Abnormal Sex-Ratio Condition in the Walnut Leaf Beetle, *Gastrolina depressa* (Coleoptera: Chrysomelidae)

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Abnormal sex-ratio (SR) condition was found in *Gastrolina depressa* in natural populations. Average hatchability of egg masses laid by SR and normal (NR) females was 47% and 92%, respectively. The male ratio in progenies was 0.3% in SR type and 48% in NR type. Egg masses laid after 16 days by normal females injected with resuspended pellet of SR ovaries tended to become SR. The SR agent(s) in *G. depressa* was sensitive only to tetracycline among four antibiotics tested. No SR colonies were found in Hokkaido, Shikoku or Kyushu. SR colonies have been found in limited areas at a northernmost limit of Fukushima and at a southernmost limit of Gifu, Central Honshu. No SR colonies were found in the Tohoku, Kinki and Chugoku districts in Honshu.

Key words: *Gastrolina depressa*, abnormal sex-ratio, microorganism, tetracyclin, geographical distribution

INTRODUCTION

Abnormal sex-ratio (SR) condition has been reported in various insects, e.g. *Drosophila* spp., mosquitoes, lady beetles, and bark beetles (MALOGOLOWKIN, 1958; IKEDA, 1970; LUS, 1947; MATSUKA et al., 1975; NIJIMA and NAKAJIMA, 1981; LANIER, 1966). The SR condition in insects is characterized by the absence of male progeny resulting from selective lethality at the embryonic stage. The causative factor of SR condition is in the cytoplasm. The SR condition can be transferred into and maintained in other individuals of the same or related species. In *Drosophila*, the SR condition is caused by infection with the SR-spiroplasma (POULSON and SAKAGUCHI, 1961; WILLIAMSON and WHITCOMB, 1974). IKEDA (1970) investigated the geographical distribution of SR condition of *D. bifasciata* in Japan, and found it to be distributed only in Honshu.

CHANG and MORIMOTO (1988) described the life tables of *Gastrolina depressa* in Ina, Nagano in central Japan and found that the number of male adults was much less than the number of females. Some female adults of *G. depressa* laid egg masses, from which only female larvae hatched.

This paper examines (1) the discovery of SR condition in *G. depressa*, (2) the transfer of SR condition by injection with SR homogenates, and (3) the effects of antibiotics to

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SR condition. In addition, we have investigated (4) the distribution and frequency of SR condition in natural populations in varied locations in Japan.

MATERIALS AND METHODS

(1) Sixteen females of *G. depressa* were collected from walnut trees in Ina, Nagano in 1987, and were reared at room temperature under natural day light from June to July. Egg masses were incubated at 20°C and hatched larvae were reared to adults in order to discriminate sexes of each individual. Rearing methods were presented in the previous paper (CHANG and MORIMOTO, 1988).

(2) The ovaries of SR females and entire pupal bodies were ground with a pestle and homogenized with 0.25 M sucrose as diluent. The homogenates were centrifuged at 13,400 g for 15 min at 4°C and the resultant pellets were resuspended in 0.5 ml of diluent. Five μ l of supernatant and pellet from SR individuals were injected into virgin females in normal sex-ratio (NR) condition using a microsyringe (Hamilton #701, needle gauge 26). Homogenates of normal individuals were injected to NR females as control. Hatchability of egg masses which were laid by the injected females was examined under 16L-8D at 25°C, and hatched larvae were reared to adult stage.

(3) Four antibiotics, streptomycin, penicillin G, kanamycin and tetracycline were injected into SR females at a dosage of 5 μ l per female at 3-day intervals. Injection was carried out after the first egg mass had been laid. This egg mass was kept at 16L-8D at 25°C and hatchability was examined for SR or NR typology. Hatched larvae were reared to adult stage for the purpose of sex examination. Tetracycline was injected at three different concentrations (500, 1,000 and 1,500 ppm). Other antibiotics were treated at 750 ppm. Same treatments were also made in NR females as control.

