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Variations in Leg Characters Among Three Biotypes of the Brown Planthopper, *Nilaparvata lugens* (Stål), in Korea

한국산 벼멸구 생태형의 계량형태적 분류

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ABSTRACT Morphometric investigations of the leg characters of both sexes of brachypterous Korean *N. lugens* biotypes were made. Simple and multivariate statistical analyses revealed that the three *N. lugens* biotypes differed from one another. The amount of variation and segregation between and among the three biotype populations were approximated by the scatter plot diagrams based on the computed discriminant scores. The variables of leg characters provided the most significant segregations of three biotype populations, thus, categorizing the three biotypes as distinct intraspecific populations of *N. lugens*

KEY WORDS *Nilaparvata lugens*, morphology, biotype

초 록 우리나라에 발생하고 있는 벼멸구 생태형의 형태적 차이를 구명하고자 생태형 1, 2, 3의 단시형 암컷과 숫컷의 다리부분의 형태를 관찰하였다. 앞다리, 가운데 다리 그리고 뒷다리의 제3부절의 형태를 51개 부위에서 조사한 다음 통계학적 분석을 위하여 정준 판별 분석법을 도입하였다. 각 생태형간의 Mahalanobis distance는 숫컷의 경우 생태형 2와 3 사이에서 가장 짧았고, 암컷은 생태형 1과 2 사이에서 가장 길었다. Scatter plot diagram상에서 각 생태형간 분리현상이 뚜렷하여 중심점이 각각 다르게 나타났고 각 생태형에 속하는 개체는 중심점 부근에 고르게 분포하였다. 각 생태형간의 Group membership 조사에서 암수 모두 각 생태형은 각각 동일한 생태형으로 분류되었다.

검 색 어 벼멸구, 생태형, 형태

The association of virulence in insect pests with host plant resistance was reported by Gallun(1972), Everson and Gallun (1980), Gallun and Khush (1980), Saxena and Barrion (1985), and several other workers. The occurrence of biotypes is an evolutionary genetic phenomenon (Saxena and Barrion 1986). Biotypes represent an infraspecific category for variant populations of similar genotype for a given biological attribute, such as the ability to colonize and thrive on erstwhile resistant cultivars. The gene-for-gene relationship exists between the genotype of virulence in the pest species and the genotype for resistance in the host plant.

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The phenotypic and genotypic asssessments have revealed that biotypes are transitory variants at the primordial stages of speciation. They are undergoing either anagenetic (phyletic) or partial to complete cladogenetic evolution. After ample time of continuous exposure to appropriate stabilized environments, each biotype eventually may emerge as a sibling species (in sympatric populations) or subspecies (in allopatric populations).

Thus the biotype concept has evolved from broad definition to a more specific categorization based on the amount of biological information concerning the resistance of host plants and the virulence of insect pest populations.

The evolution of biotypes is an exceedingly complex process which is governed by an interaction of the genetic and other biological characteristics of pest populations and the extent of cultivation of resistant plant (Barrion, 1985). The mode of interaction between the pest and host system is essentially genetic in nature. In addition, the genetic factors may involve the dominance and initial frequency of genes that confer the ability on the pest to thwart host plant resistance.

MATERIALS AND METHODS

Through seedling bulk tests, three variant populations specific to their varietal hosts were isolated from field populations: Biotype 1(B1) infested Chucheongbyeo (rice variety with no gene for reststance), Biotype 2(B2) fed on Cheongcheongbyeo (rice with *Bph* 1 resistance gene), and Biotype 3(B3) survived on Milyang 63 (rice variety with *bph* 2 gene). Each of the biotype population was maintained in well-ventilated rearing cages made of acryl with the top and both sides consisting of fine mesh screen. The three biotypes were mass reared in the Department of Entomology at Agricultural Sciences Institute, Suweon. From the caged populations of the three *N. lugens* biotypes, brachypterous males and females were randomly sampled with an aspirator and transferred directly into 70% ethyl alcohol for fixation.

Individuals of each of the biotype populations were cleared and mounted based on the technique of Saxena and Rueda (1982). About 51 morphological and morphometric attributes of the legs were examined and measured in 20 individuals each of male and female brachypterous *N. lugens* of each biotype (Table 1 and Fig. 1). Qualitative and quantitative assessments of the body parts 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.

