THE CYTOPLASMICALLY-INHERITED "SEX-RATIO" CONDITION IN NATURAL AND EXPERIMENTAL POPULATIONS OF *DROSOPHILA BIFASCIATA*

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THE cytoplasmically-inherited "sex-ratio" condition (SR) has been analyzed by many workers. BUZZATI-TRAVERSO (1941) first reported "sex-ratio" females in *Drosophila bilineata* (=*D. bifasciata*) collected in natural populations in Italy. "Sex-ratio" females of the same species were found in Pavia, Italy (MAGNI 1953) and also in Asakawa, Japan (MORIWAKI and KITAGAWA 1954). Further examples of the "sex-ratio" condition have been reported in *Drosophila prosaltans* (CAVALCANTI and FALCÃO 1954), *D. borealis* (CARSON 1956), *D. willistoni* (MALOGOLOWKIN 1958), *D. equinoxialis* (MALOGOLOWKIN 1959a), *D. nebulosa* (POULSON and SAKAGUCHI 1960b) and *D. robusta* (POULSON 1966).

The general features of the "sex-ratio" condition in the eight species mentioned above are as follows: (1) SR females produce offspring that consist almost entirely of daughters. (2) *XY* zygotes are killed in early developmental stages. (3) The SR condition is inherited only through the cytoplasm. However, there are some differences among the SR conditions originating in different species. The SR conditions in the species of the *willistoni* group are infective both inter- and intraspecifically through injection with ooplasm of SR eggs (MALOGOLOWKIN and POULSON 1957; MALOGOLOWKIN 1959a,b; MALOGOLOWKIN, CALVALHO and DA PAZ 1960). Also, the infectivity with hemolymph of SR females was demonstrated by SAKAGUCHI and POULSON (1960, 1963). POULSON and SAKAGUCHI (1960a, 1961) found that the infectious cytoplasmic agents responsible for the SR condition in these species are spirochaetes. On the other hand, the SR condition in *D. bifasciata* cannot be transferred intraspecifically by injection with abnormal ooplasm (MALOGOLOWKIN and CALVALHO 1961). The [SR] agents of *D. bifasciata* SR are transmittable only through injection of SR ovary homogenates, but the infectivity is very low compared with *D. willistoni* [SR] agents (LEVENTHAL 1968). LEVENTHAL (1968) concluded that the [SR] agents in *D. bifasciata* might be intracellular virus-like microorganisms.

The evidence that the SR conditions are affected not only by cytoplasmic factors but by nuclear genes was demonstrated in *D. prosaltans* (CAVALCANTI, FALCÃO and CASTRO 1958), in *D. willistoni* (SAKAGUCHI and POULSON 1960b; MALOGOLOWKIN 1958) and in *D. paulistorum* (MALOGOLOWKIN 1958). CAVALCANTI, FALCÃO and CASTRO (1958) concluded that these genes would be comparable to the well-known *k* gene in Paramecium which acts to suppress the

multiplication of cytoplasmic agents. The observations that both inter- and intra-specific infections of the SR agents are successful among some species but not among others, suggest that the genetic background of host species affects the multiplication of the [SR] agents.

MAGNI (1959) investigated the frequencies of SR females in natural populations of *D. bifasciata* in Italy. SR females were found in frequencies of 8.4% and 6.1% in 1955 and 1956, respectively. CAVALCANTI, FALCÃO and CASTRO (1958) reported that among 82 females of *D. prosaltans* collected in Brazil in 1949, 11 females (13.4%) produced only daughters. Moreover, MAGNI (1959) demonstrated that in experimental populations of *D. bifasciata* consisting of SR and normal (N), or SR and spontaneously cured normal (Nc), or SR and thermally cured normal (Ntc), SR females are selectively advantageous to the normal ones, while Nc and Ntc females prove superior to SR females. WATSON (1960) has analyzed statistically the mechanisms of maintenance of the SR conditions in populations on the basis of data given by MAGNI, by CAVALCANTI *et al.* and by POULSON *et al.* (*loc. cit.*).

In the present article, the author describes the results of: (1) investigations of frequencies of SR females in nature, (2) shifts of SR frequencies in experimental populations, (3) comparisons of some components of fitness between SR and normal, and (4) effects of larval crowding on both SR and normal flies, especially under competitive conditions. Finally, on the basis of these results the author considers (5) mechanisms which may account for the persistence of SR females in natural populations.

MATERIALS AND METHODS

1. Strains of *D. bifasciata* used:

(1) ST (Standard)—normal wild-type strain. This strain originated from an iso-female strain collected in Akkeshi, Hokkaido, Japan in 1952 and has been denoted as Standard (MORIWAKI and KITAGAWA 1955).

(2) SR_{ST}—"sex-ratio" strain. This strain derived from SR-J strain collected in Asakawa, Tokyo in 1953 (MORIWAKI and KITAGAWA 1957) has been backcrossed to a normal ST strain through more than 90 generations by means of small mass cultures. When SR_{ST} females mated with mutant males (MORIWAKI and KITAGAWA 1957) and other wild-type males derived from various geographic origins (MORIWAKA and KITAGAWA 1957; IKEDA, unpublished), the females consistently showed the typical SR condition. SR_{ST} females produce only 0.03% males on the average, while ST females produce about 48% males.

In the present experiments except for Experiment I under Productivity, both of the above strains were used.

(3) *or(occhio rosso)*—normal strain marked with orange eye color mutant, *or*. This strain was derived from the *or* strain which was derived from a Pavia strain in 1948 by Dr. MAGNI who kindly provided it for our laboratory.

(4) SR_{or}—"sex-ratio" strain marked with *or*. This strain was obtained from the cross $SR_{ST} \times or$. For the initiation of the experiment, the SR_{or} strain was backcrossed recurrently to *or* males for five generations.

Both of the above strains were used in Experiment I under Productivity.

