

## CELLULAR AND KARYOLOGICAL VARIATIONS IN POPULATIONS OF GREEN LEAFHOPPER *NEPHOTETTIX VIRESCENS* (DISTANT) IN THE PHILIPPINES

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### ABSTRACT

Populations of the green leafhopper, *Nephotettix virescens* (Distant), were sampled from rice fields in the Philippines (Albay, Isabela, Laguna, Nueva Ecija, and Zambales) that were infested and non-infested with tungro virus. Males were cytologically and karyologically examined, described and compared. Lacto-aceto-orcein squash preparations of the testes of all *N. virescens* sampled had meiocytes which followed the conventional sequence of reductional-equational divisions. The dominant gametic cells were primarily spermatocytes undergoing Meiosis I stages. In terms of the qualitative features of the meiotic cells of all male *N. virescens* sampled, no obvious variation was detected. However, in terms of the meiotic index, the tungro-transmitting male *N. virescens* had mean index of 86.06% whereas non-tungro-transmitters had 81.70%. All *N. virescens* exhibited normal and regular meiosis. In terms of chromosome number, all *N. virescens* have  $2n = 15$ , the karyotype formula is  $7 \text{ II} + \text{XO}$ . There were two types of spermatocytes: one with (7 IA) and the other with (7 IA + X), the latter was the predominant type. The mean relative lengths of diakinesis chromosomes of male *N. virescens* from non-tungro rice fields of five provinces did not vary significantly, while those from tungro-infested fields exhibited heterogeneous measurements, many of which were statistically varied between and among each other: Among the five different rice fields, the male *N. virescens* from Isabela and Nueva Ecija, exhibited significant differences in mean relative lengths of diakinesis autosomes 1, 2, 4 and 6 and X chromosome. Chromosomes 3, 5 and 7 were stable autosomes of both tungro-vector and non-tungro-vector *N. virescens*.

**Key words:** green leafhopper, *Nephotettix virescens*, cytogenetics

### INTRODUCTION

One of the major pests of rice is the green leafhopper, *Nephotettix virescens* (Distant) which is known as the most effective vector of the rice tungro virus in Asia.

Rice tungro is the most widespread and most devastating viral disease of rice. It was reported as a virus disease in 1965. However, its occurrence in the Philippines was suspected to occur a few years before its identification. The disease is endemic in the country and there is yet to be a lasting strategy to control it.

The current scenario in rice ecosystems in the Philippines is that tungro has so far not simultaneously affected seriously all rice growing areas. Rice tungro epidemics have been reported repeatedly in pockets. Why tungro occurs much more

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severely in certain areas but not in others is not known yet. Different researches dealing with this peculiar observation were initiated and one of them is in terms of the genetic variation of the insect vector of the tungro virus.

This study concentrated on the cytology-cytogenetics of the populations of *N. virescens* sampled from rice fields in the Philippines which were infested and non-infested with tungro virus.

The major objective of the study is to present the cellular-karyological features of the populations of *N. virescens* sampled from tungro-infested rice fields as well as those rice fields free of tungro virus. The comparison of these features will determine if the *N. virescens* populations are cytologically and cytogenetically different from each other.

## MATERIALS AND METHODS

### Field Collections of *N. virescens*

Natural populations of *N. virescens* were collected from tungro and non-tungro infested rice fields in Albay, Isabela, Laguna, Nueva Ecija and Zambales.

### Vector Infectivity of Sampled *N. virescens*

The vector infectivity of the sampled *N. virescens* was confirmed in the laboratory. Individual samples were introduced onto a susceptible variety of rice, Taichung Native 1 (TN1). Tungro infection was assessed after two weeks (incubation period) through membrane immunobinding assay (MIBA). The leaves of infected TN1 seedlings were extracted with phosphate buffer. The extracts were blotted onto nitrocellulose membrane and incubated in antiserum solution. Color reaction was observed after soaking the membrane to solution containing nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Purple indicated positive result, that is, the *N. virescens* individual was considered tungro-vector.

### Mass Rearing of *N. virescens*

Individuals confirmed to be tungro-vectors and non-tungro vectors were separately mass reared in TN1 and served as sources of newly-emerged males for the cellular-karyological investigations.

### Fixation and Preservation of Male *N. virescens*

*N. virescens* males were sorted from mass-reared populations, fixed in freshly prepared Carnoy's solution and then transferred to 70% ethanol for preservation.

