Appl. Entomol. Zool. **27** (3): 445–454 (1992)

Maintenance and Selection of Strains Exhibiting Specific Wing Form and Body Colour under High Density Conditions in the Brown Planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae)[1]

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(Received April 24, 1992; Accepted May 19, 1992)

Selections more than over 25 successive generations for specific wing form and body colour under low-density rearing conditions in *Nilaparvata lugens* failed to produce pure-bred lines with those characters. However, selections over 12 to 30 successive generations under high-density rearing conditions were successful in obtaining various lines predominantly producing specific wing form and body colour over a broad range of nymphal densities in both sexes: totally brachypterous lines with yellowish brown or highly melanized body colour and totally macropterous lines with highly melanized or yellowish brown body colour. Thus, wing form and body colouration are controlled by different genes. By successive selections based solely on wing form, two lines producing predominantly brachypterous or macropterous wing forms were obtained, but they exhibited an intermediate body colour between yellowish brown and black. When five field-collected strains were reared under high density conditions, their wing form and body colour responses to rearing density remained unchanged for more than over 20 generations. On the other hand, maintenance of these strains under low density conditions rendered them to exhibit considerable different responses from original ones, even though no special selection has been given. These results suggest that genetically controlled characters can be stably exhibited under high density conditions, while phenotype expression under low density conditions is largely influenced by the physiological situation of the individual hoppers.

Key words: wing form, body colour, wing-polymorphism, density, *Nilaparvata lugens*

INTRODUCTION

Many species of insects such as aphids, stink bugs, water striders, crickets, weevils, planthoppers etc. show wing polymorphism, in most cases dimorphism: apterous or short-winged brachypterous form for non-flier and long-winged macropterous form for flier. Expression of wing form is largely influenced by some of environmental factors, such as density, photoperiod, nutrient etc. (HARRISON, 1980). Recently, evidence is accumulating to support that wing polymorphism is fundamentally under genetic control, polygenic system being involved for the phenotype expression in most insects

[1] This work was supported in part by a Grant-in-Aid for Science Research Program B from the Ministry of Education, Science and Culture, Japan (No. 03454055).

so far studied (Roff, 1986). For elucidation of genetic mechanism of wing polymorphism, it is essential to analyze the results of crossing experiments using genuine lines in which phenotype is stably expressed in wing-form assay system employed. Otherwise, we may lead into wrong conclusion with the genes involved in the wing-form expression.

Recently, it has been shown that great variation in nymphal density and wing-form relations occurs in field populations of the brown planthopper, *Nilaparvata lugens* (Nagata and Masuda, 1980; Chu et al., 1982; Iwanaga et al., 1985, 1987; Morooka et al., 1988). When compared under uniform laboratory conditions, some populations show extremely high proportions of brachypters (brachypterous strain) and others show high proportions of macropters (macropterous strain) over a broad range of rearing density, while the remaining populations exhibit various wing-form responses to density between the two extremes (Iwanaga et al., 1985, 1987; Morooka et al., 1988).

Morooka et al. (1988) demonstrated that there was a correlation between wing form and body colour in adults, and could obtain a highly brachypterous strain by selection over ten successive generations for yellowish brown brachypters and another strain producing predominantly macropters by selection for blackish macropters. Selections based on both characters were shown to be far more effective for obtaining strains exhibiting specific wing form than those solely based on wing form in the studies by Iwanaga et al. (1985).

In this article, we show several pieces of evidence indicating that culture lines exhibiting specific wing form and body colour **can** be generated more easily by selection under high-density rearing conditions and genetically controlled characters can be stably maintained under such conditions.

MATERIALS AND METHODS

All experiments with *N. lugens* were conducted at 25 ± 1°C under 16L–8D photoperiod. The Saga, Nagasaki (Japan), North Sumatra (Indonesia), and Manila (The Philippines) strains were those used in the previous paper (Morooka et al., 1988). Two additional strains collected in Sulawesi (Indonesia) in 1982 and in Guangdong (China) in 1984 were imported to the Kyushu National Agricultural Experiment Station before they were used in the present study.