(4) Egg masses or larval colonies were collected from 41 localities in Japan from 1987 to 1989. The hatchability of egg masses was examined for judging the SR condition. Meanwhile, larval colonies were reared to adult stage and sex of each was examined.

RESULTS

The discovery of SR condition in females

IKEDA (1970) pointed out three general features of the SR condition in *Drosophila* as follows: (1) SR females produce offspring that consist almost entirely of females (2) XY zygotes are killed early in the developmental stage and (3) the SR condition is inherited only through the cytoplasm.

Table 1 shows the hatchability of eggs, mortality at the immature stage and sex ratio of F₁ progenies obtained from each field-collected female. Two types of hatchability of eggs were discovered, i.e., a low average of 47% from n1-12 and a high average of 92% from n13-16. Significant difference was found between these two groups ($p < 0.05$, STUDENT's *t*-test). Mortality of immature stage was 16% in the former type and 11% in the latter type, with no significant difference between the two. The male ratio was extremely low (average: 0.3%) in the former type, and normal (average: 48%) in the latter type. All these results suggested that the SR condition might exist in the former 12 individuals, and that hatchability could be used as an indicator of SR

Table 1. Development and sex of F₁ progenies obtained from field-collected females

<i>n</i>	No. of eggs		Hatchability (%)	Mortality in larva—adult (%)	No. of adults emerged		Male (%)
	observed	hatched			Female	Male	
1	477	200	41.9	22.0	156	0	0
2	538	228	42.4	20.6	180	1	0.6
3	388	175	45.1	10.9	156	0	0
4	204	92	45.1	8.7	84	0	0
5	365	167	45.8	12.0	147	0	0
6	502	232	46.2	12.1	203	1	0.5
7	408	189	46.3	14.8	160	1	0.6
8	477	221	46.3	25.3	164	1	0.6
9	522	242	46.4	22.3	187	1	0
10	267	125	46.8	14.4	107	0	0
11	383	193	50.4	9.8	174	0	0
12	259	141	54.4	22.7	109	0	0
Total of 12 females							
	4,790	2,205	46.5 ^{a,b}	15.5 ^a	1,827	5	0.3
13	418	363	86.8	11.8	165	155	48.4
14	325	285	87.7	11.6	131	121	48.0
15	297	280	94.3	4.3	146	122	45.5
16	218	209	95.9	17.2	81	92	53.2
Total of 4 females							
	1,258	1,137	91.6 ^{a,b}	10.7 ^a	523	490	48.4

^a Average was calculated after transformation of $\arcsin\sqrt{\bar{X}}$.^b Significant difference ($p < 0.05$, STUDENT's *t*-test).

condition. This was similar to the results of IKEDA (1970).

Transfer of SR condition

The result of artificial transfer of SR condition by injecting with homogenates of pupae or ovaries of SR individuals is shown in Table 2. When NR females were injected with SR ovarian supernatant, no transfer of SR condition occurred. All egg masses which were laid by the females injected with resuspended pellet within 15 days of injection were normal, however, four out of seven egg masses laid over 16 days after injection became SR. These four egg masses were laid by three different females injected with pellet. There was no transfer of SR condition when females were injected with SR pupal supernatant. When females were injected with pupal pellet, all individuals died before eggs were laid.

Effects of antibiotics on SR condition

The effect of four antibiotics on the hatchability of egg masses laid by SR females is shown in Table 3. Streptomycin, penicillin G and kanamycin did not affect SR condition at the concentration of 750 ppm. However, when tetracycline was injected into SR females at the concentration of 500 ppm, the hatchability of two out of 39 egg masses laid by nine females arose to 63% and 72% (67.3% in average), respectively. The two egg masses were laid by two treated females. One was the first of three egg masses, and the other was the second of seven. The male sex ratios in the two egg

Table 2. Transfer of the SR condition by injection with fractions of SR homogenates

