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# Classification of the Three Korean Biotypes of the Brown Planthopper, *Nilaparvata lugens* (Stal), By Morphological Variation

## 한국산 벼멸구 생태형의 형태적 분류

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**ABSTRACT** Morphological investigations of the abdominal lateral lobes in females and the unguitractor plates in the tarsi of legs in male and female *N. lugens* among the three Korean *N. lugens* biotypes revealed that the three biotypes varied from one another. The presence of a distinct 'cut' on the lateral lobes in brachypterous females distinguished Biotype 2 from Biotypes 1 and 3. The highest frequency of inserted unguitractor plates in fore-and mid-tarsal segments was exhibited by Biotype 2 in both sexes of each morphs.

**KEY WORDS** *Nilaparvata lugens*, biotype, morphology

**초 록** 벼멸구 생태형 1, 2, 3의 형태의 차이를 구별하고자 장시형과 단시형 암수를 대상으로 형태적 변화를 관찰하였다. 암컷은 생식 부위인 Abdominal lateral lobes의 좌우 부위를 비교 조사하였다. 수컷은 앞다리, 가운데 다리, 뒷다리 부절의 Unguitractor plate를 조사하였다. 생태형 2의 단시형 암컷은 Abdominal lateral lobes의 기부가 잘리진 것이 생태형 1과 3보다 많았다. Unguitractor plate의 경우 생태형 2는 장단시형 암수 모두 부절 안쪽으로 함입되어 있었다.

**검 색 어** 벼멸구, 생태형, 형태

Breeding for resistance was proven to be effective in controlling the epidemic outbreaks of the pest. However, with the increase in the area planted with high-yielding *N. lugens*-resistant rice varieties, a continued decrease in host-plant genetic diversity has occurred. Such a situation coupled with the prolificity and innate plasticity of the pest species has resulted in the evolution of variant populations of *N. lugens*. Theses variant *N. lugens* populations, capable of witstanding host-plant resistance, are referred to as biotypes. The existence of such host resistance-breaking biotypes has complicated the management of *N. lugens* by genetic manipulation of rice varieties.

A systematic study of *N. lugens* biotypes is important for pest mangement. It would provide tools in the analysis of *N. lugens* rice variety relationships that would serve as a foundation in programs of breeding *N. lugens*-resistant rice

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varieties. Any variation in the pest's populations or biotypes can be monitored through both differential varietal or host plant reactions as well as through the pest responses. Recent investigations at IRRI have demonstrated the existence of subtle morphological, cytological, and electrophoretic variations among *N. lugens* (Saxena & barrion 1982, 1983; Saxena & Rueda 1982; Saxena & Mujer 1984a, b). The techniques for morphological and cytological evaluations of insect biotypes have enabled the monitoring of variations even in preserved specimens, obviating the need to transport live materials.

In Korea, three biotypes of *N. lugens* were recognized. Biotype 1 infests exclusively susceptible rice varieties. Later, the presence of new *N. lugens* Biotypes 2 and 3 were observed in 1981 and 1982, respectively, by Lee et al. (1983) and Lee et al. (1984). Currently, the three Korean *N. lugens* biotypes have been identified based on their differential varietal reactions: Biotype 1 on Chucheongbyeo variety (no gene for resistance), Biotype 2 on Cheongcheongbyeo variety (*Bph* 1 resistance gene), and Biotype 3 on Milyang 63(*bph* 2 resistance gene), (Lee et al. 1982, 1985).

The study was conducted to determine if morphological variations exist among the three Korean *N. lugens* biotypes.

## MATERIALS AND METHODS

Field populations of *N. lugens* through Korea were collected from 1980 to 1982. Through seedling bulk tests, three variant populations specific to their varietal hosts were isolated: Biotype 1 (B1) infested Chucheongbyeo(rice variety with no gene for resistance), Biotype 2 (B2) fed on Cheongcheongbyeo (rice variety with *Bph* 1 resistance gene), and Biotype 3 (B3) survived on Milyang 63 (rice variety with *bph* 2 gene).

From the caged populations of the three *N. lugens* biotypes, both brachypterous and macropterous males and females were randomly sampled with an aspirator and transferred directly into 70% ethyl alcohol for fixation.