2. Media:

(1) Sterile yeast medium—This was made by a modification of OHBA's method (1961) using the following formula: water, 1000 ml; agar, 8 g; cane sugar, 50 g; Baker's live yeast, 200 g;

propionic acid (anti-mold solution), 5 ml. This medium was called 20% yeast medium and used for the experimental cage populations. Another sterile yeast medium used was called 10% yeast medium and was different from the above formula with respect to the content of yeast, i.e., 100 g of yeast to 1000 ml of water. Although this medium was not nutritionally optimal, it was employed in order to minimize contamination with bacteria and fungi.

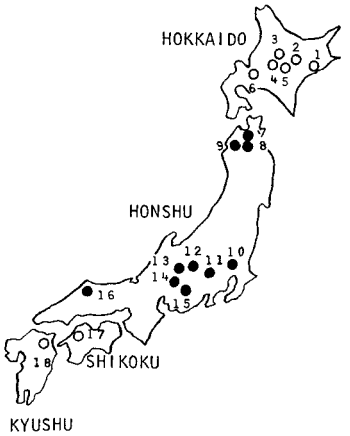
(2) Standard medium—This is different from the well known standard cornmeal-molasses-agar medium in the content of agar (8 g of agar to 1000 ml of water) because the high content of agar reduces the fecundity of *D. bifasciata*, and consequently contamination with bacteria and fungi is apt to occur easily.

Other procedures used will be described below.

RESULTS

I. The frequencies of SR females in natural populations in Japan.

D. bifasciata is found on the four main islands of Japan—Hokkaido, Honshu, Shikoku and Kyushu. To investigate the frequencies of SR females in *D. bifasciata* in Japanese populations, flies were collected in 18 localities in Hokkaido, Honshu, Shikoku and Kyushu during nine years from 1960 to 1968 (Figure 1). The collections were made during the summer in all localities with the exception of one locality, Tsukubasan, in which collections were made during the spring, since *D. bifasciata* can be found there abundantly in this season. The mean temperatures during May to September were calculated on the assumption of a decrease in temperature of 0.6°C/100 meter of elevation using as a basis the monthly aver-



Locality	Elevation	Mean temperature (May-Sept.)
HOKKAIDO		
1. Akkeshi	50 m	13.3°C
2. Meakan	400	10.9
3. Aizankei	500	14.3
4. Nishitappu	300	14.9
5. Nukabira	600	12.2
6. Nopporo	50	17.1
HONSHU		
7. Hakkoda	900	12.4
8. Yachi-Onsen	800	13.0
9. Dake-Onsen	450	15.1
10. Tsukubasan	800	18.9
11. Kumotoriyama	1900	10.4
12. Tadeshina	1300	15.9
13. Shibunoyu	1900	12.3
14. Komagatake	2000	11.5
15. Kitadake	2300	9.7
16. Daisen	750	20.7
SHIKOKU		
17. Ishizuchi	1600	13.3
KYUSHU		
18. Kuju	1300	14.7

FIGURE 1.—Collecting localities of *D. bifasciata* in Japan, their elevations and the mean temperatures. ●, localities where SR females were found.

TABLE 1

Frequencies of SR females of D. bifasciata *in natural populations of Japan*

Locality	Number of females		Percentages of SR females	Year
	Normal	SR		
Hokkaido				
Akkeshi	27	0	0	1962
	29	0	0	1964
Aizankei	98	0	0	1966
Meakan	37	0	0	1966
Nukabira	53	0	0	1966
Nishitappu	63	0	0	1964
	184	0	0	1966
Nopporo	68	0	0	1962
Honshu				
Hakkoda	14	1	6.8	1961
Yachi-Onsen	30	4(1)*	11.8	1966
Dake-Onsen	5	1(1)	..	1965
Tsukubasan	2	2	..	1961
Kumotoriyama	50	3	5.7	1961
	142	11(1)	7.2	1962
	154	18	10.5	1967
Tadeshina	45	4	8.2	1963
Shibunoyu	79	1	1.3	1960
Kitadake	35	2	5.4	1965
	113	2(1)	1.7	1967
Komagatake	44	1	2.2	1961
	216	18(4)	8.0	1962
Daisen	53	0	0	1966
	101	1	1.0	1967
Shikoku				
Ishizuchi	38	0	0	1966
	29	0	0	1967
	40	0	0	1968
Kyushu				
Kuju	24	0	0	1968

* Numbers of "intermediate" females are put in parenthesis.

age temperatures of the nearest cities for the period from 1930 to 1960. These temperatures are given in Figure 1. All of these localities are cool in summer, but in winter the minimum temperatures are below 0°C. The flora at each locality was not investigated in detail, but the woodlands consist, roughly speaking, of both coniferous trees, such as Picea, Tsuga and Abies, and deciduous trees, such as Fagus, Betula and Ulmus.

Females collected in nature were isolated as single female cultures at 20°C. Progenies which were produced were scored as SR or normal. The numbers of normal and SR females producing F_1 progenies are given in Table 1, and Figure 1 shows the localities where SR females were found.

Collections were made at six localities on Hokkaido, a total of 559 females were captured, but no SR female was found. In Ishizuchi, Shikoku, no SR female was found out of 108 females captured in three consecutive years (Table 1). Also in Kujusan, Kyushu, no SR female was found out of 24 females. Accordingly, it would seem that SR females are extremely rare if they exist at all on these islands. On the other hand, in all localities on Honshu, females carrying the SR conditions were found with a frequency of 6.0% on the average, or a total of 69 SR females *vs.* 1152 normal females. Particularly high frequencies of SR females were found in Yachi-Onsen in 1966 and Kumotoriyama in 1966 (Table 1). Otherwise, in one of the 10 localities on Honshu, Daisen (the southern part of Honshu), the frequency of SR females is extremely low, e.g., 0.6% on the average, or one SR female *vs.* 154 normal females in a total of two years' collections. The average frequency, 6.0%, is similar to the values of 8.4% and 6.1%, in *D. bifasciata* in Italy, obtained by MAGNI (1959).

