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Experimental Transfer of Abnormal "Sex-Ratio" in the Lady-Bird Beetle, *Harmonia axyridis* (PALLAS) (Coleoptera : Coccinellidae)

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An abnormal "sex-ratio" (SR) strain of *Harmonia axyridis* (PALLAS) which produced only females was selected and maintained until the 10th generation. Transfer experiments to detect the SR condition were carried out by microinjection of the supernatant from a homogenate of SR pupae into normal pupae. The transfer of the SR agent(s) from the SR to the normal females was not observed when the latter mated on the 7th day after emergence, but it was observed in beetles which mated on the 30th day after emergence. The SR agent(s) were shown to be present in the supernatant and to increase their infectious potency during the incubation period.

INTRODUCTION

The sex ratio of most insect species is usually 1 : 1 with a few exceptional cases of parthenogenesis. However, deviation from the normal sex ratio has been shown in several species of *Drosophila* having a bisexual reproductive system. Existence of strains without males has also been recognized in the lady-bird beetles, *Adalia bipunctata* L. (LUS, 1947), *Harmonia axyridis* (PALLAS) (MATSUKA et al., 1975; HU, 1979), and *Menochilus sexmaculatus* (FABRICIUS) (NIIJIMA and NAKAJIMA, 1981). It has been demonstrated that one of the abnormal "sex-ratio" (SR) in *Drosophila* was caused by helical microorganism which was maternally inherited and associated with the absence of males in the progeny of infected females (MALOGOLOWKIN and POULSON, 1957; POULSON and SAKAGUCHI, 1961). POULSON and SAKAGUCHI (1961) first regarded this microorganism as spirochete(s), however, recent morphological studies found that this organism might be spiroplasma (WILLIAMSON and WHITCOMB, 1974). And MALOGOLOWKIN and POULSON (1957) demonstrated that SR condition in *Drosophila* has been successfully transferred by injection of ooplasm from SR eggs. HODEK (1973) suggested that one to three, or even more recessive alleles, which are lethal when homozygous, were the cause of the SR condition in *A. bipunctata*. However, the factor(s) related to the SR condition in *H. axyridis* have not been reported.

In this study, to clear the agent(s) which caused the abnormal "sex-ratio" in *H. axyridis*, transmissibility of the SR condition from an SR strain into a normal one

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was investigated using a microinjection method.

MATERIALS AND METHODS

Adults of *H. axyridis* were collected from a field in Machida, Tokyo, in autumn, 1976, and a culture was started under laboratory conditions (25±1°C, dark conditions except for the observation time). Progenies from one SR female found in this rearing stock were maintained through 10 generations to examine the similar tendency of SR condition reported by MATSUKA et al. (1975). The 7th generation was subjected to injection experiments.

The insects were reared on pulverized drone honeybees (OKADA et al., 1972; MATSUKA et al., 1975).

The donor pupae were ground with a pestle and then mixed in a motor with ten times of 0.9% NaCl solution to equal the pupal weight. The suspension was then centrifuged for 15 min at 1,016×g at 12°C. The supernatant (1.5–2.0 μl per pupa) was injected into the dorsal side of a recipient pupa (2–4 days old after pupation) of a normal strain with a microsyringe.

RESULTS

The experimental design and outline of results are given in Table 1. The symbols which are used in Table 1 are continued hereafter.

Out of 262 pupae of the normal strain which were injected with the SR supernatant, 139 adult females emerged. These SR-injected females were divided into two groups: the thirty-one females of the first group were mated with normal males on the 7th day after emergence (E-1), and the twenty females of the second group on the 30th day (E-2). As a control group, thirty-one pupae of normal strain were also injected with the normal supernatant; 15 females emerged from these. The control females (control-injected females) were mated with normal males on the 30th day

Table 1. Experimental design and results of transmission of SR agent(s) into normal females of *H. axyridis*

Experiment	Period until mating after emergence	Generation investigatied	No. of eggs observed	Average hatchability (%)	No. of adults obtained	Average male percentage
SR injection	1 week (E-1)	F_1	1,562	51.4	286	40.4
(donor: SR strain; recipient: normal strain)		F_2	1,274	52.7	313	42.9
	1 month (E-2)	F_1	2,577	53.5	654	12.4
		F_2	1,413	67.9	494	43.0
Control injection	1 month (E-3)	F_1	1,284	50.7	305	45.2
(donor: normal strain; recipient: normal strain)		F_2	274	59.9	38	34.2
Normal strain	1 week (E-4)	F_1	767	80.1	249	47.0
		F_2	232	74.1	90	46.6
SR strain	(E-5)	G_2-G_{10}	16,741 (8,802)[a]	30.4 (55.3)[a]	2,154 (1,204)[a]	0.0 (21.6)[a]

[a] "Cured" SR females emerged from the 4th-7th and 10th generations.

after emergence (E-3, n=8).