Each of the biotype populations were cleared and mounted for morphological and morphometric investigations using the following procedures: 1) boil insects in 95% ethyl alcohol for ca. 10 minutes in a hot water bath, 2) macerate them in 10% lukewarm NaOH for 10~15 min, 3) wash in 95% ethyl alcohol and boil for 15~20 min in chloral-phenol(1 part chloral hydrate: 1 part phenol crystals), 4) properly orient body parts such as the legs and genitalia and mount on glass microslides using Hoyer's medium (30 g gum arabic, 50 ml distilled water, 200 g chloral hydrate, and 20 ml glycerine). Qualitative and quantitative assessments of the abdominal lateral lobes and unguitractor plates were made using 6.5X and 12.5X objectives of the phase contrast microscope equipped with a calibrated filar micrometer eyepieces. Camera lucida drawings of some selected structures were also made at 6.5X and 12.5X magnifications.

## RESULTS

### Abdominal lateral lobe variations

Among *N. lugens* females, five varied types of abdominal lobes were observed and designated as follows: Ⅰ-normal lobes, Ⅱ-right and left lobes with discontinuous cut at about 0.167mm form the posterior tips; Ⅲ-right lobe with continuous or discontinuous cut, left lobe normal; Ⅳ-right right lobe normal, left lobe with contin-

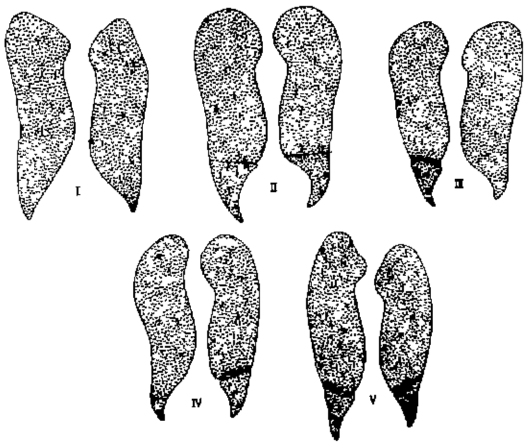


Fig. 1. Camera luchida drawings of the types of abdominal lobes in female *N. lugens* populations from Korea. Magnification, 78.75X.

uous and discontinuous cut; and V-right and left lobes with distinct cuts at about 0.167mm from the posterior tips(Fig.1). The number of insects falling under each abdominal lobe type of brachypterous females is significantly different from biotype to biotype. For instance, type I lateral lobe was most frequently encountered in Biotype 3 and Biotype 1 but least observed in Biotype 2. while type V lateral lobe was more common in Biotype 2 females than in Biotype 3 and Biotype 1 females. However, the number of macropterous females falling under each category is not significantly different from biotype to biotype.

Furthermore, the right and left abdominal lateral lobes were grouped into two categories—the 'cut' and 'uncut'. The former category indicated that a distinct horizontal cut was present at about 0.167mm from posterior tips of right and/or left lobes, while the latter category showed no 'cut' at all and was considered as the normal lateral lobe. Among the brachypterous

**Table 1. Numbers of brachypterous (BRAC) and macropterous (MAC) females of the three *N. lugens* biotypes with different types of abdominal lobes**

| Morph | Biotype | No. of individuals observed | Abdominal Lobes Types | | | | | $X^{2a}$ |
|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | IV | V | |
| BRAC | 1 | 57 | 23 | 14 | 9 | 10 | 1 | |
| | 2 | 77 | 19 | 7 | 5 | 19 | 27 | |
| | 3 | 60 | 30 | 3 | 4 | 15 | 8 | |
| | | | | | | | | 41.699** |
| MAC | 1 | 60 | 20 | 0 | 6 | 17 | 17 | |
| | 2 | 50 | 8 | 0 | 3 | 19 | 20 | |
| | 3 | 53 | 12 | 0 | 3 | 10 | 28 | 11.929 |

a**-Significantly different at 1% level by $\chi^2$ test. Avg. of 20 replicates.

