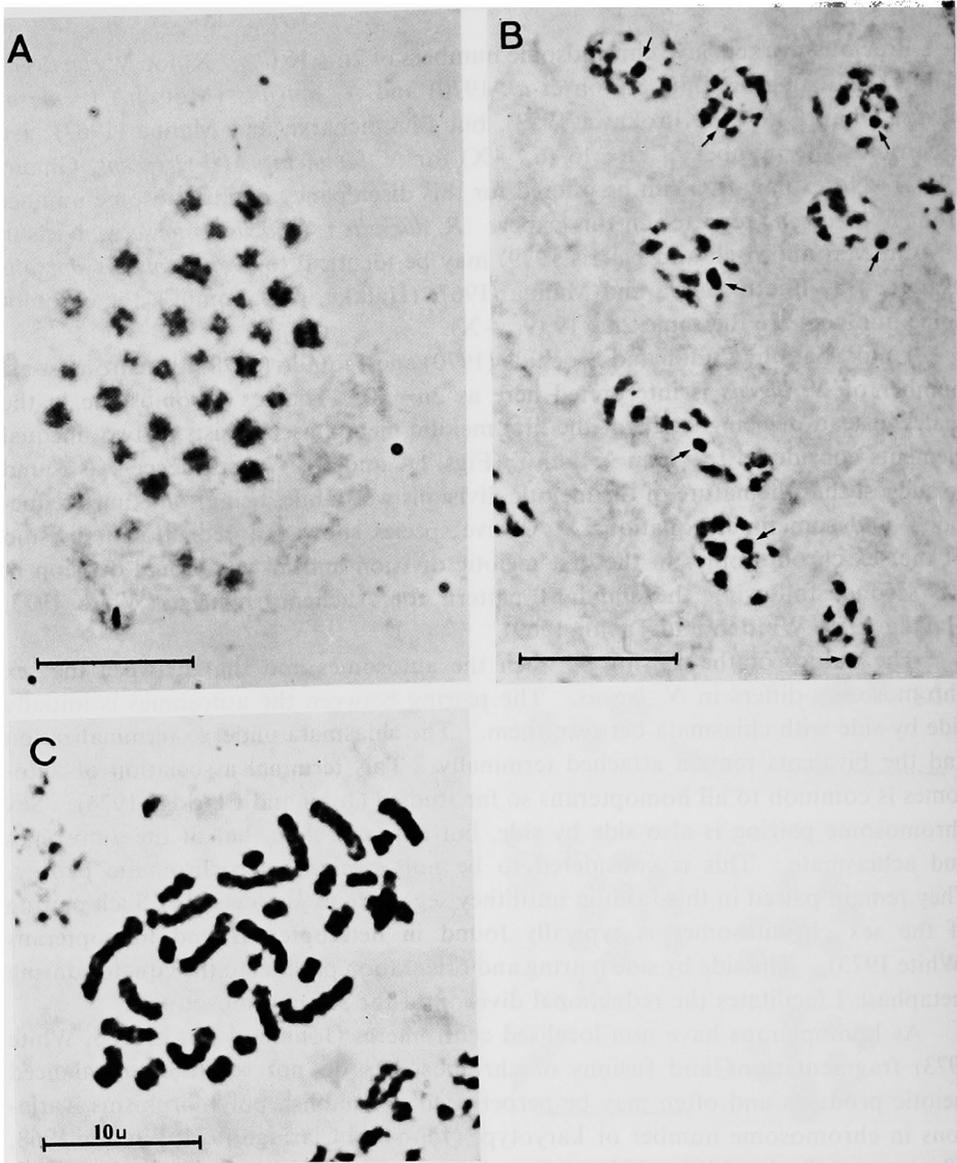


Fig. 5. A, mitotic figure from *N. lugens* male showing 30 chromosomes. B, telophase II cells in *N. lugens*. C, *N. lugens* female mitosis.

Despite the theoretical possibility of rearrangements, fusions and fragmentations, Homoptera remain remarkably conservative for chromosome number within groups (e.g. most Penatomidae have 7, Cicadellidae 9, Membracidae 11; Halkka 1959, Halkka and Heinonen 1966) and for karyotype within species. The examination of *N. lugens* from a wide geographic range reveals no instances of polymor-

phism for chromosome number or sex determining mechanism. Only three aberrant individuals were found with an extra sex chromosome.

The sex determining system in leafhoppers is usually X O, but X Y systems are known (Halkka 1959, Bhattacharya and Manna 1967, 1973b). Without comparisons with closely related species it is difficult to speculate on the origins of the X Y of *N. lugens*. The side by side, achiasmatic pairing of the sex chromosomes does not suggest an X-autosome fusion as this would be expected to give a chain of three at metaphase I (John and Claridge 1974). Of the 41 species of Delphacidae studied previously (Bhattacharya and Manna 1973a, Halkka 1959, 1962, Halkka and Heinonen 1964) chromosome numbers range from 24 to 37, with a peak at 29. The presence of 30 chromosomes in *N. lugens* might therefore suggest a fragmentation.