STATISTICAL ANALYSES

Morphometric data on the leg characters were analyzed using the Statistical Analysis System (SAS Institute, 1985): The specific analyses made for various aspects were as follows: 1) The means and standard errors were determined for each character of 20 male and female brachypterous individuals of each biotype. 2) Both sexes of the three biotypes were differentiated based on the canonical discriminant analysis of the morphometrics of the legs. 3) The distance of one biotype from the other biotypes in terms of the morphometrics of the legs was computed using the Mahalanobis expression for distance.

Table 1. Morphometric characters of the legs of adult *N. lugens*

Abbreviations	Characters
DSIL	Number of 1st dorsocentral setae on left 3rd subsegment
DSIR	Number of 1st dorsocentral setae on right 3rd subsegment
DS2L	Number of 2nd dorsocentral setae on left 3rd subsegment
DS2R	Number of 2nd dorsocentral setae on right 3rd subsegment
ADL	Number of anteroventral setae on left 3rd subsegment
ADR	Number of anterodorsal setae on right 3rd subsegment
PDL	Number of posterodorsal setae on left 3rd subsegment
PDR	Number of posterodorsal setae on right 3rd subsegment
AVL	Number of anterodorsal setae on left 3rd subsegment
AVR	Number of anterodorsal setae on right 3rd subsegment
PVL	Number of posterodorsal setae on left 3rd subsegment
PVR	Number of posterodorsal setae on right 3rd subsegment
DPL	Length of dorsal plate (μ)
ML	Length of tarsal membrane (μ)
UPL	Length of unguitractor plate (μ)
CL	Length of tarsal claw at maximum (μ)
CW	Width of tarsal claw at maximum (μ)
CL/CW	Ratio between length and width of tarsal claw
TL	Total length of 3rd subsegment (μ)

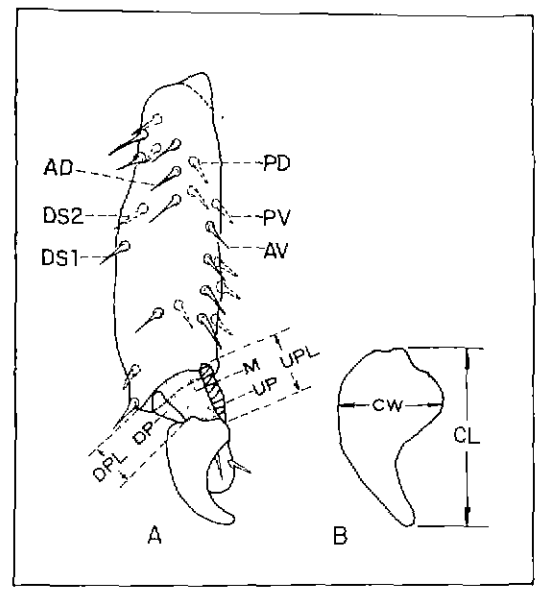


Fig. 1. Camera lucida drawing of the third subsegment of the tarsus of *N. lugens*. (A) = whole third subsegment (B) Enlarged tarsal claw, AD = anteroventral setae, AV = anteroventral setae, CL = length of tarsal claw, CW = width of tarsal claw, DP = dorsal plate, DPL = length of dorsal plate, DSI = first dorsocentral setae, DS2 = second dorsocentral setae, M = tarsal membrane, PD = posterodorsal setae, PV = posteroventral setae. UP = unguitractor plate, UPL = length of unguitractor plate.

RESULTS

Simple statistics

Foretarsus. Out of 19 foretarsal characters, the significantly mean values were noted in 5 characters in males (PVR, DPL, CL, CL/CW, and TL) and 3 characters in females (DS2L, PDL, and UPL). Among the males, B1 was significantly distinct from B2 based on characters CL and CL/CW, and from B3 in terms of CL/CW and TL. On the other hand, B2 differed from B3 in characters such as PVR, DPL, and TL. Among the females, B1 differed significantly from B2 in terms of PDL, and with B3 in terms of DS2L and UPL. Likewise, B2 difered from B3 in terms of PDL (Table 2).

Midtarsus. out of the 19 characters examined, 9 characters in males and 4 characters in females differentiated significantly the three biotypes. Two midtarsal characters, DS2R and UPL, markedly distinguished both sexes of the three botypes. B3 had higher setal counts com-

pared with B1 and B2. B1 and B2 males were differentiated significantly in terms of DS2R only. However, between B1 and B3, significant variations were shown by the following characters: DS2L, PDL, PVL, UPL, and TL. Between B2 and B3, the varying characters were DSIR, DS2L, DS2R, PDL, AVR, PVL, DPL, UPL and TL. The females, on the other hand, were differentiated based on the following characters: B1 from B2 (DS2R, PDR, and UPL); B1 from B3 (DS2R, ML, and UPL); and B2 with B3 (PDR and ML) (Table 3).