Twenty-four females out of the thirty-one SR-injected females in E-1 group oviposited, and the sex ratio (male percentage) at maturity of the offspring was 40.4% on the average (ranging from 22.1 to 66.5%). The adult females (F_1) which developed from these eggs were crossed and their eggs (F_2) investigated (E-1). The sex ratio in F_2 at maturity was 42.9% (ranging from 28.9 to 78.5%, n=18).

The male percentage of eggs produced by E-4 (referred to as the normal strain) was 47.0 (F_1) and 46.6 (F_2) on the average.

Thirteen out of twenty SR-injected females in E-2 group laid eggs. Out of these, two females produced many eggs, but no eggs hatched. Table 2 shows the hatchability, larval survivorships and sex ratio of the progenies produced by the eleven SR-injected females except for the above two females. Three SR-injected females reproduced no males in their progenies, four females produced a small number, and the other four gave a normal number. The average male percentage of all progenies produced by the SR-injected females was 12.4 (ranging from 0.0 to 48.4%). Typical individual records are shown in Table 3. The hatchability of the eggs from the three females that produced no males in E-2 was less than 50%. This low hatchability may have been due to characteristics of the SR condition (Fig. 1b). However, this phenomenon could not be used always as an indicator of the SR condition, because eggs laid by normal females often show low hatchability. In the second generation (F_2) from the SR-injected females which produced no males in F_1, the male percentage was 43.0 (ranging from 30.8 to 50.4%, n=6; E-2), i.e., almost the normal level.

In the control experiment, six out of eight females in E-3 produced both male

Table 2. Survival and sex ratio of progenies produced by SR-injected and control-injected females when mated on the 30th day after emergence

Cross no.	No. of eggs observed	Hatch-ability (%)	Viability (larval stages) (%)	No. of adults obtained		Male percentage
				♀	♂	
SR-injected (E-2)						
I-2	177	71.8	48.8	32	30	48.4
I-3	614	63.0	32.6	111	13	10.5
I-4	252	61.1	54.5	68	16	19.1
I-5	116	48.3	46.4	20	6	23.1
I-7	544	35.7	64.4	125	0	0.0
I-9	318	71.4	53.3	113	8	6.6
I-10	52	30.8	62.5	7	3	30.0
I-11	48	60.4	58.6	16	1	5.9
I-13	43	67.4	41.4	8	4	33.3
I-18	310	41.9	40.0	52	0	0.0
I-19	103	28.2	72.4	21	0	0.0
Control-injected (E-3)						
C-1	116	36.2	73.8	19	12	38.7
C-2	220	51.8	51.8	32	27	45.8
C-4	55	10.9	66.7	2	2	50.0
C-5	367	52.9	47.9	51	45	46.9
C-6	319	61.1	44.1	46	40	46.5
C-8	207	48.3	29.0	17	12	41.4

Table 3. Comparison of number of eggs (E), larvae hatched (L), females and males in SR-injected and control-injected females

Period of egg collection	Cross no. I-2				Cross no. I-4				Cross no. I-7				Cross no. C-5			
First	Nov. 20, 1977				Nov. 14, 1977				Nov. 15, 1977				Nov. 19, 1977			
Last	Nov. 26				No. 30				Dec. 1				Dec. 9			
	E	L	♀	♂	E	L	♀	♂	E	L	♀	♂	E	L	♀	♂
1st	16	5	1	2	60	45	14	11	55	28	20	0	22	13	6	6
2nd	26	19	3	6	23	14	9	4	16	9	7	0	34	18	8	10
3rd	55	34	8	9	34	25	11	1	19	7	3	0	24	16	7	6
4th	34	31	6	5	25	17	6	0	29	5	3	0	18	13	4	1
5 〃	12	11	6	3	35	16	10	0	48	9	8	0	47	23	2	7
6 〃	34	27	8	5	39	20	9	0	38	14	11	0	26	17	4	3
7 〃					36	17	9	0	35	16	9	0	39	21	4	1
8 〃									25	12	6	0	54	17	3	5
9 〃									68	19	14	0	22	13	4	1
10 〃									35	8	5	0	31	18	6	2
11 〃									39	20	12	0	50	25	3	3
12 〃									68	24	15	0				
13 〃									43	14	4	0				
14 〃									26	9	8	0				
Total	177	127	32	30	252	154	68	16	544	194	125	0	367	194	51	45

and female progenies (Tables 2 and 3), and the other two females laid no eggs. The sex ratio of these progenies (45.2% on the average, ranging from 38.7 to 50.0%) did not deviate significantly ($p>0.05$) from the normal sex ratio of this species, i.e., male percentage of 47.8 among 2,090 adults which were collected from a field of Machida, Tokyo on Nov. 28–30, 1976 (GOTOH, unpublished). The male percentage in the next generation was 34.2 (25 females and 13 males). The difference in the male percentage of F_1 progenies between the SR-injected (E-2) and the control-injected parent females (E-3) was significant ($p<0.01$)[2].