**Table 2. Comparison between 'cut' and 'uncut' lateral lobes in brachypterous (BRAC) and macropterous (MAC) females of the three *N. lugens* biotypes**

| Morph | Biotype | No. of lateral lobes[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Right lobe | | | Left lobs | | $X^2$ |
| | | Uncut | Cut | $X^2$ | Uncut | Cut | |
| BRAC | 1 | 47 | 10 | | 46 | 11 | |
| | 2 | 45 | 32 | | 31 | 46 | |
| | 3 | 48 | 12 | 12.06** | 37 | 23 | 22.41** |
| MAC | 1 | 37 | 23 | | 26 | 34 | |
| | 2 | 27 | 23 | | 11 | 39 | |
| | 3 | 22 | 31 | 4.63 | 15 | 38 | 6.18* |

a**, *-Significantly different at 1% and 5% levels by $\chi^2$ test, respectively. Avg. of 20 replicates.

females, the numbers of individuals falling under each category were significantly different at 1% level by Chi-square test from biotype to biotype. In the case of the macropterous females, the 5% significant difference was shown by the number of individual's left lateral lobes, while no significant difference was shown by the right lateral lobes among the biotypes (Table 1). Specifically, the presence of a distinct 'cut' on the right and left lateral lobes of brachypterous females distinguished B2 from B1 and B3.

### Unguitractor plate

The unguitractor plate in the tarsi of the thoracic legs of the three biotypes was observed of two types: (i) the closely-inserted ( I ) and, (ii) the loosely-attached or inserted (U) plates (Fig. 2).

In both brachypterous and macropterous males, the numbers of individuals falling under I and U plates in right and left fore-and midtarsi were significantly different at 1% level by Chi-

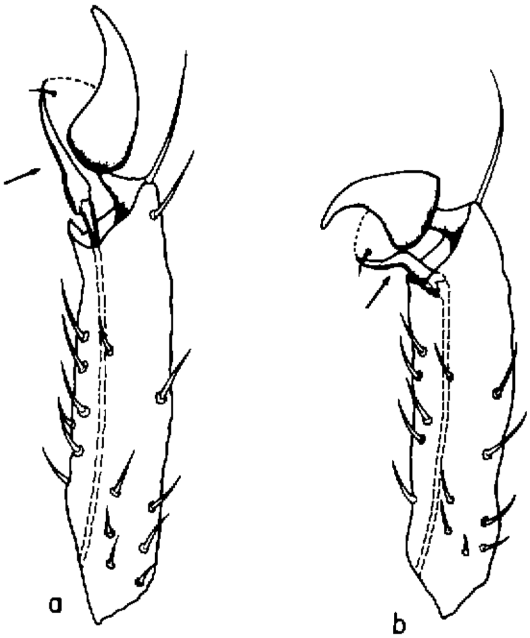


**Fig. 2.** Camera lucida drawings of the dorsal distal midtarsal segments of female *N. lugens* showing two types of attachment of unguitractor plate: (a) loosely-inserted and (b) closely-inserted. The unguitractor plates are pointed by arrow. Magnification, 200X.

**Table 3. Number of individuals with inserted (I) and uninserted (U) unguitractor plates in tarsi of the legs of brachypterous (BRAC) and macropterous (MAC) males and females of the three *N. lugens* biotypes.**

| Biotype | Leg Segments[a] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fore Tarsus | | | | | | Mid Tarsus | | | | | | Hind Tarsus | | | | | |
| | Right | | | Left | | | Right | | | Left | | | Right | | | Left | | |
| | U | I | $X^2$ | U | I | $X^2$ | U | I | $X^2$ | U | I | $X^2$ | U | I | $X^2$ | U | I | $X^2$ |
| | BRAC MALE | | | | | | | | | | | | | | | | | |
| 1 | 46 | 1 | | 43 | 4 | | 44 | 3 | | 41 | 6 | | 47 | 0 | | 47 | 0 | |
| 2 | 16 | 17 | | 20 | 13 | | 16 | 17 | | 16 | 17 | | 32 | 1 | | 33 | 0 | |
| 3 | 40 | 1 | 43.99** | 39 | 2 | 19.46** | 37 | 4 | 28.80** | 39 | 2 | 27.18** | 41 | 0 | 2.69 | 41 | 0 | – |
| | MAC MALE | | | | | | | | | | | | | | | | | |
| 1 | 38 | 2 | | 39 | 1 | | 39 | 1 | | 38 | 2 | | 40 | 0 | 1.36 | 40 | 0 | |
| 2 | 36 | 24 | | 39 | 21 | | 31 | 29 | | 35 | 25 | | 59 | 1 | | 59 | 1 | |
| 3 | 39 | 2 | 26.63** | 40 | 1 | 26.72** | 37 | 4 | 34.06** | 40 | 1 | 31.30** | 41 | 0 | | 41 | 0 | 1.36 |
| | BRAC FEMALE | | | | | | | | | | | | | | | | | |
| 1 | 40 | 16 | | 34 | 22 | | 30 | 26 | | 34 | 22 | | 51 | 5 | | 54 | 2 | |
| 2 | 35 | 47 | | 34 | 48 | | 28 | 54 | | 32 | 50 | | 64 | 18 | | 75 | 7 | |
| 3 | 38 | 22 | 12.60** | 39 | 21 | 9.13* | 36 | 24 | 10.45** | 32 | 28 | 6.77** | 60 | 0 | 16.81** | 60 | 0 | 5.44 |
| | MAC FEMALE | | | | | | | | | | | | | | | | | |
| 1 | 50 | 7 | | 47 | 10 | | 46 | 11 | | 48 | 9 | | 57 | 0 | | 57 | 0 | |
| 2 | 27 | 22 | | 29 | 20 | | 20 | 29 | | 23 | 26 | | 47 | 2 | | 48 | 1 | |
| 3 | 48 | 3 | 27.06** | 48 | 3 | 19.0** | 42 | 9 | 26.0* | 46 | 5 | 29.05** | 50 | 1 | 2.34 | 50 | 1 | 1.16 |