Hindtarsus. Significant differences in 6 out of 13 hindtarsal characters were observed among males of the three biotypes. B1 differed significantly from B2 in terms of characters DS2L, DS2R, and AVL, and from B3 in terms of characters DS1L, PDR, and TL. B2 possessed characters such as AD1, RDR, and TL which were markedly distince from B3. Among the females, just one character, DS1L, was significantly different. B1 females varied significantly from B2 and B3, while the mean values of the latter two biotypes did not differ significantly (Table 4).

Canonical Discriminant Analysis

The scatter plot diagrams based on computed discriminant scores of leg characters of males (Fig. 2a) approximated the amount of variation and segregations between and among the three *N. lugens* biotypes. The variables of leg characters of males provided the most distinctive segregations of the biotypes. Their group centroids

Table 2. Means and standard errors* in the foretarsus of legs of brachypterous adults in the three *N. lugens* biotypes

Characters	Males			Females		
	B1	B2	B3	B1	B2	B3
DSIL	4.6±0.11	4.6±0.15	4.7±0.11	4.8±0.12	4.9±0.08	4.9±0.97
DSIR	4.6±0.15	4.4±0.11	4.7±0.10	4.7±0.12	4.7±0.08	5.0±0.11
DS2L	3.6±0.11	3.7±0.10	3.8±0.11	3.7±0.11b	3.9±0.08ab	4.1±0.09a
DS2R	3.7±0.10	3.7±0.13	3.7±0.12	3.9±0.07	3.9±0.11	4.1±0.10
ADL	3.3±0.11	3.1±0.12	3.3±0.11	3.6±0.11	3.2±0.12	3.4±0.11
ADR	3.2±0.14	3.1±0.13	3.0±0.09	3.6±0.11	3.4±0.13	3.7±0.11
PDL	2.9±0.14	2.7±0.12	3.1±0.12	3.3±0.13a	2.8±0.12b	3.4±0.11a
PDR	2.9±0.10	2.8±0.08	3.0±0.09	3.1±0.12	3.0±0.07	3.3±0.12
AVL	4.2±0.12	4.3±0.16	4.3±0.11	4.6±0.11	4.7±0.15	4.8±0.16
AVR	4.4±0.15	4.3±0.16	4.4±0.13	4.6±0.17	4.4±0.11	4.6±0.15
PVL	3.7±0.12	3.7±0.15	3.9±0.09	4.3±0.10	4.3±0.11	4.3±0.10
PVR	4.0±0.13ab	3.7±0.10b	4.1±0.07a	4.1±0.14	4.1±0.10	4.3±0.11
DPL	18.7±0.62ab	19.4±0.43a	17.6±0.44b	19.6±0.70	19.2±0.81	19.5±0.61
ML	17.7±0.99	18.9±0.62	17.6±0.74	20.4±1.02	20.3±0.92	20.1±0.58
UPL	36.1±0.56	36.7±0.67	35.2±0.68	40.8±0.45a	40.6±0.66ab	38.8±0.63b
CL	58.1±0.73b	60.8±0.69a	59.5±0.75ab	72.9±0.91	71.2±0.73	70.7±0.70
CW	35.7±0.70	35.8±0.70	34.3±0.39	40.4±0.60	39.3±0.77	38.7±0.71
CL/CW	1.64±0.04b	1.71±0.03a	1.74±0.02a	1.81±0.04	1.82±0.04	1.84±0.04
TL	210.3±1.77a	209.7±2.11a	204.7±1.49b	241.2±2.08	240.8±1.64	237.1±1.65

* In a row, means followed by a common letter are not significantly different at 5% level by t-test. Avg. of 20 replicates.

Table 3. Means and standard errors* in the midtarsus of legs of brachypterous adults in the three *N. lugens* biotypes

