SHORT COMMUNICATIONS

Effect of Contagion and Cannibalism on the Abnormal Sex Ratio in *Menochilus sexmaculatus* (FABRICIUS) (Coleoptera: Coccinellidae)[1]

Masashi NOMURA[2] and Keiko NIIJIMA

Laboratory of Entomology, Faculty of Agriculture, Tamagawa University, Machida, Tokyo 194, Japan

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Abnormal sex-ratio (SR) conditions are well-known in some *Drosophila* spp. (MALOGOLOWKIN and CARVALHO, 1961). Insects of SR strains produce only or predominantly females due to high male mortality mainly at an early developmental (embryonic) stage. SR conditions in *Drosophila* are caused by a mutation on the X-chromosome (WU, 1983) or intracellular microorganism(s) (POULSON and SAKAGUCHI, 1961). SR conditions have also been found in other insect orders: Hymenoptera, Lepidoptera, Hemiptera and Coleoptera (HURST, 1993). Several coleopteran insects, e.g. the lady-bird beetles *Harmonia axyridis* (MATSUKA et al., 1975), *Menochilus sexmaculatus* (NIIJIMA and NAKAJIMA, 1981), and *Adalia bipunctata* (HURST et al., 1992), the bark beetle, *Orthotomicus latidens* (LANIER and OLIVER, 1966) and the walnut beetle, *Grastrolina depressa* (CHANG et al., 1991) show SR conditions in natural populations.

The SR condition in the lady-bird beetle, *Menochilus sexmaculatus* (FABRICIUS), was reported previously (NIIJIMA and NAKAJIMA, 1981; NIIJIMA, 1983; GOTOH and NIIJIMA, 1986). The SR strains of this beetle produced female-biased progenies and transovarially transmitted the SR trait (NIIJIMA and NAKAJIMA, 1981). The results of previous studies suggest that the SR condition is caused by microorganism(s) because it was transferred experimentally with hemolymphs (NIIJIMA and NAKAJIMA, 1981), and cured by high temperature (GOTOH and NIIJIMA, 1986; NIIJIMA and NOMURA, unpublished data) and with tetracycline treatment (GOTOH and NIIJIMA, 1986; NIIJIMA, unpublished data).

In this paper, we investigated the possibility of two ways of horizontal transmission of the SR condition in *M. sexmaculatus*, namely contagion and cannibalism.

MATERIALS AND METHODS

The SR females of *M. sexmaculatus* were originally collected at Ishigaki Island, Okinawa Prefecture, in March, 1978. Since that time, the SR condition has been maintained for 23 generations in our laboratory (NIIJIMA and NAKAJIMA, 1981). Normal sex-ratio insects (NR) were collected at Tama and Machida, Tokyo, in autumn, 1983 and 1984 respectively. The beetles were reared on pulverized drone honeybee brood powder (OKADA and MATSUKA, 1973) under laboratory conditions (25 ± 1°C, continuous darkness). Sex was usually determined at the adult stage by the color of the labrum of the mouth parts. Some beetles were dissected and the reproductive organs were examined to determine the sex.

Contagion experiment. Five virgin females from each of the SR and NR strains ranging in age from 7 to 30 days were kept together in a Petri dish (9 cm in diam.). The two strains were distinguished by coloring with liquid paint markers on the forewings. After 20 days, the females were individually mated with males of the NR strain. The females were then individually permitted to lay eggs for 15 days. Hatchability of the eggs and sex ratio of the progeny were examined. Two replicates were performed.

Cannibalism experiment. *M. sexmaculatus*, which is an aphidophagous species, often shows cannibalism under crowded conditions (NIIJIMA and NAKAJIMA, 1981). To examine the possibility that the SR agent(s) is transferred from the SR to the NR strain through cannibalism, eggs, larvae (first and second instars) and pupae of the SR strain were fed to NR larvae. After emergence, adult virgin females were kept individually for 20 days, and then they were mated with NR males. The eggs were collected after 15 days of oviposition and their hatchability and the sex ratio of the progeny were examined. The control group was fed NR larvae.

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[2] Present address: *Laboratory of Applied Entomology and Zoology, Faculty of Horticulture, Chiba University, Matsudo, Chiba 271, Japan*

RESULTS

Contagion experiment

The egg hatchability and percentage of males among the progenies produced by nine females of each strain are shown in Fig. 1. The results showed no evidence of horizontal transmission of the SR agent(s) from SR to NR females due to contagion. The sex ratio of total NR progenies (181 females: 156 males) did not show a statistically significant deviation from unity in a chi-square test ($p > 0.05$: $\chi^2 = 1.71$, df = 1).