The difference in larval viability between the SR-injected and the control-injected females was insignificant ($p>0.05$), as well as the difference of larval viability between the SR and the normal females. Since hatchability of the SR strain obviously differed from that of the normal strain (Fig. 1b), the SR agent(s) were considered to be effective in the embryo stage.

DISCUSSION

There are many reports on the infection of SR condition into normal strains through injection with the supernatant of macerated SR flies or with the ooplasm of SR eggs in *Drosophila*. Moreover, either SR supernatant or SR ooplasm obtained from *D. equinoxialis* was successfully transferred to *D. willistoni* by injection

[2] In this statistical analysis, Welch's test method was adopted because the variation of the means was significantly different ($p<0.05$).

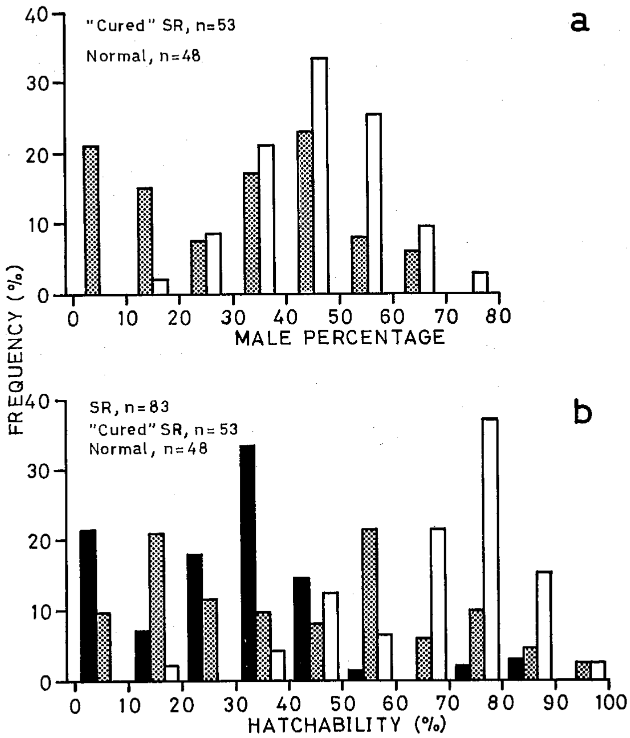

Fig. 1. Comparison of male percentage (a) and hatchability (b) among progenies produced by SR, "cured" SR and normal females.
Solid columns: SR females, semi-solid columns: "cured" SR females, open columns: normal females.

(MALOGOLOWKIN et al., 1960). In the present experiments, injection of the SR supernatant caused some infection in P_1 beetles, but the SR agent(s) were not transmitted to F_1 adults. As to the lethality transmitted by injection in *Drosophila*, reports have shown that it can be carried through more than twenty generations of their descendants (POULSON and SAKAGUCHI, 1961). The result that there was no effect of the SR agent(s) on the F_1 adults in the present experiment might be explained in two ways: either the SR agent(s) of *H. axyridis* were weaker than that of *Drosophila*, or the incubation period (one week before mating with normal males) in the F_1 progenies was too short for the SR agent(s) to be transferred subsequently. NIIJIMA and NAKAJIMA (1981) also succeeded to transmit the SR condition using a microinjection method in *Menochilus sexmaculatus*, and the condition appeared through successive generations.

The incubation period of SR agent of *D. willistoni*, which was injected in *D. neblosa*, was about two weeks (MALOGOLOWKIN and CARVALHO, 1961). From the present results, one week of incubation was too short for the SR agent(s) to have a significant effect on infection, but one month proved to be long enough. This finding suggested that SR agent(s) may increase during the incubation period.

It was demonstrated by IKEDA (1970) that the XY zygotes were killed in the early developmental stages as one of the general features of the SR in *Drosophila*. Also, hatchability was rarely observed beyond 50% in the SR females in lady-bird beetles (HU, 1979; NIIJIMA and NAKAJIMA, 1981), though the SR and the normal strain did not differ in mortality in the larval stages. These authors suggested that the males were killed during the egg stage. The present study showed the same results (Fig. 1) as the above in addition, it demonstrated that the eggs laid by the SR females hatched in spots in each egg-batch. Therefore, the males may be killed in the egg stage also in this species.

Hatchability in "cured" SR females[3] ranged from 5.5 to 90.8%, and their sex ratio ranged from 5 to 70% (Fig. 1). There is a good reason to surmise that the SR condition in *H. axyridis* was affected by the quantity of the SR agent(s) rather than the quality, as "cured" SR females occasionally emerged from the SR strain (Table 1).

The experimental results presented above were similar to those found in *Drosophila* (MALOGOLOWKIN et al., 1959), the SR agent of which was spiroplasma (WILLIAMSON and WHITCOMB, 1974). The SR agent(s) in *H. axyridis* which are present in their supernatant, therefore, may also be microorganisms as first suggested by POULSON and SAKAGUCHI (1961) for *Drosophila*.

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[3] These females were restored from SR to normal condition, in which they produced bisexual progenies.