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Further Studies of Cytoplasmic Incompatibility in Local Populations of *Laodelphax striatellus* in Japan (Homoptera: Delphacidae)

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Based on cytoplasmic incompatibility, Japanese local populations of *Laodelphax striatellus* are divided into two groups in accordance with their localities: northeastern and southwestern. Crosses between females of northeastern group and males of southwestern one were sterile whereas the reciprocal crosses were fertile. Test crosses of three newly collected populations, Ishigaki, Kagoshima, and a migrant population from the East China Sea showed that all three belonged to the southwestern group in regard to their crossing type. Some eggs, however, deposited by incompatible male and female pairs exceptionally developed to nymphs. To examine whether the nymphs were hybrids, crossing experiments were undertaken between red-eyed mutant females of the northeastern group and black-eyed wild type males of the southwestern. The mutant gene of red eye was recessive on an autosome and was inherited by F₂ or BC₁ generations through these crosses. It is, therefore, concluded that normal fertilization occurred in some incompatible eggs.

INTRODUCTION

The small brown planthopper (SBPH), *Laodelphax striatellus*, shows cytoplasmic incompatibility between two different groups of strains (NODA, 1984 a). In reciprocal crosses between different strains of SBPH, one of the crosses is fertile but the other does not give nymphal offspring in spite of successful copulation. No signs of embryonic development are observed. Chromosomal genes do not primarily participate, but a certain cytoplasmic factor which is inherited through generations is involved in this phenomenon (NODA, 1984 a). Cytoplasmic incompatibility was first reported in mosquitoes, *Culex* complex (LAVEN, 1951, 1956) and *Aedes scutellaris* (SMITH-WHITE and WOODHILL, 1954). Later it was also found in the almond moth, *Ephestia cautella* (BROWER, 1976) and in the alfalfa weevil, *Hypera postica* (HSIAO and HSIAO, 1985).

From testcrosses between local populations, Japanese SBPH populations are of two groups: one distributed in the northeastern part and the other in the southwestern part of Japan (NODA, 1984 b). The cross between the northeastern female and the southwestern male is incompatible but the reciprocal cross is compatible. Thus the Japanese local populations of SBPH show unidirectional incompatibility. However, in some cases embryonic development of the eggs laid by the incompatible pair proceeds normally to hatch nymphs.

The aim of the present study was to elucidate the following two questions. First, SBPH is sometimes caught on the East China Sea (HIRAO and ITO, 1980; NODA, 1986),

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and the evidence suggests that it is most likely to immigrate annually into western regions of the main Japanese islands. To learn the crossing type or cytoplasmic nature of these immigrants, the migrant population and two other populations from southwestern localities were crossed to determine to which group the three belonged, northeastern, southwestern or another one. Second, as mentioned, incompatible pairs between local populations sometimes, but not always, give fertile eggs which developed normally, although their number is small (NODA, 1984 b). A similar case was reported in *Culex*, but only females arose from the incompatible eggs, and the development was considered to be caused by induced parthenogenesis (gynogenesis) because the cytoplasmic incompatibility was believed to be due to the incapability of fusion of two haploid nuclei (LAVEN, 1967). Insect eggs usually start mitotic division by fusion of egg and sperm nucleus; this type of embryogenesis is called hybridogenesis. There are, however, different types of egg development, parthenogenesis, gynogenesis, and androgenesis, in which the fusion of two nuclei does not occur and development starts based on either the egg or the sperm nucleus. Testcrosses of the incompatible type of SBPH were undertaken to elucidate the cause of the occasional embryonic development.

This paper reports that migrants on the East China Sea and two other local strains from warm regions in Japan were of the same crossing type as the southwestern group and that true hybrids were produced from some incompatible crosses.

MATERIALS AND METHODS

Insect strains. The following three strains were collected in 1984: (1) the East China Sea strain (Ec): collected on the East China Sea (31° N, 126° E) at the end of June; imported with the permission of the Ministry of Agriculture, Forestry and Fisheries, Japan, No. 931 under date 1 June 1984. (2) Ishigaki strain (Ig): collected on Ishigaki Island in April. (3) Kagoshima strain (Kg): collected at Kagoshima on 23 June. Six strains used in the previous studies were also employed for testcrosses: S strain (S), Tokyo strain (To), Tsukuba strain (Tk), Odawara strain (Od), Izumo 1 strain (Iz1) and Izumo 3 strain (Iz3). The crossing types indicate that the first three strains belong to the northeastern group and the latter three strains to the southwestern.