Eight of 69 SR females, or 11.6%, produced a few sons in their F_1 progenies. These females representing the incomplete SR condition were called "intermediate" by MAGNI (1956). The number of intermediate females is shown in parentheses in Table 1 and their F_1 progenies are summarized in Table 2. The average frequency in four populations where intermediate females were found is 1.4%, or 8 intermediate females *vs.* 506 normal females. One of these eight strains, SR_{DK-1} (derived from Dake-Onsen), was tested for the sex ratio of progenies through the F_5 generation by means of single female matings with the exception of a small mass mating employed to obtain F_2 offspring. The results are summarized in Table 3. The founder female of this strain produced 52 females and 5 males in the F_1. The F_1 offspring in a small mass culture yielded 263 females and 20 males in the next generation. To obtain F_3 offspring, 41 pair matings were established. These 41 progenies fall into three groups: 37 yielded unisexual progenies; three yielded essentially normal proportions of the sexes, a total 131 females and 115 males; and only one female yielded intermediate progenies of 62 females and 12 males. F_4 progenies of the third group fall into two groups: five out of 27 females produced bisexual offspring which totaled 151 females and 165 males; and 22

TABLE 2

F_1 progenies of "intermediate" females of D. bifasciata *captured in nature*

Locality	Strain	F_1 progeny ♀♀	F_1 progeny ♂♂
Komagatake	SR_{KO-69}	17	1
	SR_{KO-80}	19	2
	SR_{KO-201}	30	2
	SR_{KO-265}	11	1
Kumotori-yama	SR_{KU-47}	57	4
Dake-onsen	SR_{DK-1}	52	5
Yachi-onsen	SR_{YC-60}	49	1
Kitadake	SR_{KT-99}	32	2

TABLE 3

Progeny tests of an intermediate strain (SR_{DK-1}) of D. bifasciata

F_1		F_2		F_3		F_4		F_5	
♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
				53	53	170	171 (6;N)	90	96(3;N)†
								179	191(5;N)
				39	23	498	441(15;N)	186	154(6;N)
								266	216(7;N)
						151	165 (5;N)	74	79(2;N)
								108	103(4;N)
								109	152(9;N)
52	5 → *	263	20	65	12				
						1146	0(22;SR)	161	0(5;SR)
								132	0(5;SR)
				39	39	....		....	
				33	0	1328	0(17;SR)	....	
				41	0	594	0(11;SR)	....	
				2230	0(35;SR)				

* These tests were done by means of single female cultures except F_2.
† Number of females tested; Normal or SR.

out of 27 females produced offspring showing the typical SR condition. F_4 of the others, either unisexual or bisexual in F_3, resulted in a sex ratio the same as that of their parents. The majority of daughters derived from an intermediate female established typical SR strains and the minority produced bisexual strains producing bisexual progenies at most within four generations.

When SR_{ST} females were backcrossed with males of a "reestablished normal" strain derived from an intermediate strain, SR_{ST} females did not produce male progeny.

II. Experiments on the SR condition under laboratory conditions.

1. Frequencies of SR females in population cages.

Three types of populations were initiated at 20°C. The populations were started at three frequencies of SR females, 80, 50 and 20%. For each population, two replicates were established. Regularly, 1000 females were introduced into a cage, and the number of males was approximately equal to the number of normal females. Starting dates and the initial frequencies of SR females are: Cages 1 and 2, Feb., 1964, 50%; Cages 3 and 4, March, 1964, 20%; and Cages 5 and 6, Dec., 1964, 80%. SR_{ST} and ST strains were used. The flies initially introduced into the cages were raised on standard medium under uncrowded conditions. During the course of these experiments, the flies were handled with an aspirator without etherization. The population cages used (made of plastic; size 12 × 23 × 38 cm) had 15 cups (3.5 cm diameter; 5.0 cm depth) containing 35 ml of 20% yeast medium. A folded paper towel was inserted in each food cup because *D. bifasci-*

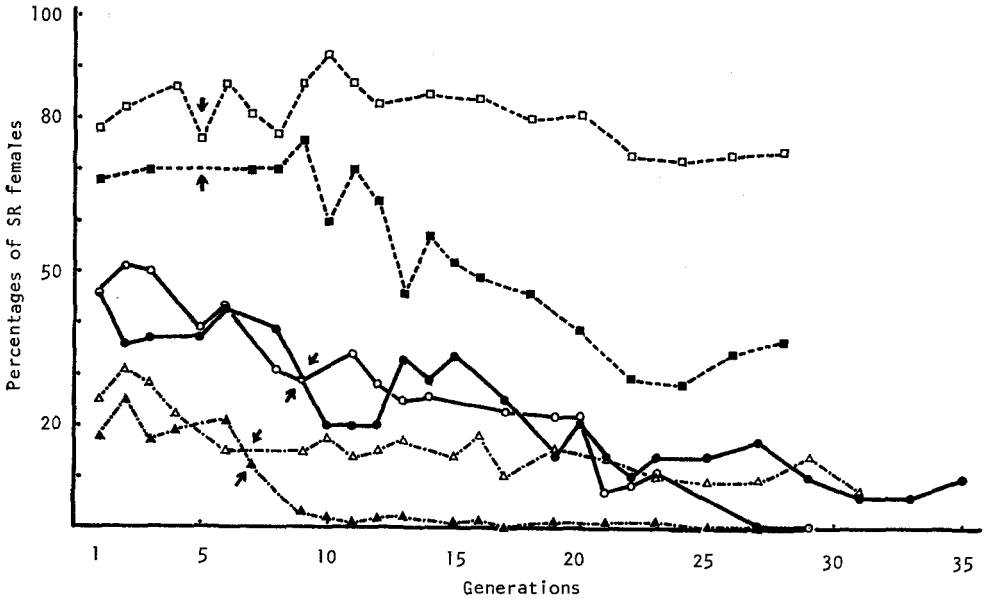


FIGURE 2.—Frequencies of SR females of *D. bifasciata* in successive generations in population cages. Arrow heads show the stages when the contamination with *D. melanogaster* occurred. —●—, C-1; —○—, C-2; —▲—, C-3; —△—, C-4; —■—, C-5; —□—, C-6.

ata have a tendency to oviposit on moist paper rather than on the food. Basing our estimation of one generation as 35 days, two cups were replaced every 5 days.

