

Abnormal Sex-Ratio Found in the Lady Beetle, *Harmonia axyridis* PALLAS (Coleoptera: Coccinellidae)

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One female of *Harmonia axyridis* PALLAS was found to yield the female progeny exclusively. This abnormal sex ratio was inherited maternally through 5 generations when the beetles were reared on pulverized drone honeybee brood. Spontaneous restoration from the abnormal condition as in the SR condition of *Drosophila* was occasionally observed.

INTRODUCTION

Genetic studies of the aphidophagous coccinellid, *Harmonia axyridis* PALLAS became much easier with the development of a semiartificial diet using the pulverized drone honeybee brood, because of eliminating the problem of collecting the prey aphids (OKADA et al., 1972; OKADA and MATSUKA, 1973). In the course of some genetic experiments on the polymorphic elytral patterns of the insect (OKADA and KONDO, 1973; LAB. ENTOMOL., TAMAGAWA UNIV., 1975), we obtained one strain which showed an abnormal sex-ratio and produced only the female progeny in some cases. The purpose of the present paper is to describe the newly found strain and to discuss its resemblance to the SR ("sex-ratio") condition of *Drosophila* (MAGNI, 1953).

MATERIALS AND METHODS

Pupae of *Harmonia axyridis* PALLAS were collected from the leaves of *Salix babylonica* L. in June, 1972 at Yurigaoka, Tama-ku, Kawasaki-shi, Kanagawa Prefecture. Descendent larvae and adults through successive generations were reared on a semiartificial diet (OKADA et al., 1972; MATSUKA et al., 1972). Lyophilized drone honeybee pupae and/or larvae were pulverized and supplied to the coccinellid larvae or adults in petridishes (9 cm in diameter). A water-saturated sponge was also placed in the dish. The petridishes were always kept in an incubator at $25 \pm 2^\circ\text{C}$ in the dark except the time for observation and replenishing food and water once a day.

RESULTS

Discovery of a female producing strain

Out of 10 pair matings of adults obtained from the field-collected pupae of *Harmonia axyridis*, 9 produced normal progenies with sex ratio of around 1, but one yielded 34 females with no male (Table 1, Cross No. 10).

The phenotypic elytral patterns of the parent which produced unisexual progeny

Table 1. NUMBER OF PROGENIES FROM THE FIELD-COLLECTED FEMALES

Cross No.	Females	Males	Male per cent
1	7	8	53.3
2	59	44	42.7
3	20	25	55.6
4	18	20	52.6
5	8	8	50.0
6	3	2	40.0
7	16	15	48.4
8	1	4	80.0
9	12	14	53.8
10	34	0	0.0

were *distincta* and *spectabilis* (KOMAI, 1956). Sixteen female offsprings out of 34 were the heterozygotes of *distincta* (D) and *spectabilis* (Sp) and 18 were the heterozygotes of *spectabilis* and *succinea* (s). Genotypes of the parents were inferred as Ds and SpSp.

Inheritance of the abnormal sex ratio (SR)

Four females with DSP elytral pattern were arbitrarily selected and crossed to normal males which had been bred in our laboratory (Cross No. 11~14). In the case of Cross No. 11, 8 egg-collections of 173 eggs in total were made, but only 39 females and no male developed (Table 2). In this cross, the male elytral pattern was the heterozygote of *axyridis* and *succinea*. Separation of elytral pattern seemed to be normal (Table 3). Three other crosses produced 33, 9 and 6 females respectively, but no male, showing the similar normal separation as to the elytral pattern. Of the 502 eggs obtained from F₁ generation (Cross No. 11~14), the hatching rate was as low as 29.9%, while a normal rate was reported as 56.5% (OKADA et al., 1972). During the

Table 2. EMERGENCE OF DAUGHTERS FROM EACH EGG-MASS DEPOSITED BY A FIRST FILIAL SR FEMALE (CROSS NO. 11)

Date of egg-collection	October, 1972			November, 1972					Total
	14th	16th	20th	9th	10th	12th	16th	18th	
No. of eggs collected	26	10	20	6	22	35	26	28	173
No. of larvae hatched	5	6	9	3	9	8	13	3	56
No. of adults emerged									
Female	5	5	8	2	6	7	6	0	39
Male	0	0	0	0	0	0	0	0	0