Characters	Males			Females		
	B1	B2	B3	B1	B2	B3
DSIL	4.2±0.14	4.2±0.12	4.4±0.11	4.5±0.11	4.8±0.14	4.7±0.11
DSIR	4.6±0.11ab	4.3±0.13b	4.7±0.10a	4.6±0.11	4.7±0.15	4.8±0.14
DS2L	3.3±0.11b	3.4±0.15b	3.9±0.07a	3.5±0.11	3.8±0.12	3.8±0.09
DS2R	3.8±0.09a	3.3±0.19b	3.9±0.14a	3.5±0.11b	3.9±0.12a	3.9±0.10a
ADL	3.3±0.15	3.3±0.11	3.4±0.11	3.7±0.15	3.3±0.11	3.7±0.11
ADR	3.2±0.16	3.2±0.12	3.3±0.13	3.5±0.15	3.6±0.17	3.7±0.11
PDL	2.8±0.12b	2.9±0.09b	3.2±0.09a	3.4±0.15	3.1±0.11	3.4±0.11
PDR	2.9±0.10	2.9±0.10	3.1±0.08	3.3±0.13a	2.9±0.11b	3.3±0.10a
AVL	4.4±0.15	4.2±0.14	4.5±0.15	4.7±0.20	4.8±0.13	5.0±0.13
AVR	4.4±0.18ab	4.7±0.16a	4.3±0.15b	4.6±0.15	5.0±0.19	4.8±0.14
PVL	3.8±0.09b	3.9±0.07b	4.1±0.07a	4.2±0.16	4.2±0.13	4.5±0.15
PVR	3.9±0.10	4.1±0.07	4.1±0.07	4.2±0.17	4.1±0.09	4.4±0.11
DPL	19.1±0.48ab	19.5±0.39a	18.1±0.54b	18.5±0.85	20.5±0.65	19.7±0.70
ML	18.7±0.96	19.0±0.49	18.9±0.56	23.2±0.70a	21.3±0.74a	18.9±0.77b
UPL	37.3±0.60a	37.2±0.56a	35.6±0.52b	43.4±0.48a	40.9±0.63b	39.6±0.53b
CL	60.0±0.52	60.3±0.96	60.2±0.69	72.4±0.89	71.1±0.99	70.4±1.05
CW	35.9±0.38	35.7±0.43	35.3±0.51	42.2±0.69	40.1±0.69	39.7±0.86
CL/CW	1.67±0.02	1.69±0.03	1.72±0.03	1.72±0.04	1.79±0.05	1.79±0.05
TL	218.8±1.98a	217.2±2.02a	210.8±1.77b	249.8±2.24	248.1±1.59	246.1±2.31

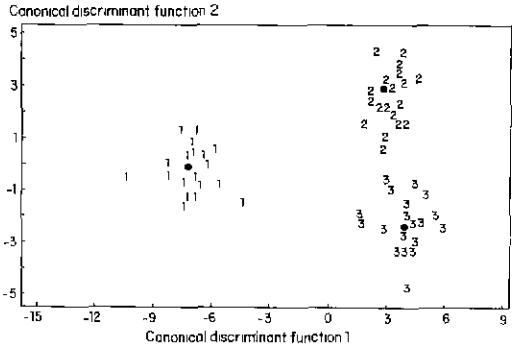
* In a row, means followed by a common letter are not significantly different at 5% level by t-test. Avg. of 20 replicates.

Table 4. Means and standard errors* in the hindtarsus of legs of brachypterous adults in the three *N. lugens* biotypes

Characters	Males			Females		
	B1	B2	B3	B1	B2	B3
DSIL	5.2±0.21	5.5±0.22	5.7±0.22	5.4±0.18b	6.3±0.23a	6.5±0.18a
DSIR	5.4±0.14	5.3±0.16	5.4±0.18	6.0±0.17	6.2±0.20	6.4±0.18
DS2L	4.6±0.22b	5.3±0.16a	4.9±0.14ab	5.2±0.17	5.2±0.17	5.2±0.17
DS2R	4.5±0.22b	5.3±0.21a	5.0±0.15ab	5.0±0.15	5.5±0.17	5.3±0.16
ADL	3.4±0.17b	3.6±0.15b	3.9±0.10a	3.9±0.17	4.3±0.26	4.1±0.10
ADR	3.9±0.12	3.7±0.14	3.7±0.10	3.9±0.18	4.4±0.20	4.0±0.10
PDL	3.8±0.18	3.9±0.12	3.9±0.17	4.1±0.10	4.4±0.15	4.4±0.15
PDR	3.5±0.14b	3.8±0.17b	4.0±0.15a	4.2±0.17	4.5±0.15	4.5±0.17
AVL	3.8±0.12b	4.2±0.16a	4.0±0.14ab	4.3±0.13	4.3±0.16	4.3±0.11
AVR	3.9±0.11	4.3±0.17	4.1±0.10	4.6±0.11	4.2±0.09	4.4±0.15
PVL	3.8±0.14	4.1±0.19	4.0±0.11	4.4±0.13	4.8±0.18	4.3±0.13
PVR	3.8±0.11	4.0±0.10	4.1±0.12	4.4±0.11	4.3±0.11	4.5±0.17
TL	259.8±2.25a	256.8±1.99a	251.9±1.83b	297.9±3.05	295.9±2.11	297.2±2.11