Estimation of the frequency of SR females in the population cages was made by establishing single female cultures. Three cups placed for a week in every cage were used as a source of larvae for a population sample. Larvae were removed from the cups and placed in a vial (3 × 10.5 cm) containing standard medium and a heavy yeast suspension. This technique allowed larvae to grow and develop under uncrowded conditions. Approximately 270–300 adult females which emerged from the cultures were placed individually with males in food vials. Their progenies were scored as SR or normal. A check on the frequency of SR females was made once a generation as a rule until the 15th generation; after the 16th generation the sample was extracted once every few generations.

Figure 2 shows the changes in the frequencies of SR females in six populations. In C-1 and C-2 initiated with 50% SR females, the frequencies of SR females decreased gradually to approximately 10% after 20 generations. In C-2, however, the SR females were suddenly eliminated during the period from the 23rd to 27th generations. This rapid elimination was presumably caused by nonparasitic mites which heavily infested only this cage at that time. In C-3, one of the two cages started with 20% SR females, the frequencies of SR females rapidly dropped from about 20% to almost zero within 10 generations. After that, SR females maintained a low frequency but they were eliminated entirely by the 26th generation. In the other cage started with 20% SR females, C-4, SR females decreased slowly to an approximate equilibrium similar to that in C-1 (about

10% SR). The last two cages, C-5 and C-6, started with 80% SR females gave apparently different results. The proportions of SR females in C-6 did not differ significantly from its initial value after 30 generations. C-5 showed the same tendency as C-6 in earlier generations, but after the 10th generation, it dropped swiftly to a frequency below 50%. During these experiments, a slight contamination with *D. melanogaster* occurred in all cages in the early generations (the stages are shown by arrows in Figure 2). This contamination did not seem to affect the frequency of SR females in almost all of the cages. However, the effect of *D. melanogaster* could not completely be excluded in the case of C-3, because the frequencies of SR females in this cage were rapidly decreased at almost the same time as the contamination. The data presented lends strong support to the interpretation that the SR females are more readily eliminated through competition with normal flies, with other species of Drosophila, or with other organisms.

2. Comparisons of components of fitness.

To investigate the differences in components of fitness between SR and normal strains, the following characters were measured: (a) rate of development, or the average time between the egg laying and eclosion, (b) fertility and time of sexual maturity after eclosion, (c) productivity, measured by the number of daughters produced in a given reproductive life.

(a). Rate of development.

The strains used are SR_{ST} and ST. Well-fed parental flies were placed in oviposition vials containing 10% yeast medium for an hour. Forty hours after oviposition, the first instar larvae were introduced into vials (3×10.5 cm) with 10 ml of 10% yeast medium following OHBA's method (OHBA 1961). Fifty larvae were introduced into each vial. For the SR strain, 10 replicates were established, while for the normal strain 20 replicates were established in order to obtain data on similar numbers of females, since only about half the larvae of the normal strain are females. The rate of development was measured as the average time between the egg laying and eclosion. The number of flies emerging was recorded every four hours from the time when the first fly appeared to the 504th hr after the beginning of oviposition. By the 504th hr, about 90% of flies have emerged. Another set of experiments was set up as replicates. These two sets are referred to as Experiments I and II.

The results of both experiments and the combined data are given in Table 4. The cumulative emergence curves obtained from the combined data are shown in Figure 3. These experiments were carried out at 20 ± 0.5°C, but at the 5th day in Experiment I and at the 2nd day in Experiment II after oviposition, the temperature accidentally rose to 21 ± 0.5°C for about 24 hr. A significant difference is not observed between the rate of development of SR and ST females. *D. bifasciata* shows a tendency to emerge in the early morning rather than either in the daytime or at night. This tendency is reflected in the depressed parts of the curves in Figure 3. It seems to be a characteristic of *D. bifasciata* that males grow faster than females.

TABLE 4

Mean times of development in hours of SR and ST females and ST males of D. bifasciata

Experiment	Strain and sex	Total number of flies emerged	Number of flies recorded	Mean development time in hours
I	SR♀	322	291	468.6
	ST♀	318	271	470.6
	ST♂	311	280	464.6
II	SR♀	343	319	477.0
	ST♀	355	329	477.8
	ST♂	340	329	468.0
I + II*	SR♀	665	610	473.0
	ST♀	673	600	474.5
	ST♂	651	609	466.4

* Combined data of Experiments I plus II.

(b). Fertility.

The fertility and the time of attainment of sexual maturity after the eclosion were examined in SR and normal females. SR_{ST} and ST strains were used. Flies were raised under uncrowded conditions on standard medium and several drops of yeast suspension were added during larval stages. Virgin females were collected within 12 hr after eclosion and stored on live yeast seeded standard medium