Table 3. ELYTRAL PATTERN FOUND IN THE PROGENY OF CROSS NO. 11

Elytral pattern	<i>distincta axyridis</i>	<i>distincta succinea</i>	<i>spectabilis axyridis</i>	<i>spectabilis succinea</i>	Total
Female	11	11	7	10	39
Male	0	0	0	0	0
Ratio observed	0.28	0.28	0.18	0.26	1.00
Ratio expected	0.25	0.25	0.25	0.25	1.00

embryological development, the number of dead eggs was approximately equal to those hatched. Growth and emergence of the hatched larvae took place normally.

These results indicate that the abnormal sex-ratio (SR) was inherited maternally regardless the elytral pattern.

Further transmittance and restoration from SR

When 10 F_2 females from Cross No. 11 were mated with the normal males, 4 females (Cross No. 21~24) deposited no egg, 5 females (Cross No. 15~19) yielded unisexual progeny, but one (Cross No. 20) produced bisexual progeny, 35 males and 30 females (Table 4). Hatchability of the eggs laid by this female was also high as that of the normal strain. Mating both sexes derived from this cross (4 examples) produced the normal bisexual progeny. On the other hand, 10 F_2 females obtained from Cross No. 12 yielded only female progeny, when crossed to the normal males (Cross No. 25~34). These results indicated that the restoration from SR condition to normal character took place in some cases.

Table 4. DEVELOPMENT AND SEX OF F_3 PROGENY OBTAINED FROM THE SR STRAIN (Cross No. 11)

Cross no.	No. of eggs		Hatchability (%)	No. of adults emerged		Male per cent
	observed	hatched		Female	Male	
15	69	35	50.7	18	0	0
16	72	10	13.9	7	0	0
17	30	16	53.3	10	0	0
18	67	6	9.0	2	0	0
19	326	42	12.9	23	0	0
20	199	144	72.4	30	35	53.8
21-24	0	0	—	0	0	—

Table 5. DEVELOPMENT AND SEX OF SR (F_4) AND NORMAL STRAINS

Cross no.	No. of eggs		Hatchability (%)	No. of adults emerged		Male per cent
	observed	hatched		Female	Male	
35	132	60	45.5	34	0	0
36	83	30	36.1	15	0	0
37	24	7	29.2	2	0	0
38	48	9	18.8	0	0	—
39	264	197	74.6	77	2	2.5
40	100	53	53.0	6	1	14.3
41	386	86	22.3	14	0	0
42	540	117	21.7	37	4	9.8
43	145	48	33.1	3	0	0
44	29	26	89.7	8	4	33.3
45	483	153	32.3	64	0	0
46	528	171	32.4	38	25	39.7
Total	2762	957	34.6	298	36	10.8
Total of 12 normal strains	3968	2707	68.2	536	465	46.5

Seventeen females of F_4 generation from Cross No. 19, 28 and 29 were crossed to the normal males. Eggs were laid by twelve females out of these within 40 days after copulation (Cross No. 35~46, Table 5). The normal females were treated similarly in the control experiments (Cross No. 47~58). The SR strain was inferior to the normal one in the fecundity, i.e., there were less proportion of egg-laying individuals (12 out of 17) and less eggs per individual within the experimental period. Six of these