Treatment	Days after injection									
	1-5			6-10			11-15			After 16
	No. ♀♀ exam.	Egg masses laid	NR	SR	No. ♀♀ exam.	Egg masses laid	NR	SR	No. ♀♀ exam.	Egg masses laid
SR ovaries										
supernatant	12(1) ^a	27	27	0	10	40	40	0	3	11
pellet	14(7)	11	11	0	8	21	21	0	4	7
Control:										
NR ovaries										
supernatant	4(1)	10	10	0	4	9	9	0	2	5
pellet	5(1)	10	10	0	3	8	8	0	1	3
SR pupae										
supernatant	5	—	—	0	5	9	9	0	4	15
pellet	18(18)	—	—	0	—	—	—	0	—	—
Control:										
NR pupae										
supernatant	6(2)	10	10	0	3	11	11	0	2	3
pellet	5(5)	—	—	0	—	—	—	0	—	—

^a Numerals in parentheses indicate dead ♀♀ before egg laying.^b Four egg masses were laid by 3 females.

Table 3. Effect of antibiotics on the hatchability of egg masses laid by SR females

Antibiotics	No. ♀ exam.	No. egg masses laid	No. SR egg masses (Average hatchability %)	No. NR egg masses (Average hatchability %)
Streptomycin ^a	SR 9(1) ^b	84	84 (44.4)	0
	NR 5	58	0	58 (87.6)
Penicillin G ^a	SR 7	30	30 (45.4)	0
	NR 6	42	0	42 (89.6)
Kanamycin ^a	SR 6(1)	36	36 (43.1)	0
	NR 7(2)	18	0	18 (85.1)
Tetracycline				
500 ppm	SR 9	39	37 (47.2)	2 (67.3) ^c
1,000 ppm	SR 15(6)	21	21 (33.9)	0
1,500 ppm	SR 4(2)	3	3 (46.8)	0
500 ppm	NR 5(1)	19	0	19 (88.5)
1,000 ppm	NR 4(1)	9	0	9 (86.7)

^a Concentration of antibiotics was 750 ppm.

^b Numerals in parentheses indicate dead ♀♀ before egg laying.

^c Frequency of males was 9.8% and 20.0%.

Fig. 1. Collection localities and SR frequency of *G. depressa*.

masses were 9.8% and 20% six and five males had emerged, respectively. When tetracycline was injected at the concentrations of 1,000 and 1,500 ppm, some females died before eggs were laid.

Geographical distribution and frequency of SR condition in natural populations

G. depressa is distributed in the four main islands of Japan; Hokkaido, Honshu, Shikoku and Kyushu. Table 4 and Fig. 1 show the collection localities of *G. depressa*

Table 4. Distribution and frequency of SR colonies in natural populations at different localities in Japan (1987, 1988 and 1989)