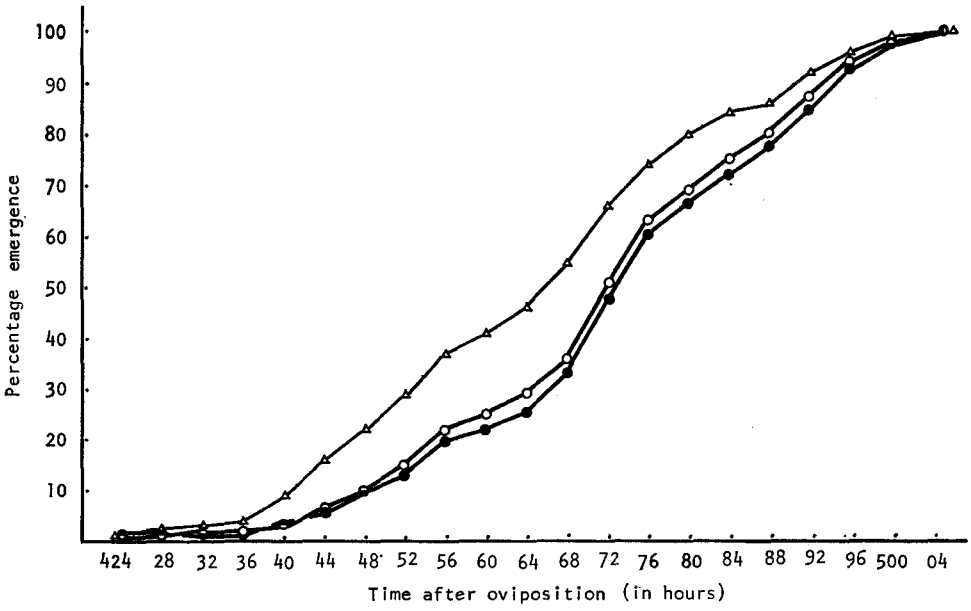


FIGURE 3.—Cumulative curves of the rate of development obtained from the combined data of Experiments I and II (see text). —▲—, ST male; —○—, ST female; —●—, SR female.

TABLE 5

Comparison of the mating abilities of SR and ST females of D. bifasciata

Days after eclosion	SR		ST	
	Number of females tested	Number of ♀♀ producing offspring	Number of females tested	Number of ♀♀ producing offspring
1	80	31	80	35
2	80	54	80	57
3	80	72	80	69
4	80	66	80	69
5	85	75	80	75
6	84	74	80	73
7	71	64	80	70

in 3 × 10.5 cm culture vials (about 30 flies per vial) until tested. The females, stored for 1, 2, 3, 4, 5, 6, or 7 days, respectively, were placed individually with two males (6–7 days old) for only 24 hr in culture vials with standard medium. After 24 hr, the males were removed and the females were left to produce offspring. About 10 days later, these cultures were scored as fertile or sterile. About 80 females were tested in each treatment. The experiments were carried out at 20°C and without etherization.

The results are given in Table 5. The percentages of fertile females reached a plateau at approximately 80 to 90% fertility on the third day after eclosion. No difference was observed in either female fertility or time of attainment of sexual maturity between SR and ST strains.

(c). Productivity.

Productivity was measured as the number of daughters produced per female; two sets of experiments were performed.

Experiment I: In this experiment, two kinds of crosses were made. Cross A was made between 1 SR_{or} ♀, 1 ST ♀ and 2 *or* ♂♂ in each vial. In another (designated as Cross B), 1 SR_{ST} ♀ and 1 *or* ♀ were placed with two *or* ♂♂ in each vial. The offspring marked with *or* from Cross A were produced by SR females and flies with normal phenotype, by ST females. In Cross B, the phenotypes of offspring were reversed. Virgin females were collected within 12 hr after eclosion and were mated with *or* males in food vials for two days. On the second day after eclosion, the parental females were introduced into culture vials containing standard medium as mentioned above. They were transferred to fresh vials every two days until the 24th day after eclosion. The number of offspring was recorded from 40 parental females surviving until the 24th day. The comparison of productivity was made between the number of daughters given by ST females in Cross A and those from SR_{ST} females in Cross B. The offspring of mutant females were discarded because they comprised only about one-third of those produced by the normal females.

The results are shown in Table 6-A. The average number of daughters per female in a given reproductive life (18 days) are 144.9 for the ST strain and 128.0 for SR_{ST}. The difference between them is significant at the 5% level.

TABLE 6

Number of offspring produced by SR and ST females of D. bifasciata

A. Experiment I

Age of female (days)	Normal			SR		
	Number of offspring		Number of daughters per female	Number of offspring		Number of daughters per female
	♀♀	♂♂		♀♀	♂♂	
2–4	59	0	1.5	0	0	0.0
4–6	486	450	12.2	560	0	14.0
6–8	759	644	19.0	603	0	15.1
8–10	846	801	21.2	765	0	19.1
10–12	639	550	16.0	557	0	13.9
12–14	841	867	21.0	707	0	17.7
14–16	680	660	17.0	696	0	17.4
16–18	758	712	19.0	631	0	15.8
18–20	726	663	18.2	559	0	15.0
Total	5794	5347	144.9	5118	0	128.0
			B. Experiment II			
4–8	1934	1827	16.7	1496	0	12.9
8–12	3688	3362	31.8	3010	0	26.0
12–16	3364	3303	29.0	2858	0	24.6
16–20	2840	2712	24.5	2305	1	19.9
20–24	2358	2309	20.3	2205	0	19.0
Total	14184	13513	122.3	11874	1	102.4

Experiments I and II are different from each other with respect to the strains and the experimental schemes (see text).

Experiment II. Strains used were SR_{ST} and ST. Flies were raised on standard medium and heavy yeast suspensions were added during larval stages. Virgin females were collected within 12 hr after eclosion, and 24 hr later they were mated with 5–6 day old males for three days. On the fourth day after eclosion, ST females were placed singly with two males of their own strain in individual vials containing standard medium seeded with live yeast. The parental flies were transferred to newly prepared food vials every fourth day until the 24th day after eclosion. In the case of the SR strain, two SR_{ST} females were introduced with three ST males into each food vial in order to equalize the larval density between cultures of SR and those of normal strains, since only about half the eggs laid by SR females yielded viable larvae, while most of normal eggs yield viable larvae. Other procedures were as same as those in the case of ST.

The number of offspring was counted and recorded on 116 parental females surviving until the 24th day. The results are summarized in Table 6-B. The average number of daughters produced per ST female (122.3 daughters) during a given reproductive life (20 days) is larger than that (102.4 daughters) produced by a SR female. The difference is significant at the 1% level.

