Differences in timing of the emergence of the overwintering generation between rice and water-oats populations of the striped stem borer moth, *Chilo suppressalis* (Lepidoptera: Crambidae)

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Abstract

The striped stem borer moth, *Chilo suppressalis*, consists of two host-associated populations: a rice-feeding population and a water-oats-feeding population. We investigated the seasonal occurrence of each population using sex pheromone traps in paddy fields and adjacent water-oats vegetation. Trapped males were individually classified into their respective populations by morphometric analyses of genitalia. Although the first flight of the water-oats population was long, with 1 to 3 poorly resolved peaks during April to June, that of the rice population was much shorter, with a sharp peak in early June. This result supports previous observations that adults of the overwintering generation of the water-oats population occur about two months earlier than those of the rice population; however, it is uncertain how this difference enhances reproductive isolation between the two populations because the occurrence overlaps.

Key words: *Chilo suppressalis*; host race; pheromone trap; water-oats; reproductive isolation

INTRODUCTION

The striped stem borer moth, *Chilo suppressalis* (Walker) was one of the most serious rice pests in East and Southeast Asia until the 1960s (Miyashita, 1982). The larvae of this species feed on rice, *Oryza sativa* and water-oats, *Zizania latifolia*, a common aquatic weed that grows on ponds or waterways in the vicinity of rice paddy fields (e.g. Marumo, 1930).

After comparing the ecology of individuals feeding on rice and water-oats, many researchers believe that this species is in the course of diverging into two populations: a rice population and a water-oats population (Samudra et al., 2002; Matsukura et al., 2006; Ueno et al., 2006). In addition to apparent differences in body size between the two populations (Maki and Yamashita, 1956), morphometric differences in male genitalia have been detected (Matsukura et al., 2006). Furthermore, differences in mating time between populations have been reported by different investigators (Konno and Tanaka, 1996; Samudra et al., 2002; Ueno et al., 2006). For example, under laboratory conditions (25±1°C and 15L9D photoperiodic condition), moths of the rice population mate from 2 to 6 h with an average of 3.7 h into scotophase, whereas moths of the water-oats population mate from 5 to 9 h with an average of 7.4 h (Samudra et al., 2002).

Differences in seasonal occurrence between the two populations have also been suggested. In most parts of Japan, these species have two periods of adult emergence per year: from late May to mid-June (first flight season) and from late July to late August (second flight season) (Kishino, 1974). Several authors have inferred from light trap monitoring that the first flight season of individuals feeding on water-oats was earlier than that of individuals feeding on rice plants (Maki and Ya-
mashita, 1956; Takasaki et al., 1969; Koga and Miyahara, 1973); however, the population could not be identified based on individual trapped adults; therefore, these investigators presumed two different populations based on differences in body size, even though body size distribution widely overlaps between the two populations (Maki and Yamashita, 1956).

In our previous study, we created a morphometric discrimination method that classifies the population of individual adult males at high accuracy according to a proportional difference in genitalia (Matsukura et al., 2006). Using this discrimination method, we verified differences in seasonal occurrence between rice and water-oats populations of *C. suppressalis*.

### MATERIALS AND METHODS

#### Census places.
The seasonal occurrence of *C. suppressalis* was investigated in Dochi (D) (35°57'N, 140°00'E) and Nogizaki (N) (35°56'N, 139°57'E) in Moriya City, Ibaraki Prefecture. These places contained both rice paddy fields and water-oats vegetation. The water-oats in Dochi were grown on a waterway and fallow paddy fields, and those in Nogizaki were on a river that was divided from the paddy fields by a river bank. The distance between the two census locations was ca. 4 km.

#### Monitoring the seasonal prevalence of occurrence.

During the 2002 to 2004 seasons, we captured adult males using sex pheromone traps made with sticky traps (30x24 cm; Sankei Chemical Co. Ltd., Tokyo). One trap was placed at two sites in Dochi (D1 and D2), and also at two sites in Nogizaki (N1 and N2). D1, D2, and N1 were adjacent to water-oats vegetation. N2 was ca. 200 m from N1, located in the rice paddy field in which rice plants were transplanted from late April to early May. We monitored the occurrence of *C. suppressalis* at D1 in 2002 and 2004, D2 in 2003 and 2004, N1 from 2002 to 2004, and N2 in 2004. A rubber septum soaked with a 0.6 mg synthetic sex pheromone mixture containing (Z)-11-hexadecenal, (Z)-13-octadecenal, and (Z)-9-hexadecenal at a ratio of 48:6:5 (Shin-Etsu Chemical Co. Ltd., Tokyo) was used as an attractant. Although this pheromone blend was identified as a sex pheromone for insects collected from rice (Tatsuki et al., 1983), the blend also showed comparable attractiveness to males of the water-oats population (Tsuda and Ichihashi, 1995). We replaced sticky traps almost weekly, and rubber septa monthly. Pheromone traps were fixed about 50 cm above ground level from April to October in every year. The numbers of trapped males were checked approximately every 3 days, and the numbers of trapped males per day were calculated from a 3-day moving average.

#### Identifying the population of trapped males.

Trapped males were individually classified into a population by the discriminant function method (Matsukura et al., 2006). This discriminant function was constructed from morphometric data of the overwintering generation of the two populations; however, this method was applied to all generations. Moreover in this study, we adopted the simple discriminant function consisting of four variables: the length of two of three lines of the valva (Va and Vb), the length of the juxta (JL), and the width of the juxta (JW) as a descriminant method, as described in Matsukura et al. (2006), because it seemed to be suitable for analyzing large numbers of samples. Only males trapped in 2004 were classified into their respective populations.