For maintaining the strains at high-density rearing conditions, every generation 50 females and 50 males, were sampled so as to match the percentage of brachypterous form and grade of black pigmentation in both sexes to those of pre-sampled population, and enclosed in a rectangular solid vessel (11.0×12.0 cm in basal width, 18.0 cm in ht.) with about 950 rice seedlings of the Reiho variety, and 500 to 700 first-instar nymphs sampled at random from the vessel were reared in a same type vessel to adult emergence. The rearing density can be regarded to be essentially the same as that at a 150 density plot in the cylindrical vessel used in the following wing-form assay experiments. In experiments at low-density rearing conditions, 10 females and 10 males were enclosed in a rectangular solid vessel, and 50 to 70 first-instar nymphs were used for rearing.

To compare the relationships of adult wing form and body colour to nymphal density among these strains, 10, 20, 50 and 150 first-instar nymphs were reared in each cylindrical vessel (5.4 cm in dia., 22.0 cm ht.) with about 130 rice seedlings. Upon adult emergence, wing form and colour grade were determined based on the

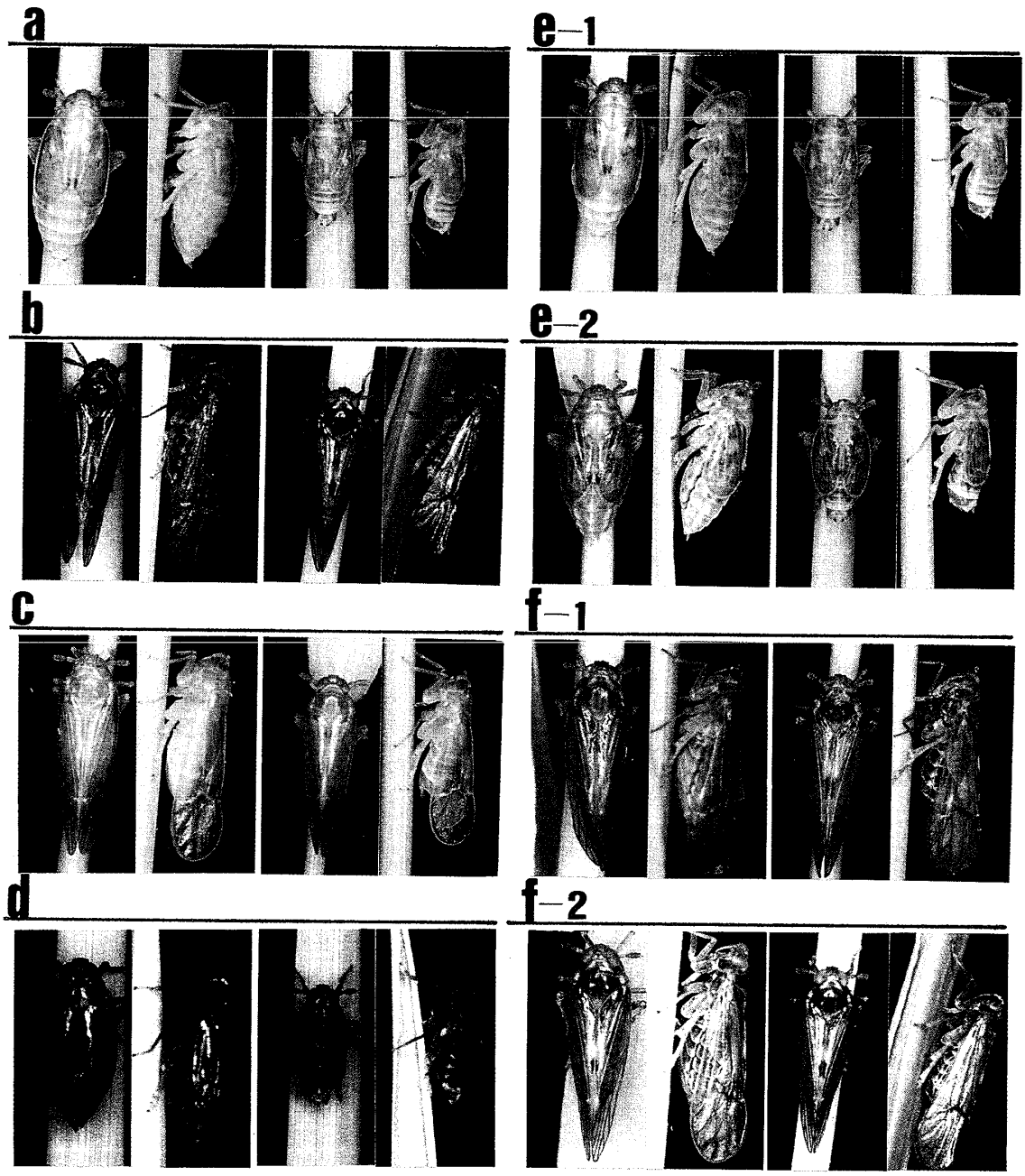

Fig. 1. Photographs of various *N. lugens* lines obtained after over 12 to 30 generations of successive selection for specific wing form and body colour as given in the respective names of the lines. The left pairs of each set of photographs are females, and the right pairs are males. a. Yellowish brown brachypters (grade I); b. Blackish macropters (grade III); c. Yellowish brown macropters (grade I); d. Brackish brachypters (grade III); e. Brachypters with colour of grade I (e-1) or II (e-2); f. Macropters with colour of grade II (f-1) or III (f-2). See text for grade of colouration.