A**, *-Significantly different at 0.01 and 0.05 level by $\chi^2$ test.

square test from biotype to biotype. However, in terms of their hindtarsal plates, the frequencies of insects falling under each category did not differ significantly (Table 3).

Among the brachypterous females of the three biotypes, the right tarsi of all the legs were significantly different at 1% level while the left fore-and mid-tarsi were significantly different at 5% level by Chi-square test. Insignificant difference was observed in left hindtarsi. In macropterous females, the right and the left foretarsal and the left midtarsal unguitractor plates in the three biotypes differed significantly at 1% level, whereas the differences in right mid-tarsal plates were significant at 5% level by Chi-square test. Differences were insignificant in the right and left unguitractor plates of hindtarsi in the three biotypes.

The inserted unguitractor plates occurred mainly in midtarsi followed by foretarsi and least in hindtarsi of the three biotypes. As in males, the females of biotype 2 exhibited the highest frequencies of inserted unguitractor plates in fore-and mid-tarsal segments.

## DISCUSSION

Biotypes are a major threat to the stability of resistant rice varieties. Failure to recognize their existence in nature can have far reaching and frustrating consequences in pest management.

A systematic study of *N. lugens* biotypes is essential to pest management. Any variation in the pest's biotypes can be monitored through both differential varietal or host plant reactions as well as through the pest's morphological, cytological, behavioral and physiological attributes. The morphological and cytological attributes of adults provided effective criteria for differentiating *N. lugens* biotypes.

Alterations in the ecological and physiological traits of a species are frequently followed by subtle changes in their morphological characteristics (Bey-Bienko 1958). Morphology is the end product of physiological activity, initiated by the genome and modified by the environment (Eastop 1973). Also, a change in the physiology of the immature stage of development is likely to result in a change in morphology of adult.

Morphological variations has been recorded among biotypes or races of several insect species: the fruit fly, *Drosophila robusta* Sturtevant (Stalker & Carson 1947); the European corn borer, *Ostrinia nubilalis* (Hübner) (Kim et al. 1967); the pea aphid, *Acyrthosiphon pisum* (Harris)(Thottapilly et al. 1977), and the greenbug, *Schizaphis graminum* (Rondani) (Fargo et al. 1986). Saxena & Rueda (1982) made in-depth evaluations and multivariate analyses of the morphology and morphometrics of the sensory appendages(antenna, leg, and rostrum) of *N. lugens* biotypes. They found distinct segregations of the three biotypes of brachypterous and macropterous *N. lugens*. Contrary to their findings, Sogawa (1978) found no significant differences among biotypes of *N. lugens* in genitalia and leg except the frequency of number of spines on the hind basitarsus. Also, Liquido(1978) did not observe any morphological differences among biotypes in terms of leg and genitalia characters. In the case of the three Korean biotypes of *N. lugens*, this study demonstrated that the lateral lobes of females and unguitractor plates in the tarsi of legs distinctly segregated the three biotype populations.

*N. lugens* in Korea is a polymorphic species composed of integradations of biotype populations which exhibited subtle morphological variations. Such variations are *de novo* isolating mechanisms leading toward sibling speciation. The occurrence of three distinct *N. lugens* biotypes in Korea is an evident fact of sympatric speciation by destructive selection (Thoday & Gibson 1962) and host specialization (Bush 1969).

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