### Slide Preparations

The testis of *N. virescens* was extracted and macerated on a glass slide with a drop of lacto-aceto-orcein using a bent needle. Cover glass was placed over the squashed tissues. Uniform pressure was applied to the slide preparation to ensure that the cells were flattened and the chromosomes well spread. The prepared slide was passed over a flame several times and alternate staining and destaining were applied until the desired preparation was obtained.

### Microscopic Observations and Analysis

The macerated testicular tissues of *N. virescens* were observed under low power objective (10x) of Bausch and Lomb research microscope. The cells were scanned

and the details of cellular components particularly the nuclear components were observed under the oil immersion (100x) objective. The qualitative features of the cells and chromosomes were described and recorded. The quantitative characteristics of the autosomes and sex chromosomes such as number and relative lengths were determined and recorded. Photomicrographs of cells and chromosomes of *N. virescens* were taken and arranged to show the sequential stages particularly of the first meiotic division.

### Statistical Analysis

The mean relative lengths of autosomes and sex chromosomes of *N. virescens* from tungro- and non-tungro-infested rice fields of five provinces in the Philippines were tabulated and compared statistically using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

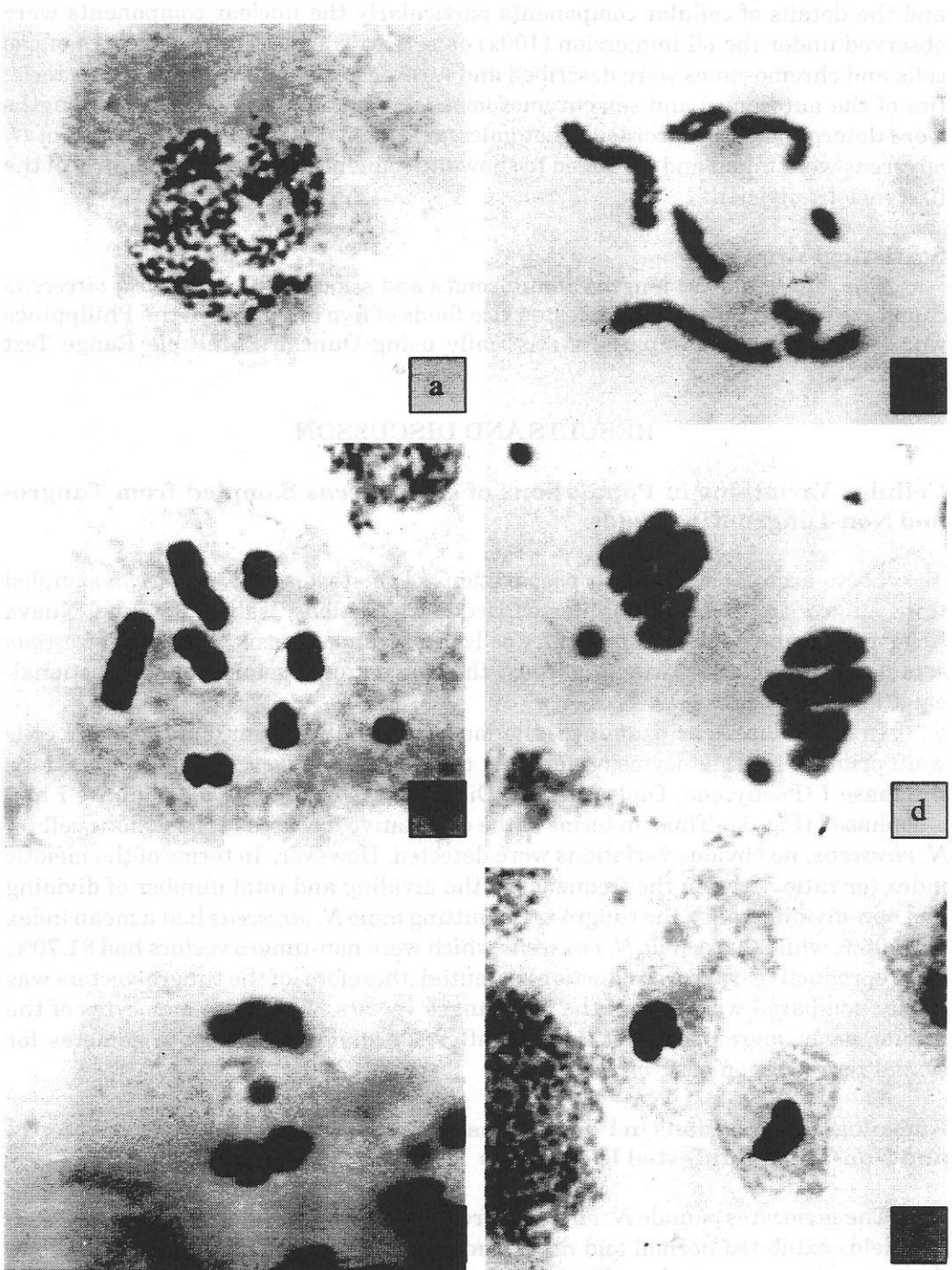
### Cellular Variations in Populations of *N. virescens* Sampled from Tungro- and Non-Tungro-Rice Fields