Table 6. COMPARISON OF NUMBERS OF EGGS (E), LARVAE HATCHED (L), MALES AND FEMALES FOUND IN THREE TYPES OF SR STRAINS

Period of egg collection	"Complete SR" (Cross no. 45)				"Incomplete SR" (Cross no. 39)				"Progressive SR" (Cross no. 42)				Normal (Cross no. 55)			
	First April 28, 1973				April 20, 1973				April 26, 1973				April 14, 1973			
	Last May 22				May 9				May 22				May 18			
	E	L	♀	♂	E	L	♀	♂	E	L	♀	♂	E	L	♀	♂
1st	28	9	4	0	32	25	7	0	12	12	2	1	15	14	9	2
2nd	18	9	4	0	10	8	2	0	12	12	4	1	15	11	4	3
3rd	29	9	2	0	39	34	9	0	9	5	1	0	16	15	4	3
4th	37	11	1	0	32	18	9	0	7	7	2	2	19	16	7	6
5"	23	10	3	0	6	3	0	0	11	7	3	0	11	8	2	2
6"	43	20	12	0	39	29	15	1	3	2	2	0	18	15	10	2
7"	26	13	7	0	24	19	5	0	21	14	7	0	12	12	2	2
8"	22	10	7	0	7	4	2	0	6	4	3	0	8	5	1	1
9"	15	2	1	0	47	32	19	0	24	4	2	0	15	15	2	1
10"	45	23	12	0	10	8	1	0	47	4	1	0	30	24	5	5
11"	18	10	7	0	18	17	8	1	18	0	0	0	35	29	3	3
12"	37	6	2	0					34	0	0	0	17	15	0	2
13"	22	6	2	0					8	0	0	0	11	10	0	1
14"	39	11	0	0					7	2	0	0	29	27	6	5
15"	36	0	0	0					35	3	3	0	25	23	6	3
16"	5	2	0	0					48	2	0	0	36	32	6	1
17"	12	0	0	0					35	3	1	0	19	16	4	3
18"	28	2	0	0					28	1	0	0	38	28	5	5
19"									33	1	1	0	39	24	4	6
20"									22	6	2	0	16	12	7	3
21st									39	11	3	0	54	30	6	4
22nd									36	0	0	0	24	17	4	5
23rd									5	2	0	0	38	34	8	4
24th									21	7	0	0	44	28	8	6
25"									19	8	0	0	23	17	6	9
26"													40	39	4	1
27"													53	41	4	3
28"													24	22	7	8
29"													42	36	10	3
30"													36	21	3	4
31st													27	13	1	2
Total	483	153	64	0	264	197	77	2	540	117	37	4	824	654	148	108

SR strains produced no males and 5 produced males albeit of a small number.

Several types of SR were noted among these crosses presented in Table 5. The first one was "complete SR", in which the hatching rate was half as high as the normal and only female adults developed (Cross No. 45 in Table 6). The second one, "incomplete SR" (Cross No. 39), produced few males from egg batches of which the hatching rate was somewhat higher than the above. The third one, "progressive SR" (Cross No. 42), produced bisexual progeny with a normal hatching rate during the initial ovipositing period and later produced only females as the hatching rate decreased.

Among 8 offsprings of the complete SR (Cross no. 45) examined, 6 females gave unisexual progenies but 2 females which came from the same egg-batch "cured" to produce male progeny. Only one out of 6 female offsprings of the incomplete SR (Cross no. 39) produced no male, 4 produced progeny of both sexes and the remaining one did not lay eggs. In the case of progressive SR, the individuals obtained during the initial bisexual period produced both sexes. On the contrary, only one out of 5 daughters from SR-period produced males, 2 of those were the complete SR and 2 females gave no egg.

Similar results were obtained when F_4 SR females were mated with the males emerged from the field-collected pupae. Transmittance of the SR condition from the 4th to the 5th generation was also unstable to some extent as indicated in earlier generation.

DISCUSSION

The abnormal sex-ratio condition (SR) of *Drosophila* has been analyzed by many workers. The general feature of the SR has been shown by IKEDA (1970) as follows: (1) SR females produce offsprings that consist almost entirely of females, (2) XY zygotes are killed in early developmental stages, (3) the SR condition is inherited only through the cytoplasm. The low hatching rate found in the SR strain of *Harmonia axyridis* might be due to the death of male zygotes with XY (YOSHIDA, 1944) during the embryological development as is the case in *Drosophila*.

The SR condition found in *Drosophila* is infective through injection with some body constituents. The etiological agents of SR has been established as spirochetes in *D. willistoni* and some other species (SAKAGUCHI and POULSON, 1963), while virus-like microorganisms have been inferred to be responsible for the SR in *D. bifasciata* (IKEDA, 1970). Whether a similar microorganism exists in *Harmonia* should be tested through injection experiments, which are in progress.