The regular division of the sex chromosomes during meiosis of *N. lugens*, should give 50% X and 50% Y bearing sperm yielding a balanced sex ratio. This was confirmed by the single pair crosses giving a sex ratio of 1:1. An ecological explanation for reported imbalance of sex ratio in the field should therefore be sought in this species.

#### Abstract

The karyotype of male *N. lugens* has previously been reported as  $2n=29 (14_{11}+X)$  giving an X O, X Y sex determining mechanism. However, this investigation revealed the alleged single X chromosome in the male consists of two elements of unequal length which separate reductionally in the first meiotic division and equationally in the second. From this, and by comparison with other X O species it was determined that *N. lugens* has an X Y sex determining system with a chromosome number of  $2n=30 (14_{11}+X Y)$  in the male.

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#### References

- Bhattacharya, A. K. and Manna, G. K. 1967. A study of germinal chromosomes during meiosis in fifty species of Homoptera (Hemiptera). Proc. 54th Ind. Sci. Congr. Pt. 111, 418.
- and — 1969. Males with different sex chromosome constitutions in a homopterans species, *Parabolocratrus albomaculatus*. Proc. 56th Ind. Sci. Congr. Pt. III, 445.
- and — 1973a. Gonial chromosomes, their behaviour and metrical data in nine species of Fulgorids (Homoptera). Cytologia 38: 281–290.
- and — 1973b. Morphology, behaviour and metrical studies of the germinal chromosomes of

- ten species of Membracidae (Homoptera). *Cytologia* **38**: 657–665.
- Ghauri, M. S. K. 1971. Revision of the genus *Nephotettix* Matsumura (Homoptera: Cicadellaoidea: Euscelidae) based on the type material. *Bull. Ent. Res.* **60**: 481–512.
- Halkka, O. 1959. Chromosome studies on the Hemiptera, Homoptera, Auchenorrhyncha. *Ann. Acad. Sci. Fen., Series A. IV Biologica* **43**: 1–72.
- 1962. The chromosomes of the Membracidae. *Hereditas* **48**: 215–219.
- and Heinonen, L. 1964. The chromosomes of seventeen Nearctic Homoptera. *Hereditas* **52**: 77–80.
- Halkka, O. and Heinonen, L. 1966. Chromosome numbers in 20 American or Sudanese leafhoppers, with notes on the role of cytology in Homopteran taxonomy. *Ann. Ent. Fenn.* **32**: 11–19.
- John, B. and Claridge, M. F. 1974. Chromosome variation in British populations of *Oncopsis* (Hemiptera: Cicadellidae). *Chromosoma (Berl.)* **46**: 77–89.
- and Lewis, K. R. 1965. *The Meiotic System*. *Protoplasmatologia V1/F/1*. Wien: Springer.
- Kurokawa, H. 1953. A study of the chromosomes in 12 species of Homoptera. *La Kromosomo* **15**: 543–546.
- Liquido, N. J. 1978. Morphology, cytology and breeding behaviour of *Nilaparvata lugens* (Stål.) (Delphacidae, Homoptera). M. Sc. Thesis, University of the Philippines, Los Baños, Laguna, Philippines.
- Maramorosch, K. and Harris, K. F. 1979. *Leafhopper and Plant Disease Agents*. Academic Press.
- Mitsuhashi, J. 1966. Chromosome numbers of the green leafhopper, *Nephotettix cincticeps* Uhler (Homoptera, Cicadellidae). *Appl. Ent. Zool.* **1**: 103–104.
- Saitoh, K., Kudoh, K. and Mochida, O. 1970. A study of the male meiotic chromosomes in two Homopteran species of economic importance. *Sci. Rep. Hirosaki Univ.* **17**: 31–37.
- Vilbaste, J. 1962. Über die Arten *Rhopalopyx preyssleri* (H.-S.) und *Rh. adumbrata* (C.R. Sahlb.) (Homoptera, Issidae). *Notulae Entomologica* **42**: 62–66.
- White, M. J. D. 1973. *Animal Cytology and Evolution*. Third edition, Cambridge University Press.
- Whitten, M. J. 1969. An unusual chromosome system in a leafhopper (Homoptera: Auchenorrhyncha). *Chromosoma (Berl.)* **24**: 37–41.
- and Taylor, W. C. 1968. Chromosomal polymorphism in an Australian leafhopper (Homoptera: Cicadellidae). *Chromosoma (Berl.)* **26**: 1–6.
-