3. Larval competition.

Larval competition is one of the important components of fitness in Drosophila

populations. In carrying out the competition studies, the first instar larvae of SR_{ST} and ST strains (not more than 45 hr old) were introduced into 3 × 10.5 cm vials containing 10 ml of 10% yeast medium at densities of 50, 100, 200 and 400 per vial (for convenience, they are referred to as D50, D100, D200 and D400). Larvae were collected in the same manner as mentioned under "Rate of development". In this case, however, the parental flies were allowed to oviposit for four hours in order to collect enough larvae within a given time. The population at each density consisted of both SR and ST larvae in a ratio of 1 : 1, 1 : 2, or 1 : 4. (These combinations are referred to as Series C, D, or E, respectively.) In other words, the proportions of females of SR to ST are 2 : 1 in Series C, 1 : 1 in Series D, and 1 : 2 in Series E, because all of SR larvae are females, whereas for the ST strain only half of them are females. In addition to the above series, two types of populations were set up at each density. One of them consisted of only ST larvae and another was composed of only SR_{ST} larvae (they were designated as Series A and B, respectively). In D50, Series D and E were not set up. For a given series, D50 was replicated 30 times, D100 and D200 replicated 10–15 times while D400 was replicated 5–6 times.

In the case where SR and ST larvae were introduced together into a vial, the frequency of SR females emerging from cultures was estimated by following formula:

$$\text{Expected frequency of SR females} = \frac{X}{YR + X}$$

where X is the number of SR larvae initially introduced, Y means the number of ST larvae initially introduced and R shows the proportion of ST females emerged from Series A at a given density.

$$\text{Observed frequency of SR females} = \frac{Z - Wr}{Z}$$

where Z is the total number of females and W is the number of males emerged from mixed population and r is the ratio of females to males given from Series A at a given density. The experiments were carried out at 20°C. The results are shown in Table 7. The average number of flies emerging decreases with increasing larval density. In D50, the percent emergence is about 66%, while in D400 where the larval density is highest, the values are approximately 35%. On the other hand, the percentages of pupation are almost equal regardless of larval densities. However, the proportions of pupae which pupated in the food increased with increasing larval density as shown in Table 8. The increase of proportions of pupae embedded in the food are responsible for the reductions of percent emergence, because the pupae in the food fail to emerge from puparia.

As seen in Table 7, there is no difference in preadult viability between SR and ST strains at any densities when the larvae of both strains were introduced separately (i.e., in the case of Series A and B). The sex ratio of the ST strain given in Series A at four different larval densities is not significantly different from the normal sex ratio (52% females on the average). The numbers of SR females that emerged from mixed populations (Series C, D and E) both at D200 and at D400, are significantly smaller than the expected value (P always 0.05 or lower).

TABLE 7

Competition between SR and ST strains of D. bifasciata *at various larval densities*

Number of larvae per vial		Number of vials	Total number of larvae	Percentage of pupation	Number of adults		Percent emerging	Percentage of SR females	
SR	ST				♀♀	♂♂		Expected	Observed
0	50	30	1500	80.8	516	483	66.6	....	....
50	0	30	1500	80.9	1011	2	67.5	....	....
25	25	30	1500	81.2	739	253	66.1	65.94	63.43
0	100	10	1000	88.7	340	319	65.9	....	....
100	0	10	1000	85.3	628	0	62.8	....	....
50	50	10	1000	80.5	504	145	64.9	65.96	69.34
33	66	15	1485	83.6	541	311	57.4	49.22	41.82**
20	80	15	1500	86.1	490	369	57.3	32.64	19.74***
0	200	10	2000	68.8	603	560	58.2	....	....
200	0	10	2000	72.6	1137	0	56.9	....	....
100	100	10	2000	65.1	775	286	53.1	65.86	60.21***
66	132	10	1980	86.4	665	348	51.2	49.28	43.56**
40	160	10	2000	83.8	570	398	48.4	32.53	25.58***
0	400	6	2400	79.6	391	390	32.5	....	....
400	0	6	2400	74.9	776	0	32.3	....	....
200	200	6	2400	80.5	619	254	36.4	66.67	58.86***
133	266	6	2394	78.4	469	266	30.6	50.03	43.14**
80	320	5	2000	89.5	418	301	36.0	33.36	27.80*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

In the tests at D100, different results were obtained. In Series D, the proportions of SR females are significantly lower than expected at the 1% level, and in Series E, the value is also significantly lower at the 0.1% level. However, in Series C the proportion of SR females does not differ significantly from expectation

TABLE 8

Number of pupae pupating either on the wall of vial or in food

Number of larvae per vial		Total number of larvae	Number of pupae		Percent pupation
SR	ST		On the wall	In the food	
33	66	1485	964	290	83.6
20	80	1500	961	331	86.1
Total		2985	1925	621	85.3
66	132	1980	1147	564	86.4
40	160	2000	1161	515	83.8
Total		3980	2308	1079	85.3
133	266	2394	977	905	78.4
80	320	2000	904	886	89.5
Total		4394	1881	1791	83.6

($P > 0.10$). In this last case, some pupae which pupated in the food were picked up with a needle and placed on the wall of vials before the beginning of eclosion. The differences within the results at D100 may have arisen because of such treatment.

4. Comparisons of a quantitative character.

(a) Comparisons of wing length among flies raised at various levels of larval density and at three different temperatures.

To investigate the effects of density and temperature on a body character, comparisons of wing length between SR and ST females raised at various densities and temperatures were performed. Both SR_{ST} and ST females were maintained at 15°C, 20°C and 25°C at various densities. The females resulting from the D50, D100, D200 and D400 series at 20°C in the competition experiments (Series A and B) were used for the measurements of wing length at 20°C. Females raised both at D100 and at D400 at 15°C, and at D100 at 25°C were obtained by the methods used in the competition experiment.

The left wings of 50 females in each treatment were drawn on paper with a projection microscope and the lengths from the humeral crossvein to the distal tip of the wing along the third longitudinal vein were measured with a ruler (1 unit = 1/500 meter). One millimeter is equivalent to 13.1 ruler units.