Prefecture, locality (Year)	Colonies examined	NR	SR (%)
Hokkaido			
Matsumae (1989)	28	28	0 (0.0)
Tohoku (Northeastern Honshu)			
Aomori Pref., Hirosaki (1987)	10	10	0 (0.0)
Ikarigaseki (1989)	24	24	0 (0.0)
Iwate Pref., Kanegasaki (1989)	28	28	0 (0.0)
Akita Pref., Tazawako (1989)	32	32	0 (0.0)
Yamagata Pref., Tsuruoka (1989)	31	31	0 (0.0)
Kanto and Chubu (Central Honshu)			
Niigata Pref., Kawaguchi (1989)	27	27	0 (0.0)
Fukushima Pref., Tadami (1989)	32	32	0 (0.0)
Kaneyama (1989)	28	23	5 (17.9)
Tochigi Pref., Minamikawachi (1989)	21	21	0 (0.0)
Gunma Pref., Shimonita (1988)	28	14	14 (50.0)
Saitama Pref., Ogano (1989)	10	5	5 (50.0)
Kanagawa Pref., Sagamiko (1989)	26	8	18 (69.2)
Nagano Pref., Kamiyamada (1987)	12	5	7 (58.3)
Ina (1988)	24	9	15 (62.5)
Nagano (1988)	24	12	12 (50.0)
Iiyama (1988)	17	5	12 (70.6)
Yamanashi Pref. Minobu (1988)	16	4	12 (75.0)
Nirasaki (1988)	16	3	13 (81.3)
Shizuoka Pref., Fukori (1988)	18	18	0 (0.0)
Toyama Pref., Fuchu (1989)	30	28	2 (6.7)
Ishikawa Pref., Tsurugi (1989)	18	17	1 (5.6)
Kanazawa (1989)	10	10	0 (0.0)
Fukui Pref., Katsuyama (1989)	24	17	7 (29.2)
Gifu Pref., Yatsuo (1989)	19	16	3 (15.8)
Mino (1989)	32	31	1 (3.1)
(1989)	26	25	1 (3.8)
Sunami (1988)	15	15	0 (0.0)
Fujihashi (1989)	16	11	5 (31.1)
Kuze (1989)	15	15	0 (0.0)
Kinki and Chugoku (Western Honshu)			
Shiga Pref., Eigenji (1989)	15	15	0 (0.0)
Mie Pref., Iitaka (1989)	32	32	0 (0.0)
Nara Pref., Yoshino (1989)	15	15	0 (0.0)
Tottori Pref., Hino (1989)	34	34	0 (0.0)
Shimane Pref., Hirose (1989)	31	31	0 (0.0)
Okayama Pref., Okayama (1988)	24	24	0 (0.0)
Soja (1988)	24	24	0 (0.0)
Yamaguchi Pref., Mikawa (1988)	17	17	0 (0.0)
Shikoku and Kyushu			
Kagawa Pref., Shionoe (1988)	32	32	0 (0.0)
(1989)	38	38	0 (0.0)
Tokushima Pref., Kamiyama (1989)	26	26	0 (0.0)
Fukuoka Pref., Ukiha (1989)	35	35	0 (0.0)

colonies and frequencies of SR condition in natural populations. No SR colonies were found in Hokkaido, Shikoku or Kyushu. SR colonies were found in central Honshu, in limited areas at a northernmost limit of Fukushima and at a southernmost limit of Gifu. The frequency was high in the mountainous areas in central Honshu and low in the outer limits. No SR colony was found in the Tohoku, Kinki and Chugoku districts of Honshu.

DISCUSSION

MAGNI (1953) reported that the hatchability of eggs laid by SR females of *Drosophila bifasciata* was about 50% and the male ratio of progenies was only 0.5% in an Italian population. LANIER (1966) also found that hatchability of eggs from SR females in the bark beetle, *Orthotomicus latidens*, was about 50%, whereas hatchability of eggs from normal females was 92%. For *G. depressa*, the average hatchability of SR and NR egg masses was 47% and 92%, respectively (Table 1). The hatchability of *G. depressa* in SR condition was similar to that in *D. bifasciata* and *O. latidens*. The male ratio in the progeny was 0.3% in *G. depressa*, which was also near to that of *D. bifasciata*. These facts suggested that SR in *G. depressa* is similar to *Drosophila* and the bark beetle, and that the male embryo is killed by SR agent.

MALOGOLOWKIN (1958) reported that the SR condition in *Drosophila willistoni* appeared after a two-week latent period when the normal virgin females were injected with SR ooplasm. In the present study, the latent period of SR agent(s) in *G. depressa* was about 16 days (Table 2). This suggested that a propagative microorganism, which may exist in the cytoplasm, might cause the SR condition after a latent period. LEVENTHAL (1968) pointed out that the SR agents in *D. bifasciata* might be intracellular virus-like microorganisms. In this study, pellet obtained by centrifuging at 13,400 g for 15 min can transfer SR condition, which suggests that the SR agent(s) in *G. depressa* is a microorganism larger than viruses. The SR agent in *Drosophila* is known to be a spiroplasma that disappears from the hemolymph of SR flies after penicillin G treatment (POULSON and SAKAGUCHI, 1961; WILLIAMSON and WHITCOMB, 1974). The SR agent in *Culex pipiens*, *Menochilus sexmaculatus* and *Hypera postica* is sensitive to tetracycline hydrochloride (YEN and BARR, 1974; GOTOH and NIJIMA, 1986; KLOSTERMEYER, 1978). In the present experiment, the SR agent(s) in *G. depressa* was sensitive only to tetracycline among the four antibiotics used, which suggested that SR condition of this species was caused by mycoplasma or related organisms. However, we could not hitherto detect such a SR agent under an electron microscope. Successive observation to investigate whether the individuals of the next generation were SR or NR was impossible due to the obligatory diapause of this species.