Cannibalism experiment

Most 4th stadium larvae feeding on 1st and 2nd stadium larvae or pupae of the SR strain died of malnutrition, although a few survived to the adult stage and produced progenies. This malnutrition was not caused by the SR agent(s) because NR larvae feeding on NR larvae also died of the same symptoms. On the other hand, NR larvae feeding on SR eggs throughout their larval period did not show symptoms of malnutrition. The NR larvae apparently fed on more than 100 eggs during their larval stage.

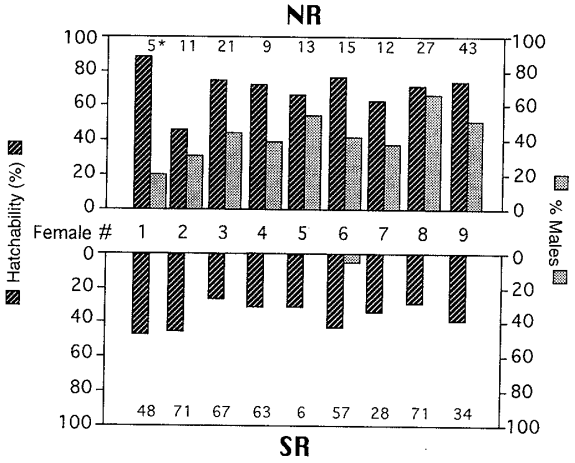

Fig. 1. Hatchability of eggs and percentages of males in progenies of NR (upper panel) and SR (lower panel) females of *Menochilus sexmaculatus* under contagion conditions. * No. of adults obtained.

Hatchability of eggs and sex ratios in the F_1 progenies obtained from four randomly selected females which fed on SR eggs (E-2 and E-3) or SR larvae (L-10 and L-33) are shown in Tables 1 and 2, respectively. The lack of deviation from a 1 : 1 sex ratio in progenies (chi-square test at 5%; E-2: $\chi^2 = 0.01$, df = 1; E-3: $\chi^2 = 0.01$, df = 1; L-10: $\chi^2 = 0.01$, df = 1; L-33: $\chi^2 = 0.21$, df = 1) suggested that the SR agent(s) was not transferred through digestive organs of the beetles after cannibalism.

We further investigated the effects of cannibalism on F_2 progenies (Table 3). The F_1 females were incubated for 60 days before being allowed to mate with NR males. Although this incubation period seems was apparently sufficient for SR agent(s) to increase, the F_2 progenies did not show the SR condition.

DISCUSSION

Experimental studies on blood transfer (NIIJIMA, 1983) and antibiotic treatments (GOTOH and NIIJIMA, 1986) have suggested that the SR agent(s) of *Menochilus sexmaculatus* is most probably a microorganism, as in the case for *Drosophila* (MALOGOLOWKIN and CARVALHO, 1961). On the basis of this assumption, the transmission of the SR agent(s) was studied in the present study. Unlike insect-pathogenic viruses, the SR agent(s) was not infectious by body contact. An SR agent of *Drosophila*, which is a spiroplasma (POULSON and SAKAGUCHI, 1961), is not infectious either (MAGNI, 1954).

At present, insect species shown to have SR strains are all herbivorous except for lady-beetles, which are sarcophagous insects. WERREN et al. (1994) suggested that cannibalism may be a vehicle for transmitting SR agent (Rickettsia-like bacteria) of *Adalia bipunctata*

Table 1. Hatchability and sex ratios of progenies obtained from normal adults fed on SR eggs

Egg batch numbers	Female #E-2				Female #E-3			
	No. of eggs	Hatch-ability (%)	No. of adults	% Male	No. of eggs	Hatch-ability (%)	No. of adults	% Male
1–5	92	76.1	21	52.4	39	69.2	14	42.9
6–10	91	76.9	28	42.9	57	65.0	12	75.0
11–15	74	62.2	17	41.2	57	73.7	16	50.0
16–20	80	60.0	12	66.7	81	77.8	27	44.4
Total	337	69.4	78	48.7	234	72.2	69	50.7

Table 2. Hatchability and sex ratios of progenies obtained from normal adults fed on SR larvae

Egg batch numbers	Female #L-10				Female #L-33			
	No. of eggs	Hatch-ability (%)	No. of adults	% Male	No. of eggs	Hatch-ability (%)	No. of adults	% Male
1–5	34	76.5	7	28.6	62	75.8	8	37.5
6–10	54	63.0	5	80.0	60	63.3	1	0
11–15	48	66.7	2	100	74	66.2	4	50.0
16–20	68	51.5	0	—	83	50.6	1	100
21–23	42	40.5	3	33.3	50	42.0	5	40.0
Total	246	58.9	17	52.9	329	56.5	19	42.1