The mean wing lengths (m) in units and coefficients of variation (C.V.) of SR and ST females raised at D100 at 25°C are: SR♀, $m = 33.78 \pm 0.13$, C.V. = 2.72; ST♀, $m = 33.48 \pm 0.13$, C.V. = 2.81. The other data are summarized in Table 9. Reduction in wing length is correlated with increasing larval density. A similar relationship between the wing size and the intensity of larval crowding has been reported in *D. melanogaster* and *D. virilis* by OHBA (1961) and in *D. pseudoobscura* by SOKOLOFF (1966). Temperature effects at various densities are not simple. For D100, the mean wing length at 20°C is significantly greater than that at 25°C at the 0.1% level in both SR and ST strains, whereas the value for ST females at 15°C is significantly greater than that at 20°C. For D400, the mean wing length is greater at 20°C than at 15°C at the 0.1% level.

TABLE 9

Mean wing length of SR and ST females of D. bifasciata *raised at various larval densities at different temperatures and their coefficients of variation (C.V.)*
13.1 units = 1 mm

Larval density	Strain	15°C		20°C	
		Mean in units	C.V.	Mean in units	C.V.
50	SR	.....	...	36.56 ± 0.12	2.40
	ST	.....	...	36.39 ± 0.15	3.73
100	SR	35.88 ± 0.19	3.77	36.05 ± 0.12	2.28
	ST	36.40 ± 0.13	2.73	35.99 ± 0.10	1.93
200	SR	.....	...	35.36 ± 0.15	2.95
	ST	.....	...	35.47 ± 0.15	3.03
400	SR	32.07 ± 0.18	4.61	33.02 ± 0.17	3.75
	ST	33.15 ± 0.22	3.97	34.30 ± 0.22	4.50

So far as the present results are concerned, a linear relationship between temperature and wing length is not observed.

Paired comparisons between SR and ST females when the density and temperature are kept constant, show the following relationships: (1) There is no significant difference in wing length between both strains at D50, D100 and D200 at 20°C and at D100 at 25°C. (2) The mean wing length of ST females is significantly greater than that of SR females at D400 both at 15°C ($P < 0.05$) and at 20°C ($P < 0.001$) and also at D100 at 15°C ($P < 0.05$). Here we must consider that only half the ST larvae are females, while all of SR larvae are females. Hence, these differences of wing length between SR and ST females may be caused by the difference in larval constitutions. The fact that the sex ratio of the normal strain did not deviate from a normal sex ratio (approximately 1 : 1) at any density would suggest that the differences in wing length under some conditions might result from cytoplasmic differences between both strains because both strains are similar to each other with respect to genetic background (the SR_{ST} strain had been backcrossed to the ST strain for more than 90 generations). The effects of these factors would be expressed phenotypically under very severe conditions.

(b) Comparisons of wing length among flies sampled from experimental and natural populations and raised under various laboratory conditions.

To obtain information on the cage populations, the adult females were sampled from the four cage populations (C-1, C-4, C-5 and C-6) on the second generation after the population study had ceased, and wing length was measured. The mean wing lengths (m) in units and coefficients of variation (C.V.) are: C-1, $m = 33.94 \pm 0.19$, C.V. = 3.93; C-4, $m = 34.73 \pm 0.16$, C.V. = 3.17; C-5, $m = 33.28 \pm 0.27$, C.V. = 5.78; C-6, $m = 34.60 \pm 0.18$, C.V. = 3.80.

To compare the wing length of females raised in cage populations with that of females reared in culture vials, normal females were obtained at both D200 and D400 at 20°C on 20% yeast medium and their wing lengths were measured. The values are: D200, $m = 36.46 \pm 0.13$, C.V. = 2.53; D400, $m = 35.20 \pm 0.15$, C.V. = 2.93.

In addition, to obtain some data on flies developed in nature, the wing lengths of females captured in four natural populations, Kitadake (KD), Kumotori-yama (KU), Daisen (DS) and Ishizuchi (IS), were measured. The values are: KD, $m = 36.99 \pm 0.33$, C.V. = 6.39; KU, $m = 35.75 \pm 0.33$, C.V. = 6.61; DS, $m = 35.82 \pm 0.23$, C.V. = 4.65; IS, $m = 35.39 \pm 0.35$, C.V. = 5.44.

In each case, the mean wing length was obtained from the data on left wings of 50 females with an exception of that for the Ishizuchi natural population from which only 29 females were available for the measurements.

The relationships among average wing lengths derived from cages, natural populations, 20% yeast medium and 10% yeast medium at 20°C are shown in Figure 4. The flies reared on 20% yeast medium have significantly larger wings than those raised on 10% yeast medium at all larval densities ($P < 0.001$). Comparing the mean wing length among flies derived from different cage populations, the values are not significantly different between C-4 and C-6, but these two

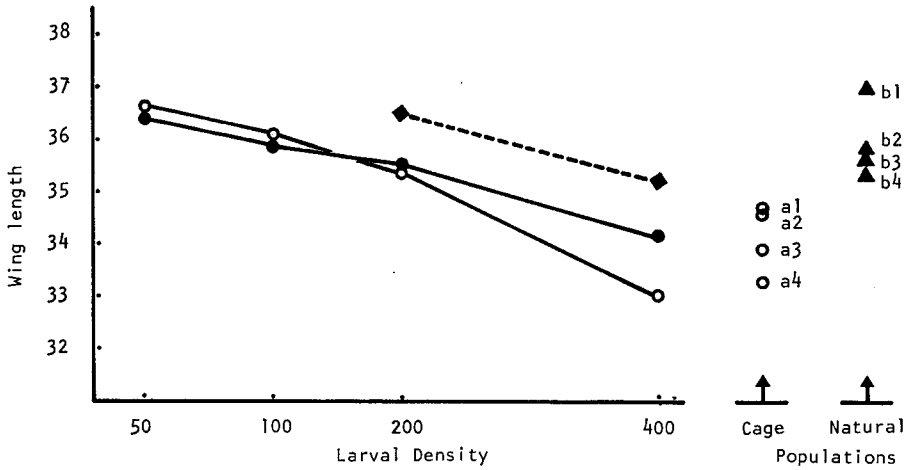


FIGURE 4.—Comparison of wing lengths of females sampled from experimental and natural populations and reared at different larval densities. —◇—, 20% yeast medium, ST; —●—, 10% yeast medium, ST; —○—, 10% yeast medium, SR.