criterion described previously (MOROOKA et al., 1988). Briefly, colour types were scored as follows: grade I, yellowish brown, having no blackish parts on the abdomen; grade III, highly blackish, having no yellowish brown parts on the abdomen; grade II, intermediate between I and III.

For high-density selection experiments, 50 females and 50 males with specific wing-form and/or colour grade selected within 24 hr after emergence were enclosed for mating in a rectangular solid vessel, and the offsprings sampled at random from the vessel were reared at 50 or 150 density plots in cylindrical vessels as mentioned above. For low-density selection experiments, 10 females and 10 males were enclosed for mating and 20 offsprings were reared in each vessel. This procedure was repeated every generation.

RESULTS

Successive selections for specific wing form and body colour under low-density rearing conditions

Body colour of ventral abdomens of adults in various lines obtained in the present study is presented in Fig. 1. Yellowish brown colour as in a and c was scored as grade I, and highly blackish one as in b and d was scored as grade III. The others as in e-2 and f-1 were grouped as grade II.

First, selections for macropters with blackish colour near grade III from the Saga strain emerging from 20 density plots were repeated. As shown in Fig. 2, the percentage of brachypterous form and mean colour grade in both sexes fluctuated greatly over 25 successive generations. Also, successive selections for brachypters with yellowish brown colour near grade I failed in producing genuine lines with those characters.

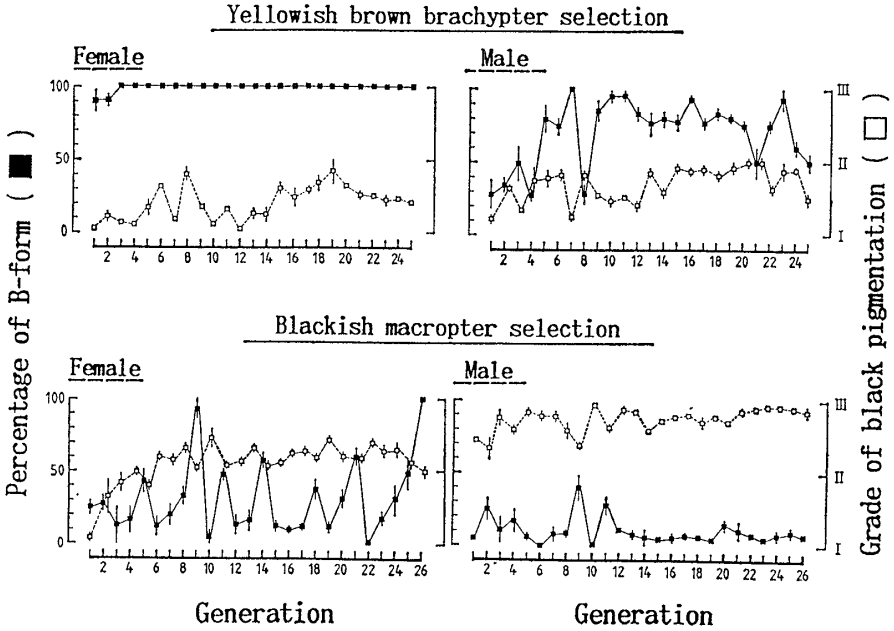

Fig. 2. Changes in % of brachypterous form (B-form) and grade of black pigmentation at 20 density plots by successive selections for adults with special wing form and body colour, as indicated in the figures, in the Saga strain. Values are averages±S.E. (six replicates).