The SR individuals of *G. depressa* were only found in central Honshu. The frequency was higher in the mountainous areas in central Honshu and lower at the outlying regions (Table 4 and Fig. 1). One possible hypothesis to explain this distributional cline is that the mountainous areas in Honshu are abundant in food resources of walnut trees, favoring *G. depressa* propagation. The population density was higher in Ina than in Okayama, Chugoku district (CHANG, unpublished). At a high population density, even an extreme bias in sex ratio may not seriously affect the mating chance of females or population increase. SR individuals therefore may not be excluded in such populations.

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REFERENCES

- CHANG, K.-S. and N. MORIMOTO (1988) Life table studies of the walnut leaf beetle, *Gastrolina depressa* (Coleoptera: Chrysomelidae), with special attention to aggregation. *Res. Popul. Ecol.* **30**: 297–313.
- GOTOH, T. and K. NIJIMA (1986) Characteristics and agent(s) of abnormal sex ratio (SR) in two aphidophagous coccinellid species. In *Ecology of Aphidophaga. II* (I. HODEK ed.). Dr. W. Junk Publ., Hague, pp. 545–550.
- IKEDA, H. (1970) The cytoplasmically-inherited “sex-ratio” condition in natural and experimental populations of *Drosophila bifasciata*. *Genetics* **65**: 311–333.
- KLOSTERMEYER, L. E. (1978) Eastern and western alfalfa weevils, *Hypera postica* (GYLLENHAL): Distribution in Nebraska, chromosome comparisons, and effects of antibiotics on cross incompatibility. Ph.D. dissertation. University of Nebraska, Lincoln, 73 pp.
- LANIER, G. N. (1966) Interspecific mating and cytological studies of closely related species of *Ips* DEGEER and *Orthotomicus* FERRARI (Coleoptera: Scolytidae). *Can. Entomol.* **98**: 175–188.
- LEVENTHAL, E. (1968) The sex ratio condition in *Drosophila bifasciata*; Its experimental transmission to several species of *Drosophila*. *J. Invertebr. Pathol.* **11**: 170–183.
- LUS, Ya. Ya. (1947) Some aspects of the population increase in *Adalia bipunctata* L. 2. 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. (1958) Maternally inherited “sex-ratio” conditions in *Drosophila willistoni* and *Drosophila paulistorum*. *Genetics* **43**: 275–286.
- MATSUKA, M., H. HASHI and I. OKADA (1975) Abnormal sex-ratio found in the lady beetle, *Harmonia axyridis* PALLAS. *Appl. Ent. Zool.* **10**: 84–89.
- NIJIMA, K. and K. NAKAJIMA (1981) Abnormal sex-ratio in *Menochilus sexmaculatus* (FABRICIUS). *Bull. Fac. Agric. Tamagawa Univ.* **21**: 59–67 (in Japanese).
- POULSON, D. F. and B. SAKAGUCHI (1961) Nature of “sex-ratio” agent in *Drosophila*. *Science* **133**: 1489–1490.
- WILLIAMSON, D. L. and R. F. WHITCOMB (1974) Helical wall-free prokaryotes in *Drosophila*, leafhoppers and plants. *Colloq. Inst. Natl. Sante Rech. Med.* **33**: 283–290.
- YEN, J. H. and A. R. BARR (1974) Incompatibility in *Culex pipiens*. In *The Use of Genetics in Insect Control* (R. PAL and M. J. WHITTEN, eds.). Elsevier, Amsterdam, pp. 97–118.