* In a row, means followed by a common letter are not significantly different at 5% level by t-test. Avg. of 20 replicates.

were evidently separated. As in males, the clusters of each female biotypes in the canonical variate plane were tight in terms of their leg characters. The group centroid of each biotype was distinct from the other (Fig. 2b). Thus, the amount of variation in leg characters within individuals of each biotype populations was low, however, between and among biotypes, the amount of variation was distinctly high. The canonical discriminant analysis categorize the three biotypes as distinct intraspecific populations of *N. lugens*



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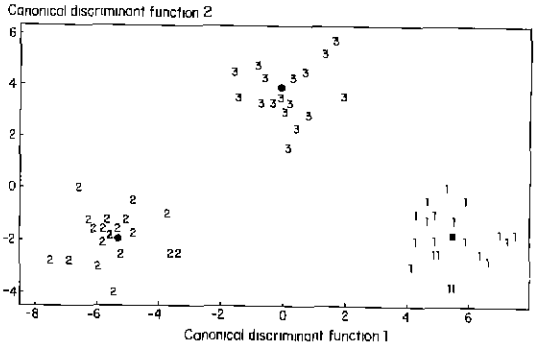

B

Fig. 2. Scatter plot diagrams based on computed discriminant scores of leg characters of brachypterous males (A) and females (B) of B1, B2, and B3.

Mahalanobis distance

Comparisons between biotypes were provided by the Mahalanobis' distance (Table 5).

The higher the values of Mahalonobis distance, the higher was the degree of differentiation between biotypes. The leg characters provided the highest distances between males of B1, B2, and B3; B1 vs. B3 > B1 vs. B2 > B2 vs. B3. Thus, the leg characters of B2 were closely resembling those of B3, while leg characters of B1 were distinctly different from B3. In the case of females, the distances were highest between B1 vs. B2, while B3 resembled B1 and B2.

Table 5. Relationship between biotype of brachypterous males and females *N. lugens* based on the Mahalanobis distance

Biotype pairs	Mahalanobis distance*	
	Male	Female
B1 vs B2	10.52	10.98
B1 vs B3	11.13	7.78
B2 vs B3	4.99	7.80

Group Classification of Biotypes

The males' as well as females' leg characters of the three biotypes exhibited 100% group classification. Thus, all the 20 individuals examined per biotype possessed leg characters exclusive for the biotype. Thus, the predicted group memberships of the three biotypes showed that their leg character significantly distinguished each biotype populations from the others.

DISCUSSION

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

Table 6. Predicted group membership of *N. lugens* biotypes based on the leg characters of brachypterous adults

Sexes	Actual group	Predicted group membership(%)		
		Biotype 1	Biotype 2	Biotype 3
Male	Biotype 1	100	0	0
	Biotype 2	0	100	0
	Biotype 3	0	0	100
Female	Biotype 1	100	0	0
	Biotype 2	0	100	0
	Biotype 3	0	0	100

(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.

At the genetic and specific levels, the qualitative morphological characters are most convenient and useful diagnostic parameters. The distinction in such characters becomes less evident in infraspecific taxa such as sibling or cryptic species, subspecies, host races, and biotype. However, these infraspecific taxa may be revealed through statistical analysis of fine structure evaluations of groups of specimen from various sources (Kim et al. 1966). A biotype or group usually appears as morphologically distinct from the others if a combination of characters is used in a multivariate space. Univariate statistical analysis hardly provide morphological distinction (Fargo et al. 1986). Morphological variations have been recorded among biotype or races of several insect species: fruit fly, *Drosophila robusta* Sturtevant (Stalker & Carson 1947); the European corn borer, *Ostrinia nubilalis* (Hubner) (Kim et al. 1967); the pea aphid, *Acyrthosiphon pisum* (Harris) (Thottapilly et al. 1977), other aphid species, (Singh et al. 1981); 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*. They found distinct segregations of the three biotypes of brachypterous and macropterous *N. lugens*. Utilizing these techniques and criteria of evaluation, the three *N. lugens* biotypes from Korea were found to differ significantly from each other in leg characters. *N. lugens* in Korea is a polymorphic species comprising intergradations of biotype populations which exhibit subtle morphological variations. These morphological variations among *N. lugens* biotypes are evidences of *de novo* genetic isolating mechanisms.

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