#### Investigating injury to rice.

To check the density of *C. suppressalis* classified into the rice population, which has generally decreased during last several decades, we sampled larvae from rice stems in both census locations on October 5, 2003. Although already harvested, rice stubble in the paddy fields was available for investigation. We randomly sampled the stubble, and counted the numbers of injured hills, injured stems, and larvae.

### RESULTS

#### Seasonal prevalence of catches

There were roughly two main occurrence periods at all sites and in all years (Fig. 1); however, the start of the first flight season differed among sites. Male catches were observed from early May at site N2, whereas they were observed from mid- or early April at other sites (D1, D2, and N1). At all sites, the first flight season lasted until late June or early July.

The second flight season was shorter than the first flight season. The second flight season began in early August at all sites except N1 in 2003,
where an obvious peak was observed in July, and the second flight season ended in mid- or late September (Fig. 1).

**Estimated population of trapped males**

We estimated the population of individual trapped males by morphometric discrimination. The populations of some trapped males could not be identified because they had lost or injured their genitalia. The numbers of males estimated as belonging to the water-oats population (estimated water-oats population) were greater at all four sites than those estimated as belonging to the rice population (estimated rice population) (Fig. 2).

**Seasonal occurrence of each estimated population**

Figure 3 shows the seasonal occurrence of estimated rice and water-oats populations at each site. In Dachi, the estimated water-oats population was caught from April to late June, and from early August to late September. In contrast, no clear peak of the estimated rice population was observed at either D1 or D2 because of the extremely low pop-
ulation density of the estimated rice population.

The seasonal occurrence of both the water-oats and rice populations at N1 was similar to that of D1 and D2; however, that at N2 differed from the others (Fig. 3). In N2, the first flight season of the estimated water-oats population began not in April but early May, and ended not in late but early June. A clear and sharp occurrence of the estimated rice population was also observed in early June, overlapping the estimated water-oats population (Fig. 3). In early September, both the estimated water-oats and rice populations had a short occurrence, but the size of the estimated water-oats population was much greater than that of the estimated rice population (Fig. 3).

Larval density and injury of rice

No larvae were collected from rice stubble in Dochia, whereas fourteen larvae were gathered from Nogizaki (Table 1). Furthermore, the percentages of injured hills and stems were lower in Dochia than in Nogizaki, indicating the very low density of the rice population in Dochia.

DISCUSSION

This study detected a clear difference in the starting time of the first flight season between rice and water-oats populations of *C. suppressalis*. The peak of the first flight season of the rice population was observed in early June (Fig. 3, N2), a result consistent with previous reports that the first flight season of the rice population is from early to late June in most parts of Japan (e.g. Maki and Yamashita, 1956; Kishino, 1974; Miyashita, 1982). In contrast, the occurrence of the water-oats population began in April (Fig. 3). Such an early occurrence of the water-oats population was also pointed out in previous studies (Maki and Yamashita, 1956; Ishida et al., 2000). All lines of evidence indicate that the overwintering generation of the water-oats population begins to occur one or two months earlier than the rice population.

Not only the starting time of the first flight season, but also the duration differed between the two populations. Most of the rice population occurred in early June, whereas continuous occurrence lasting for nearly three months from April to late June was observed for the water-oats population (Fig. 3), suggesting that the water-oats population has greater variation in its first flight season, or passes two generations from April to June. The long flight season of the water-oats population causes an overlap of flight seasons between the two populations.

The differences in seasonal occurrence between the two populations may be the result of an adaptation to the phenology of each host plant. In general, adaptation to host plant phenology is very important for phytophagous insects, because the plants supply foods (i.e. sprouts, fruits, and stem) during a particular season (Tauber et al., 1986). In the present census locations, water-oats usually sprout in early April, whereas young rice plants are not transplanted to paddy fields until early May. The starting periods for the first flight season of the rice and water-oats populations correspond well with the appearance of each host plant in the field.

Generally, several ecological obstacles cause and maintain sympatric host race formation (e.g. Prokopy et al., 1988; Pashley et al., 1992; Thomas et al., 2003). In particular, differences in seasonal occurrence greatly contribute to sympatric host race formation, because differences in the reproductive season reduce opportunities for cross mating between populations. Such differences were observed in the apple maggot fly, *Rhagoletis pomonella* (Bush, 1969; Feder and Filchak, 1999; Filchak et al., 2000) and the European corn borer, *Ostrinia nubilalis* (Thomas et al., 2003), for example; however, while the adult occurrence period is

<table>
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<tr>
<th>Site</th>
<th>Hills</th>
<th>Stems</th>
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<tr>
<td></td>
<td>No. investigated</td>
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</tr>
<tr>
<td>D</td>
<td>78</td>
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<td>N</td>
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divided more clearly between populations in these other species (Bush, 1969; Thomas et al., 2003), the first flight seasons observed in *Chilo suppressalis* populations widely overlapped (Fig. 3). Such an overlap between rice and water-oats populations is also observed in their mating time (Konno and Tanaka, 1996; Samudra et al., 2002), suggesting that not one strong but multiple ecological obstacles collectively maintain host race formation in *Chilo suppressalis*. Further ecological and biological comparisons between the two populations are needed to clarify the mechanism of host race formation in *Chilo suppressalis*.

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