Successive selections for specific wing form and body colour under high-density rearing conditions

As shown in Fig. 3, successive selections from the Saga strain for macropters with blackish colour, or for brachypters with yellowish brown colour at 50 density plots were far more effective in producing adults with the characters selected for than those at 20 density plots (ref. Fig. 2). Further, selections were carried out with the progenies emerging from 150 density plots in the middle of generations, which were found to be efficient to obtain pure-bred lines. Progenies after 10 generations of selection for yellowish brown brachypters at 150 density became totally brachypterous with grade I colour (ref. Fig. 1 a), while those selected for blackish macropters became totally macropterous with grade III colour (ref. Fig. 1 b).

After selections for blackish macropters had been conducted for 18 generations at high-density rearing conditions in a line used in Fig. 3, the line was also exposed for selections for specific wing form and body colour at low-density rearing conditions. As shown in Fig. 4, the selections over 11 generations under these conditions could not produce lines exhibiting characters for which selections were intended.

In early generations of successive selection for yellowish brown brachypters under high-density rearing conditions in the Saga strain, a few yellowish brown macropters occurred and such individuals were selected for. After 12 generations of selection under high-density rearing conditions, a line showing totally macropterous character with yellowish brown colour of grade I was obtained (Fig. 5, ref. Fig. 1 c). On the other hand, when brachypters with blackish body colour near grade III, which were obtained during early generations of selection for blackish macropters, were continuously selected, a totally brachypterous line with a blackish colour of grade III was established after 30 generations (Fig. 5, ref. Fig. 1 d).

Two sets of experiments were carried out to determine the efficiency of selection, when wing form was used alone as a selecting factor under high rearing conditions. As shown in Fig. 6, selection over 25 generations for brachypters from the Saga strain induced a line showing totally brachypterous form, while selection for macropters took 14 generations to produce a predominantly macropterous line. Colour of the former strain was scored grade II or grade I, and that of the latter one was grade II or grade III (ref. Fig. 1 e and f).

All lines obtained under high density conditions predominantly exhibited their specific wing form and body colour at all densities tested in our assay system using cylindrical vessel.

Maintenance of wing form and body colour characters in various strains under high-density rearing conditions

Following experiments were carried out to clarify the changes of phenotype expression in field-collected strains when maintained at high-density and low-density rearing conditions without giving any special selection. As shown in Fig. 7, all of five strains reared at high density conditions exhibited same types of responses to density in wing form and colouration as those of their ancestors, even after 43 generations as in the case of the North Sumatora strain. On the other hand, rearing of the Manila, Sulawesi and Guangdong strains at low density conditions only for three or four generations considerably changed the characters of their progenies: the proportion of brachypters increased, while the grade of black colouration tended to decline.

Thus, it was concluded that the characters of all strains kept under high-density

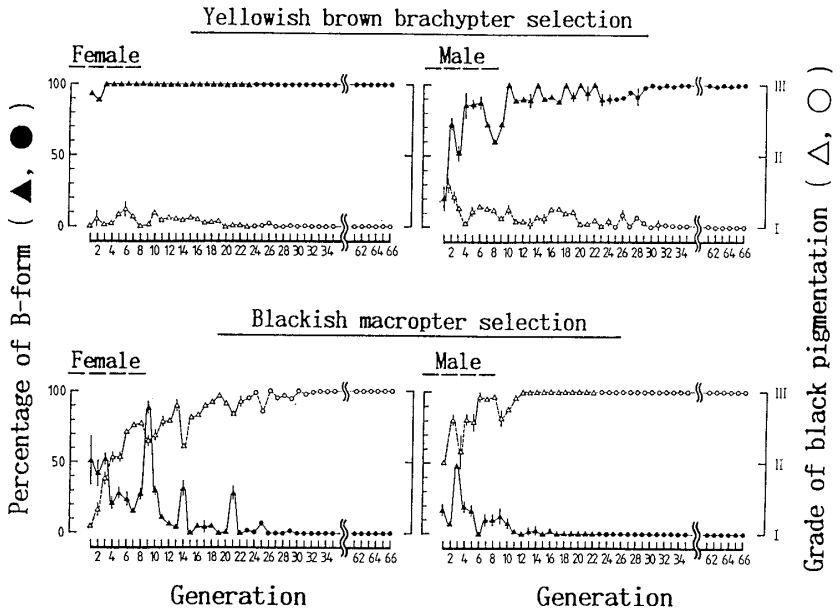

Fig. 3. Changes in % of brachypters and grade of black pigmentation at 50 (▲, △) and 150 (●, ○) density plots by successive selections for adults with special wing form and body colour, as indicated in the figures, in the Saga strain. After 22 or 23 generations of selection, rearing density was increased from 50 to 150. See Fig. 1 a and b for the lines selected for yellowish brown brachypters and blackish macropters, respectively. Values are averages± S.E. (six replicates).