Lacto-aceto-orcein squash preparations of the testes of *N. virescens* sampled from tungro- and non-tungro-infested rice fields in Albay, Isabela, Laguna, Nueva Ecija and Zambales showed testicular cells undergoing meiosis. All the *N. virescens* samples had meocytes which followed the conventional sequence of reductional-equational divisions.

In all *N. virescens* examined, the most commonly encountered gametic cells were primary spermatocytes undergoing the different stages of Meiosis I, namely: Prophase I (Pachytene, Diplotene and Diakinesis); Metaphase I, Anaphase I and Telophase I (Fig. 1). Thus, in terms of the qualitative features of the meiotic cells of *N. virescens*, no obvious variations were detected. However, in terms of the meiotic index (or ratio between the frequency of the dividing and total number of dividing and non-dividing cells), the tungro-transmitting male *N. virescens* had a mean index of 86.06%, while those male *N. virescens* which were non-tungro vectors had 81.70%. The reproductive (sperm-production) potential, therefore, of the tungro-vectors was higher compared with that of the non-tungro vectors. The more meocytes of the former mean more sperms to develop after spermatogenesis, more gametes for sexual reproduction of *N. virescens*.

### Karyological Variations in Populations of *N. virescens* Sampled from Tungro- and Non-Tungro-Infested Rice Fields

The meocytes of male *N. virescens* from both tungro- and non-tungro-infested rice fields exhibited normal and regular meiosis. In terms of chromosome number, there were two types of spermatocytes observed: one with seven bivalent autosomes (7 IA) and the other type had seven bivalent autosomes plus an X-chromosome (7 IA + X), indicating that the karyotype of the heterogametic male *N. virescens* is 7 II + XO. Of the two types of gametes, *N. virescens* from all rice fields had more of the (7 IA + X) type (Fig. 1c).



**Figure 1.** First meiotic stages in spermatocytes of *Nephrotettix virescens* (Distant) a) pachytene, b) diplotene, c) diakinesis, d) metaphase I, e) anaphase I and f) telophase I. Magnification 1000x (oil immersion objective).

In terms of the relative length of chromosomes, the diakinesis chromosomes of *N. virescens* (Fig. 1c) exhibited the most stable dimension compared to pachytene chromosomes of many species, particularly in plants. In this study the relative lengths of diakinesis chromosomes of *N. virescens* from tungro- and non-tungro-infested rice fields (Table 1) and tungro-infested rice fields (Table 2) were determined and compared (Table 3).

The mean relative lengths of all the diakinesis chromosomes of male *N. virescens* from non-tungro-infested rice fields of five provinces did not vary significantly (Table 1). On the other hand, those from tungro-infested rice fields exhibited heterogeneous measurements, many of which were statistically varied between and among each other (Table 1). The chromosomes of tungro-vector *N. virescens* were heterogeneous whereas those of non-tungro vectors were homogeneous. Table 3 shows the comparisons between the tungro- and non-tungro-vector *N. virescens* from five different rice fields. *N. virescens* from Isabela and Nueva Ecija showed significant differences in terms of the mean relative mean length of diakinesis chromosomes between the tungro-vector and non-tungro vector *N. virescens*. The significant differences involved autosomes 1, 2, 4 and 6 and X-chromosome. Except for autosome 4, the varying autosomes were relatively longer in tungro-vector *N. virescens* while the X-chromosome was relatively shorter compared with the vector *N. virescens*. Chromosomes 3, 5 and 7 were stable autosomes. The autosomes and X-chromosome exhibiting varying relative lengths can be utilized as chromosomal indices in comparing *N. virescens* populations when considering their tungro-vector potential.

### SUMMARY AND CONCLUSION

Green leafhopper, *Nephotettix virescens* (Distant) sampled from rice fields in the Philippines (Albay, Isabela, Laguna, Nueva Ecija and Zambales) were cytologically and karyologically examined, described and compared.