Testcrosses for crossing potentiality. The procedures were described in a previous paper (NODA, 1984 b). Each nymph of a test strain was isolated and adults a few days old were crossed. A male and a female were introduced in a test tube containing rice seedlings, and the test tubes were placed at 25°C. Newly-hatched nymphs were observed two weeks later. Some females of incompatible pairs were then dissected to check the presence of sperm in their female spermathecae.

Testcrosses for the cause of occasional embryonic development. As for the cause of development in eggs deposited by incompatible pairs, the author assumed four possible types of reproduction: parthenogenesis, gynogenesis, androgenesis and hybridogenesis. To determine which of these account for the development of incompatible eggs, red-eyed recessive mutant gene in SBPH was used as a marker for testcrosses. First, mode of inheritance of the red-eye-gene characteristics of To strain was investigated by the cross between Iz1 female and To male and between Ec female and To male. Phenotypes of the eye color of F₂ and BC₁ generations were noted. Second, based on the results of the testcrosses for inheritance manner, To female and Ec male were crossed and the eye color of F₁ progeny from these incompatible pairs was examined. Then,

Table 1. Crosses in three newly collected local strains of *L. striatellus*

♂ \ ♀	Newly collected			Laboratory cultured				
	Ig	Ec	Kg	Iz3	Od	Tk	To	S
Ig		22-19	20-19	16-15	27-25	18-0	23-1	24-0
Ec	28-24		25-23	23-21	26-26	24-4	21-1	22-0
Kg	26-23	25-24		28-25	29-27	29-0	25-0	27-0
Iz3	15-14	12-10	19-18					
Od	19-19	24-20	19-19					
Tk	23-21	18-16	30-29					
To	30-28	23-18	29-26					
S	19-18	12-9	13-13					

Each pair of values presents the number of pairs tested (left) and producing nymphs (right). The quadrant of broken lines shows incompatible crosses.

the resultant adults of this F₁ generation were crossed *inter se* to obtain F₂ or were crossed with To strain to obtain BC₁, and the eye color of the offspring were examined.

RESULTS AND DISCUSSION

Crossing potentiality in newly collected strains

Three newly collected strains, Ec, Ig and Kg, were crossed to five strains which had been used in the previous studies (NODA, 1984 b). The newly collected strains were compatible with each other and with the southwestern strains, Od and Iz3. Most males of these three strains, however, did not fertilize the eggs of the northeastern strains, Tk, To and S. Incompatible crosses are shown in the quadrant of broken lines in Table 1. This indicates that these three strains belong to the southwestern group, and supports the previous suggestion that two local Japanese populations of SBPH which account for unidirectional incompatibility seem present in central Japan (NODA, 1984 b).

Female spermathecae were observed under a microscope and it was ascertained that copulation had taken place in most of the incompatible pairs: e.g., 18 out of 19 pairs of Tk female × Kg male, 10 out of 11 pairs of S female × Ec male, and all 11 pairs of To female × Kg male. On the other hand, some females in the compatible pairs did not produce offspring (Table 1); some of these had sperm in their spermathecae while others did not. Infertility of compatible pairs is, therefore, due partly to copulation failure.

Again, nymphs hatched from eggs produced by some incompatible pairs as noted in the previous studies (NODA, 1984 b), though their number was small. Some offspring obtained from To female × Ec male and Tk female × Ec male were back-crossed to their maternal or paternal strains. Incompatibility was recognized in the crosses between females of the F₁ and males of the paternal strains (or paternal group) (Table 2). This indicates that the crossing potentiality of the F₁ individual is determined by that of its maternal parent, and the cytoplasmic condition was transmitted from the mother without alteration. Thus, the reason some eggs deposited by incompatible pairs were fertile cannot be ascribed to the change of crossing type of the maternal parent. In

Table 2. Results of crossing F₁ from incompatible pairs with parent groups

Female	Male	No. pairs tested	No. pairs producing nymphs
To ♀	× Ec ♂		
F ₁	Ec	5	0
Ec	F ₁	4	2
To	F ₁	4	4
Tk ♀	× Ec ♂		
F ₁	Ec	5	0
F ₁	Tk	4	3
Ec	F ₁	5	4
Tk	F ₁	5	5
F ₁	Ec	5	2 ^a
F ₁	Tk	4	3
Ec	F ₁	6	4
Tk	F ₁	7	7
F ₁	Od	5	0
F ₁	To	5	5
Od	F ₁	4	4
To	F ₁	5	4
F ₁	F ₁	4	4

^a In two batches, one and six nymphs were observed respectively.