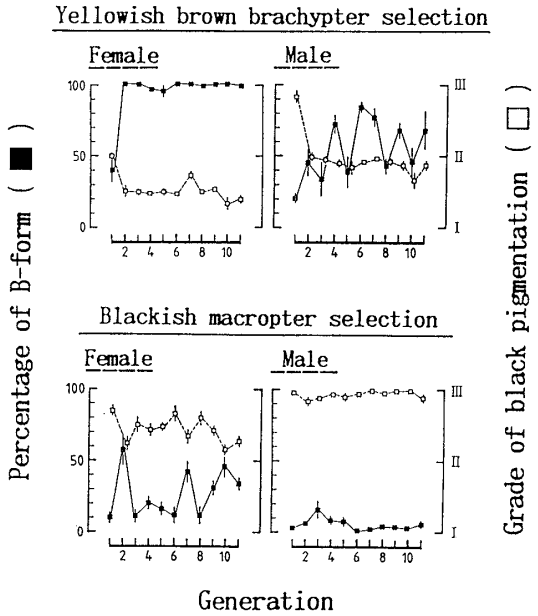

Fig. 4. Changes in % of brachypters and grade of black pigmentation at 20 density plots by successive selections for adults with special wing form and body colour, as indicated in the figures, in the line derived from the Saga strain, which were obtained after 18 generations of selection for blackish macropters under 50 density plots, as shown in Fig. 3. Values are averages±S.E. (six replicates).

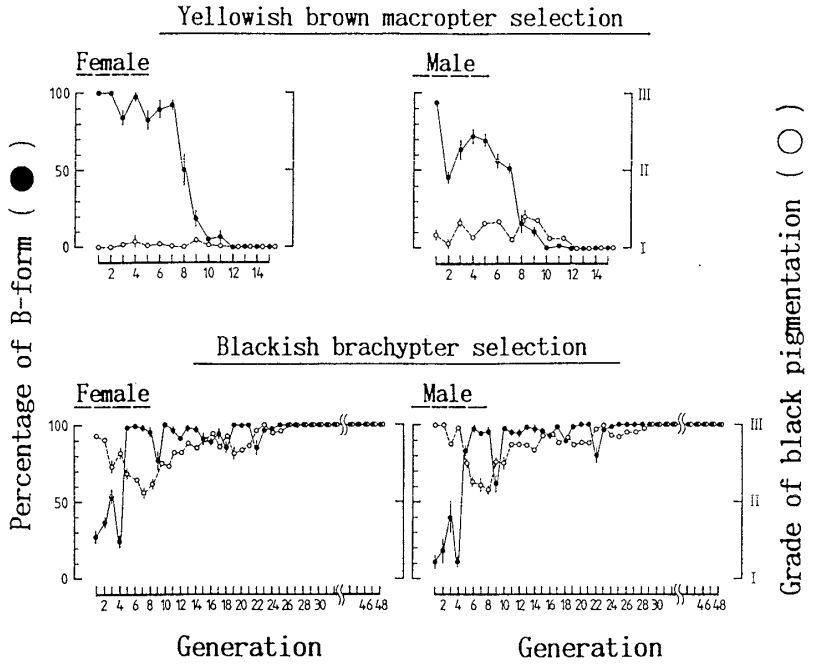

Fig. 5. Changes in % of brachypters and grade of black pigmentation at 150 density plots by successive selections for adults with special wing form and body colour, as indicated in the figures, in the lines derived from the Saga strain. See Fig. 1 c and d for the lines selected for yellowish brown macropters and blackish brachypters, respectively. Values are averages± S.E. (six replicates).