Testicular cells of *N. virescens* from both tungro-infested and non-tungro-infested rice fields showed that:

1. meiosis followed the conventional sequence of reductional-equational divisions;
2. the dominant gametic cells were primary spermatocytes undergoing Meiosis I stages, namely; Prophase I (Pachytene, Diakinesis and Diplotene), Metaphase I, Anaphase I, and Telophase I;
3. the chromosome number was  $2n = 15$ , the karyotype formula was  $7 \text{ II} + \text{XO}$  so there were two types of spermatocytes:  $(7 \text{ IA})$  and  $(7 \text{ IA} + \text{X})$ , the latter was the dominant type.

Significant cellular features exhibited by the tungro-vector versus the vector are as follows:

1. Mean meiotic index of tungro-vector *N. virescens* was 86.06% while the non-vectors had 81.70%.
2. The male *N. virescens* from Isabela and Nueva Ecija exhibited significant differences in mean relative lengths of diakinesis autosomes 1, 2, 4 and 6 and X-chromosome.

**Table 1.** Comparison of the relative mean lengths of the diakinesis chromosomes of male green leafhopper *Nephotettix virescens* (Distant) sampled from non-tungro-infested ricefields.

| Chromosome Number | Albay  | Isabela | Laguna | Nueva Ecija | Zambales |
|-------------------|--------|---------|--------|-------------|----------|
| 1                 | 0.188a | 0.203a  | 0.203a | 0.233a      | 0.211a   |
| 2                 | 0.163a | 0.159a  | 0.170a | 0.171a      | 0.193a   |
| 3                 | 0.139a | 0.135a  | 0.134a | 0.142a      | 0.147a   |
| 4                 | 0.128a | 0.126a  | 0.125a | 0.114a      | 0.117a   |
| 5                 | 0.116a | 0.122a  | 0.118a | 0.106a      | 0.108a   |
| 6                 | 0.104a | 0.105a  | 0.104a | 0.087b      | 0.087a   |
| 7                 | 0.099a | 0.085a  | 0.090a | 0.081a      | 0.073a   |
| X                 | 0.067a | 0.064a  | 0.056a | 0.067a      | 0.064a   |

\* In a horizontal row, means with the same letter are not significantly different at 5% by DMRT.

**Table 2.** Comparison of the relative mean lengths of the chromosomes at diakinesis of male green leafhopper *Nephotettix virescens* (Distant) sampled from tungro-infested ricefields.

| Chromosome Number | Albay  | Isabela | Laguna | Nueva Ecija | Zambales |
|-------------------|--------|---------|--------|-------------|----------|
| 1                 | 0.192b | 0.238a  | 0.213a | 0.183b      | 0.247b   |
| 2                 | 0.166b | 0.210a  | 0.168b | 0.162b      | 0.147c   |
| 3                 | 0.138a | 0.130a  | 0.131a | 0.112a      | 0.127b   |
| 4                 | 0.121c | 0.113a  | 0.116a | 0.097b      | 0.120c   |
| 5                 | 0.112b | 0.102a  | 0.106a | 0.093a      | 0.111b   |
| 6                 | 0.101b | 0.090a  | 0.102b | 0.093a      | 0.098b   |
| 7                 | 0.097b | 0.067a  | 0.086b | 0.072a      | 0.078b   |
| X                 | 0.079c | 0.049b  | 0.060a | 0.056a      | 0.071c   |

\* In a horizontal row, means with the same letter are not significantly different at 5% by DMRT.

**Table 3.** Comparison of the relative mean lengths of the chromosomes at diakinesis of male green leafhopper, *Nephotettix virescens* (Distant) sampled from non-tungro- (H) and tungro- (T) infested rice fields.