MALOGOLOWKIN et al. (1958) have reported that the SR condition appeared after about 2-week latent period when the normal virgin females of *D. willistoni* were injected with SR ooplasm. The offsprings obtained during the latent period showed a normal sex-ratio of close to 1 : 1 and then the females were produced mainly or exclusively. These phenomena resemble "progressive SR" of *Harmonia axyridis*. Multiplication of some reproductive systems which may exist in the cytoplasm of *H. axyridis* might be achieved during the latent period to raise the SR condition. Spontaneous restoration from the SR condition was also elucidated as the result of unequal segregation of the cytoplasmic particles during cellular division. Instability of transmittance of the SR condition indicates that it might be affected by environmental factors including diet. As it is known that the SR of *Drosophila melanogaster* can be "cured" at a high temperature (SAKAGUCHI and POULSON, 1963), further investigation of the SR

condition in *Harmonia* is necessary.

The frequencies of SR in natural populations of *Drosophila* has been investigated in several species and regions. For example, 6.0% of the SR females in average have been found in 10 natural populations of *D. bifasciata* in Honshu Island, Japan (IKEDA, 1970). In *Harmonia axyridis*, a single female was followed in this report. However, we have noticed several other females producing female progenies in our rearing experiments. LUS (1947) reported the existence of the strains without males in another coccinellid beetle, *Adalia bipunctata* and also showed that the transmittance of unisexuality was unstable among *Adalia* populations. The SR condition may not be exceptional in beetles.

REFERENCES

- IKEDA, H. (1970) The cytoplasmically-inherited "sex-ratio" condition in natural and experimental populations of *Drosophila bifasciata*. *Genetics* **65**: 311-333.
- KOMAI, T. (1956) Genetics of ladybeetles, *Adv. Gen.* **8**: 155-188.
- LABORATORY OF ENTOMOLOGY, TAMAGAWA UNIVERSITY (1975) Variation and inheritance of elytral patterns in a lady beetle, *Harmonia axyridis*. *Heredity (Tokyo)* **29**: 72-79. (in Japanese)
- LUS, YA. YA. (1947) Some aspects of the population increase in *Adalia bipunctata* L. The strains without males. *Dokl. Akad. Nauk SSSR* **57**: 951-954. (in Russian)
- MAGNI, G. E. (1953) 'Sex-ratio': a non-Mendelian character in *Drosophila bifasciata*. *Nature* **172**: 81.
- MALOGOLOWKIN, C., D. F. POULSON and E. Y. WRIGHT (1958) Experimental transfer of maternally inherited abnormal sex-ratio in *Drosophila willistoni*. *Genetics* **44**: 59-74.
- MATSUKA, M., D. SHIMOTORI, T. SENZAKI and I. OKADA (1972) Rearing some coccinellids on pulverized drone honeybee brood. *Bull. Fac. Agric. Tamagawa Univ.* **12**: 28-38.
- OKADA, I., H. HOSHIBA and T. MAEHARA (1972) An artificial rearing of a coccinellid beetle, *Harmonia axyridis* PALLAS, on pulverized drone honeybee brood. *Bull. Fac. Agric. Tamagawa Univ.* **12**: 39-47. (in Japanese)
- OKADA, I. and S. KONDO (1973) Elytral patterns of a ladybird, *Harmonia axyridis* Pallas. *Heredity (Tokyo)* **27**: 57-60. (in Japanese)
- OKADA, I. and M. MATSUKA (1973) Artificial rearing of *Harmonia axyridis* on pulverized drone honeybee brood. *Environ. Entomol.* **2**: 301-302.
- SAKAGUCHI, B. and D. F. POULSON (1963) Interspecific transfer of the "sex-ratio" condition from *Drosophila willistoni* to *D. melanogaster*. *Genetics* **48**: 841-861.
- YOSHIDA, T. (1944) Chromosome studies in the Coleoptera, I. A study of chromosomes in ten species of Coccinellidae and Chrysomelidae. *Jap. J. Gen.* **20**: 107-115. (in Japanese)