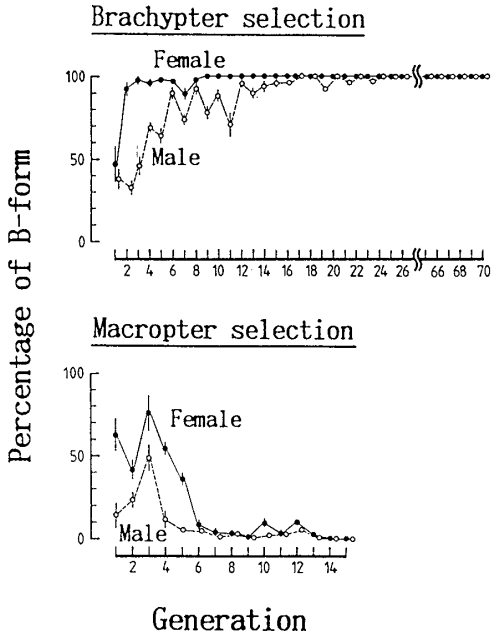

Fig. 6. Changes in % of brachypters at 150 density plots by successive selections solely for adults with special wing form, as indicated in the figures, in the Saga strain. See Fig. 1 e and f for the lines selected for brachypters and macropters, respectively. Values are averages±S.E. (six replicates).

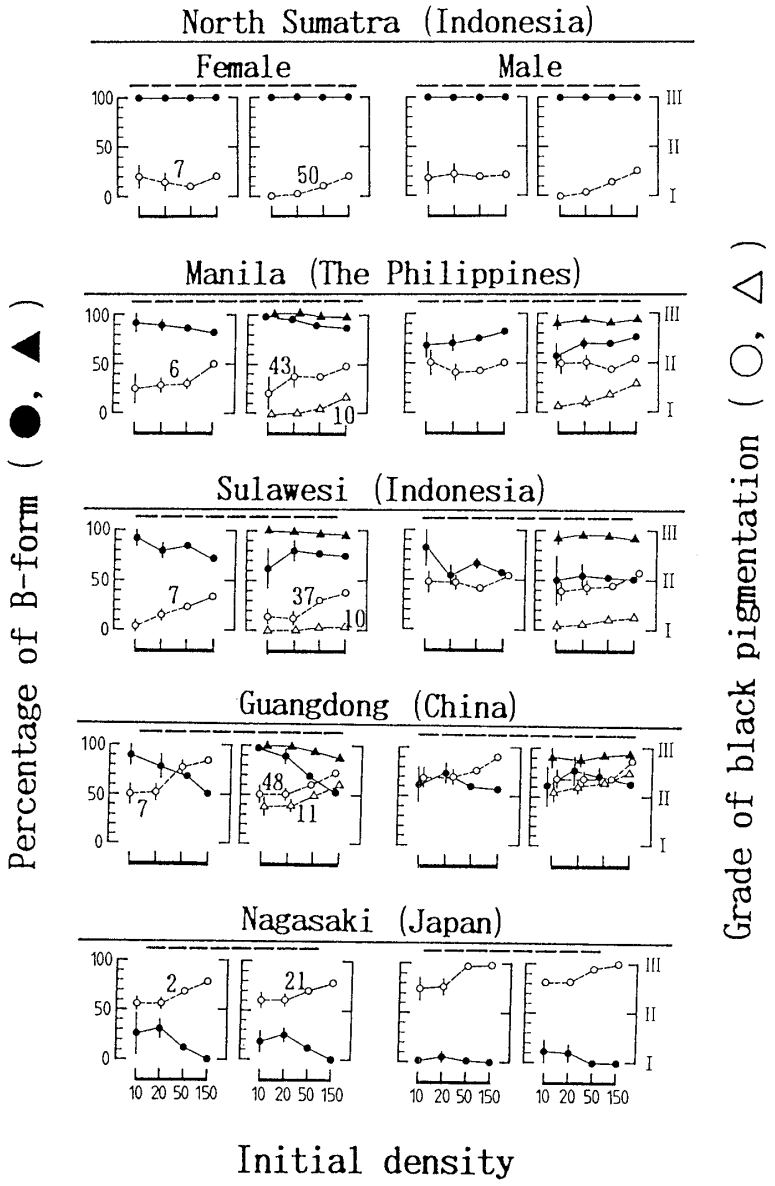

Fig. 7. Comparison of responses in adult wing form and body colour to nymphal densities in five field-collected strains reared under high density conditions (○, ●) without giving any special selection for wing form and body colour. The number given in the figure is the generation. Parts of three strains maintained under high density conditions for generations as given in respective left figures of each sex were successively reared under low density conditions in following generations (△, ▲). Values are averages±S.E. (three replicates).

rearing conditions could be maintained unchanged.