Table 3. Genetic analysis of red-eyed form

	♀	♂	No. pairs tested	No. black-eyed		No. red-eyed	
				♀	♂	♀	♂
I.	Iz1	To	—	128	100	0	0
	F ₁ (Iz1 × To)	To	—	163	145	151	130
	F ₁ (Iz1 × To)	F ₁ (Iz1 × To)	—	562	652	201	199
II.	Ec	To	3	many	many	0	0
	F ₁ (Ec × To)	F ₁ (Ec × To)	{15	262	256	82	76
			{17	205	227	95	91 ^a
			{12	240	292	94	101

^a Chi-square $p < 0.01$, when expected segregation ratio was assumed as 3/1. Chi-square p in the other crosses was more than 0.1.

this respect, LAVEN (1967) showed that parthenogenetic females produced by incompatible pairs of *Culex* retain the original crossing type.

Cause of initiation of embryogenesis in incompatible eggs

Recessive inheritance of the red eye mutant gene of To strain was proved by the testcrosses as shown in Table 3. The color of eyes was examined in F₁, F₂ and BC₁ of Iz1 female × To male (Table 3, I) and in F₁ and F₂ of Ec female × To male (Table 3, II). These results can be explained in terms of a single recessive red eye gene on an autosome. To examine how the incompatible eggs go into embryonic development, this recessive mutant gene on the chromosome was employed for test crosses. The

Table 4. Eye color of offspring from incompatible pairs

	♀	♂	No. pairs tested	No. black-eyed		No. red-eyed	
				♀	♂	♀	♂
I.	To	Ec	1	2	2	0	0
	F ₁ (To × Ec)	To	2	25	31	17	33
II.	To	Ec	1	2	1	0	0
	F ₁ (To × Ec)	To	1	22	23	23	30
III.	To	Ec	1	0	1	0	0
	To	F ₁ (To × Ec)	1	10	7	9	4
IV.	To	Ec	1	11	14	0	0
	F ₁ (To × Ec)	F ₁ (To × Ec)	10	193	172	60	63

To and Ec are red- and black-eyed form, respectively. Succeeding generations were obtained in the above 4 pairs out of 36 incompatible pairs (To female × Ec male) three weeks after pairing.

principle of the test cross is as follows. When red-eyed To females and black-eyed Ec males are cross-mated, the egg nucleus has the red eye gene and the sperm nucleus has the black eye gene. In parthenogenesis and gynogenesis, only the egg nucleus bearing the red eye gene participates in embryogenesis, so that all offspring should have red eyes. In androgenesis, all offspring will be black-eyed because only the sperm nucleus participates in development. F₁ individuals having black eyes may also be obtained by hybridogenesis. Discrimination of the latter two types of development is possible by checking the eye colors of F₂ or BC₁, because all offspring from androgenesis contain no chromosomal material from the female parent and so have black eyes as against hybridogenesis results in segregation of red eye and black eye phenotype in F₂ and BC₁ generations.

Four pairs out of 36 incompatible matings of To female × Ec male gave offspring. Hatched planthoppers had black eyes without exception (Table 4), so that the postulated parthenogenesis and gynogenesis were neglected. Some of the offspring were crossed with each other (Table 4, IV) or back-crossed to To strain (Table 4, I-III), and red-eyed individuals appeared in F₂ and BC₁ generations. This clearly indicates that hybridogenesis took place at the onset of development in the eggs of incompatible crosses. YEN and BARR (1973) stated that a true hybrid is produced by chance from incompatible crosses of *Culex*, but the frequency in the laboratory culture is too low to exclude possible contamination. The present observation on SBPH confirms true hybrid potential from incompatible pairs. The cause of exceptional nymphs in incompatible crosses is the karyogamy between female nucleus and male nucleus. Cytoplasmic incompatibility factors, transmitted transovarially, do not react with rare frequency.

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