Table 3. Hatchability and sex ratios of progenies obtained from daughters of normal adults fed SR eggs or larvae

Female No.	Oviposition period (days)	No. of eggs	Hatch-ability (%)	No. of adults	% Males
L-10-13	10	184	45.7	10	50.0
L-33-8	9	172	62.8	33	42.4
L-33-14	7	122	82.0	48	41.7
E-3-3	5	130	73.8	33	36.4

to other NR groups. We, therefore, investigated the possibility of oral transfer of the SR agent(s) through food. Since GOTOH (1982) suggested that it took about 20 days to increase SR agent(s) in the beetles after transmission of SR agent(s) by the injection method, the emerged virgin females which fed on SR beetles were individually kept for 20 days before mating. These cannibalism experiments (Tables 1 and 2), however, showed that the SR agent(s) of *M. sexmaculatus* is not transferred horizontally. In *Drosophila willistoni*, NR larvae feeding on the extract of SR flies were reported to show the SR condition (two out of 39 female flies; CARVALHO and DA CRUZ, 1962). In another case on *Drosophila*, NR larvae feeding on the SR extract never showed the SR condition (CARVALHO and DA CRUZ, 1962). Such discrepancy threw some doubt on the likelihood of horizontal transmission of SR agents in *Drosophila* spp. In the case of *M. sexmaculatus*, SR insects were found only in Ishigaki Island, Okinawa Pref. Therefore, the SR agent(s) may have at most a low incidence of horizontal transmission between host beetles and may maintains themselves in limited regions.

Recently, WERREN et al. (1994) reported that the infectious microorganisms responsible for a male-killing phenomenon in the two-spotted lady-bird beetle, *Adalia bipunctata*, is closely related to bacteria of the genus *Rickettsia*, based on the sequence of 16S rDNA amplified from a DNA extract of SR beetle ovaries. Thus, the SR agent of *A. bipunctata* is different from *Drosophila*'s spiroplasma. There are many reports of other rickettsia-like microorganisms giving rise to sexual alterations in arthropods; for example, *Wolbachia* (ROUSETT et al., 1992) and *Orientia tsutsugamushi* (URAKAMI and TAMURA, 1996). Although horizontal transfer of SR agent(s) was not demonstrated in the present study, it will be value to apply molecular techniques to *M. sexmaculatus* to characterize its SR agent(s). If the SR-mechanism of *M. sexmaculatus* is identified, it will be useful for sex-ratio control when this lady beetle is used as a predator of aphids in the field.

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A Component of a Synthetic Aggregation Pheromone of *Riptortus clavatus* (THUNBERG) (Heteroptera: Alydidae), That Attracts an Egg Parasitoid, *Ooencyrtus nezarae* ISHII (Hymenoptera: Encyrtidae)[1]

Nobuo MIZUTANI,[2] Takashi WADA,[2] Hiroya HIGUCHI,[2] Mikio ONO[3] and Walter Soares LEAL[4]

[2] *Kyushu National Agricultural Experiment Station, Nishigoshi, Kumamoto 861–11, Japan*

[3] *Fuji Flavor Co., Ltd., Hamura, Tokyo 190–11, Japan*

[4] *National Institute of Sericultural and Entomological Science, Tsukuba 305, Japan*

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The bean bug, *Riptortus clavatus* (THUNBERG), is an economically important pod-sucking pest in soybean production in Southern Japan (TABARU and NAGAI, 1981; SETOGUCHI et al., 1986). The adult males release a pheromone which attracts both adult sexes and conspecific nymphs, in particular, the second stadium nymphs (NUMATA et al., 1990; LEAL et al., 1995). This pheromone was identified and found to be comprised of three components: (*E*)-2-hexenyl (*Z*)-3-hexenoate, (*E*)-2-hexenyl (*E*)-2-hexenoate and myristyl isobutyrate (E2HZ3H, E2HE2H and MI for short, respectively) with a mixture ratio of 1 : 5 : 1 (LEAL et al., 1995). A 100 mg synthetic mixture of these components was equivalent to ten live males in the attraction of adults and second stadium nymphs in a field experiment (LEAL et al., 1995).

This synthetic aggregation pheromone also attracted females of an egg parasitoid, *Ooencyrtus nezarae* ISHII, in a field experiment (LEAL et al., 1995). *O. nezarae* is the predominant parasitoid of *R. clavatus* eggs in soybean fields of Southern Japan (TAKASU and HIROSE, 1985; MIZUTANI et al., 1996). Thus, it is likely that the parasitoid female utilizes the host pheromone as an attractant kairomone (LEAL et al., 1995).

O. nezarae has a comparatively wide host range. Nine bug species besides *R. clavatus* are known to be

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