DISCUSSION

In a previous work (Morooka et al., 1988), a predominantly macropterous line with blackish colour was established after 10 generations of selection for blackish

macropters at a rearing density of 20 hoppers per a cylindrical vessel, while a highly brachypterous line with yellowish brown colour was obtained by selection for yellowish brown brachypters at a rearing density of 50 hoppers. However, during the course of selection, wing form and colour grade were found to be varied in these established lines. In this article, we have clearly demonstrated that selections under high-density rearing conditions (150 hoppers per vessel) were far more successful in obtaining pure-bred lines with the characters for which the selections had been carried out, than those under low-density rearing conditions. We further demonstrated that patterns of wing form and body colour responses to density were maintained for over 50 generations if the hoppers were reared under high density conditions.

These results suggest that wing form and body colour responses to density can be stably manifested under high density conditions. Under low density conditions such as 10 and 20 density plots, hoppers of most strains showed considerable variation in the percentage of brachypterous form and colour grade, while those from 50 and 150 density plots gave essentially definite values in these responses, as demonstrated by smaller variations of these values in all of field collected strains (Fig. 7). These facts appear to indicate that phenotype expression at low density is largely influenced by circumstantial conditions of the individual hoppers.

In selection experiments conducted in the present study, it should be taken into consideration that selections have been carried out twice at each generation, firstly for adults with specific phenotype, and secondly for first-instar nymphs deriving from the parents. The lines with different wing form and body colour established in the present experiments also differ in pre-oviposition period and fecundity: brachypterous lines tend to have shorter pre-oviposition period and higher fecundity than macropterous lines (Morooka, unpublished). So, small mass selection of first-instar nymphs deriving from adults raised under low density may work to obtain progenies from parents with shorter pre-oviposition period and higher fecundity, rendering a great genetic drift for the selection intended. On the other hand, large mass selection under high density conditions would be far more fruitful for obtaining progenies with selected character, because of smaller variation in phenotype expression of the parents, and also of larger gene pool for following generation. Collection of larger number of first-instar nymphs from a same sample size is expected to allow less genetic drift which will work to select more representative individuals of the population, not being influenced by the bias selection. Further experiments are now in progress to know if the mechanism of effective establishment of pure-bred lines and maintenance of specific character in various strains under high rearing conditions can be explained by these two reasons.

In a previous paper (Morooka et al., 1988), we demonstrated a negative correlation between the proportion of brachypters and the degree of black pigmentation in *N. lugens* adults. In this study, we could produce lines predominantly exhibiting a specific wing form either with a yellowish brown body, or with a highly melanized body. This indicates that wing form and body colour are regulated by different genes, locating at close loci on the same chromosome. A series of genetic analysis of wing form and body colour using the lines with these specific characters is now in progress.

ACKNOWLEDGMENTS

We thank Dr. Shingo Oya for allowing us to analyze four field-collected strains imported from other countries, and Mrs. Lisa Tsukamoto for correcting the English.

REFERENCES

Chu, Y. I., R. Kou and Y. S. Lee (1982) Studies on the geographical variation of the brown planthopper (*Nilaparvata lugens* Stål) in Taiwan. *Chin. J. Entomol.* **2**: 1–55 (in Chinese with English Summary).

Harrison, R. G. (1980) Dispersal polymorphism in insects. *Annu. Rev. Ecol. Syst.* **11**: 95–118.

Iwanaga, K., S. Tojo and T. Nagata (1985) Immigration of the brown planthopper, *Nilaparvata lugens*, exhibiting various responses to density in relation to wing morphism. *Entomol. Exp. Appl.* **38**: 101–108.

Iwanaga, K., F. Nakasuji and S. Tojo (1987) Wing polymorphism in Japanese and foreign strains of the brown planthopper, *Nilaparvata lugens*. *Entomol. Exp. Appl.* **43**: 3–10.

Morooka, S., N. Ishibashi and S. Tojo (1988) Relationships between wing-form response to nymphal density and black colouration of adult body in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae). *Appl. Entomol. Zool.* **23**: 449–458.

Nagata, T. and T. Masuda (1980) Insecticide susceptibility and wing-form ratio of the brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) and the white backed planthopper, *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae) of Southeast Asia. *Appl. Entomol. Zool.* **15**: 10–19.

Roff, D. A. (1986) The evolution of wing dimorphism in insects. *Evolution* **40**: 1009–1020.