| Chromosome Number | Populations | Condition |       | Statistical Result |
|-------------------|-------------|-----------|-------|--------------------|
|                   |             | H         | T     |                    |
| 1                 | Albay       | 0.188     | 0.192 | NS                 |
|                   | Isabela     | 0.203     | 0.238 | **                 |
|                   | Laguna      | 0.203     | 0.213 | NS                 |
|                   | Nueva Ecija | 0.233     | 0.183 | NS                 |
|                   | Zambales    | 0.211     | 0.247 | NS                 |
| 2                 | Albay       | 0.163     | 0.166 | NS                 |
|                   | Isabela     | 0.159     | 0.210 | **                 |
|                   | Laguna      | 0.170     | 0.168 | NS                 |
|                   | Nueva Ecija | 0.171     | 0.162 | NS                 |
|                   | Zambales    | 0.193     | 0.147 | NS                 |
| 3                 | Albay       | 0.139     | 0.138 | NS                 |
|                   | Isabela     | 0.135     | 0.130 | NS                 |
|                   | Laguna      | 0.134     | 0.131 | NS                 |
|                   | Nueva Ecija | 0.142     | 0.112 | NS                 |
|                   | Zambales    | 0.147     | 0.127 | NS                 |
| 4                 | Albay       | 0.128     | 0.121 | NS                 |
|                   | Isabela     | 0.127     | 0.113 | **                 |
|                   | Laguna      | 0.125     | 0.116 | NS                 |
|                   | Nueva Ecija | 0.114     | 0.097 | NS                 |
|                   | Zambales    | 0.117     | 0.120 | NS                 |
| 5                 | Albay       | 0.116     | 0.112 | NS                 |
|                   | Isabela     | 0.122     | 0.102 | NS                 |
|                   | Laguna      | 0.118     | 0.106 | NS                 |
|                   | Nueva Ecija | 0.106     | 0.093 | NS                 |
|                   | Zambales    | 0.108     | 0.111 | NS                 |
| 6                 | Albay       | 0.104     | 0.101 | NS                 |
|                   | Isabela     | 0.105     | 0.090 | NS                 |
|                   | Laguna      | 0.104     | 0.102 | NS                 |
|                   | Nueva Ecija | 0.087     | 0.093 | **                 |
|                   | Zambales    | 0.087     | 0.098 | NS                 |
| 7                 | Albay       | 0.099     | 0.097 | NS                 |
|                   | Isabela     | 0.085     | 0.067 | NS                 |
|                   | Laguna      | 0.090     | 0.086 | NS                 |
|                   | Nueva Ecija | 0.081     | 0.072 | NS                 |
|                   | Zambales    | 0.073     | 0.078 | NS                 |
| X                 | Albay       | 0.067     | 0.079 | NS                 |
|                   | Isabela     | 0.064     | 0.049 | NS                 |
|                   | Laguna      | 0.056     | 0.060 | NS                 |
|                   | Nueva Ecija | 0.067     | 0.056 | **                 |
|                   | Zambales    | 0.064     | 0.071 | NS                 |

NS - not significant, \*\* - significant at 5% by DMRT

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|    |       |       |            |
|----|-------|-------|------------|
| NS | 0.238 | 0.233 | Albay      |
| NS | 0.218 | 0.203 | Ibabao     |
| NS | 0.183 | 0.233 | Neiva Eaja |
| NS | 0.247 | 0.211 | Zambales   |
| NS | 0.168 | 0.163 | Albay      |
| NS | 0.210 | 0.188 | Ibabao     |
| NS | 0.168 | 0.170 | Iaguna     |
| NS | 0.163 | 0.171 | Neiva Eaja |
| NS | 0.147 | 0.193 | Zambales   |
| NS | 0.138 | 0.139 | Albay      |
| NS | 0.130 | 0.138 | Ibabao     |
| NS | 0.131 | 0.134 | Iaguna     |
| NS | 0.113 | 0.142 | Neiva Eaja |
| NS | 0.127 | 0.147 | Zambales   |
| NS | 0.121 | 0.128 | Albay      |
| ** | 0.113 | 0.127 | Ibabao     |
| NS | 0.116 | 0.128 | Iaguna     |
| NS | 0.097 | 0.114 | Neiva Eaja |
| NS | 0.130 | 0.117 | Zambales   |
| NS | 0.113 | 0.118 | Albay      |
| NS | 0.103 | 0.123 | Ibabao     |
| NS | 0.106 | 0.118 | Iaguna     |
| NS | 0.093 | 0.106 | Neiva Eaja |
| NS | 0.111 | 0.108 | Zambales   |
| NS | 0.101 | 0.104 | Albay      |
| NS | 0.090 | 0.103 | Ibabao     |
| NS | 0.103 | 0.104 | Iaguna     |
| ** | 0.083 | 0.087 | Neiva Eaja |
| NS | 0.098 | 0.087 | Zambales   |
| NS | 0.097 | 0.099 | Albay      |
| NS | 0.087 | 0.088 | Ibabao     |
| NS | 0.088 | 0.080 | Iaguna     |
| NS | 0.073 | 0.081 | Neiva Eaja |
| NS | 0.078 | 0.073 | Zambales   |
| NS | 0.079 | 0.087 | Albay      |
| NS | 0.043 | 0.081 | Ibabao     |
| NS | 0.080 | 0.088 | Iaguna     |
| ** | 0.056 | 0.067 | Neiva Eaja |
| NS | 0.071 | 0.084 | Zambales   |

NS - not significant. \*\* - significant at 5% by DMRT