○, Mean wing length of females sampled from cage population. a1 . . C-4; a2 . . C-6; a3 . . C-1; a4 . . C-5.

▲, Mean wing length of females captured in nature. b1 . . Kitadake; 2b . . Daisen; b3 . . Kumotoriyama; b4 . . Ishizuchi.

values are significantly larger than those both of C-1 at the 5% level and of C-5 at the 0.1% level. While the value of C-1 flies seems to be greater than that of C-5 flies, no statistical difference is obtained ($0.1 > P > 0.05$). The flies from cage populations are always significantly smaller than those from 20% yeast medium cultured at D400 (P always 0.05 or lower). On the other hand, the flies from C-1, C-4 and C-6 are either greater or approximately equal to ST females from 10% yeast medium cultured at D400 at 20°C. The flies from C-5 cage are apparently smaller than those from 10% yeast medium cultured at D400 ($P < 0.01$) and the coefficient of variation is highest among flies derived from cage populations and is comparable to those for natural populations. In the C-5 population, as mentioned previously, the frequencies of SR females changed most drastically. The facts that the mean wing length for C-5 is smallest and the C.V is the highest among four cage populations, would suggest that the flies within this population were under severe competitive conditions.

The flies captured in nature have wing lengths which are apparently greater than those from population cages. The mean wing lengths of flies from KU and IS populations are similar to that for D400 on 20% yeast medium. Females collected in the DS population show an intermediate value between those obtained for D200 and D400 on 20% yeast medium. In the case of KD natural population, wing length is most comparable to that for D200 on 20% yeast medium. However, the C.V. value is evidently greater than the values given for the most crowded conditions in both experiments on 10% yeast medium and on 20% yeast medium.

DISCUSSION

In *Drosophila bifasciata*, a species found on the four main islands of Japan, flies were collected at 18 localities in Kyushu, Shikoku, Honshu and Hokkaido between 1960 and 1968. In all of ten localities in Honshu, SR females were found in frequencies ranging from 1% to 12%. These values are comparable to the values of 8.4% and 6.1% obtained by MAGNI (1959) in Italian natural populations of *D. bifasciata* and also similar to the frequency (13.4%) given for the Brazilian populations of *D. prosaltans* (CAVALCANTI, FALCÃO and CASTRO 1958). However, in Daisen, located in the southern part of Honshu, the frequency of SR females is extremely low, 0.6% on the average of two years' collections. On the other hand, in any locality on other islands (Kyushu, Shikoku and Hokkaido), no SR female was found. Hence, it would appear that the SR females are extremely scarce if they exist at all in these three islands. The distributions of SR females will be discussed later in this report.

Eight of 69 SR females, or 11.6%, showed the intermediate SR condition. Intermediate females have also been found in natural populations of *D. bifasciata* in Italy (MAGNI 1956). MAGNI concluded from his investigations that these intermediate females were produced as a result of unequal segregation of cytoplasmic particles during cellular division. If the females did not receive enough cytoplasmic particles to kill all of the *XY* zygotes, they would become either intermediate or normal females. MAGNI's conclusion would seem to be applicable to the present results. When males of the re-established normal strain were crossed repeatedly to SR_{ST} females, a disturbance of the SR condition is not found. The hypothesis that intermediate females are produced by the presence of genes unfavorable for the multiplication of cytoplasmic particles should be excluded.

To document the frequency distribution of SR females, three types of experimental populations were started in a 20°C constant temperature room. In five of the six populations, i.e., in C-1, C-2, C-3, C-4 and C-5, the frequencies of SR females decreased gradually and, especially in two of them (C-1 and C-4), an approximate equilibrium at 10% was achieved. However, in one population, C-6, started with 80% SR females, the frequency of SR females did not depart much from the initial value. In C-5 and C-6, the number of viable larvae must be smaller as compared with those of four other populations, for the initial ratios of normal females were lower than those of other populations. As shown in Table 6, the mean number of daughters of normal females is significantly greater than that of SR females. Under such relatively lower larval densities, it seems that SR females could be maintained at the initial high frequency as seen in C-6 and in the earlier generations of C-5. On the other hand, the rapid decrease of SR females in C-5 could be caused by genetic drift which was accompanied by the increase of the proportion of normal flies. It appears, therefore, that this change resulted in the increase of larval densities in the population cage and consequently the relative frequencies of SR females would be gradually decreased because of the severe larval competition. During the present experiments, contamination with *D. melanogaster* or with mites occurred. The contamination may have acted

as one remotely possible cause of drastic changes in C-2 and in C-3. The data presented here suggest that the SR females would be more readily decreased through competition with normal flies, with other species of Drosophila, or with other organisms.

MAGNI (1959) demonstrated that the SR females in *D. bifasciata* are eliminated from the experimental populations by mixing them with thermally cured females or with spontaneously cured females which show normal sex ratio, whereas the SR females are superior to the normal ones in experimental populations, at least in early generations. He then concluded that the SR females carrying the [SR] cytoplasm would be selectively favored over the normal ones. The differences between MAGNI's results and the present results are likely to be a consequence of the differences in both breeding techniques and strains used. MAGNI used the breeding technique described by BUZZATI-TRAVERSO (1955), in which a population is maintained in ordinary culture vials. This method tends to emphasize not only larval but also adult competition because all the newly emerging flies are transferred into culture vials to allow them to produce offspring. As a consequence, the densities of adult flies become extremely high in vials as compared with those of cage populations.

The rate of development and the fertility appear to be similar in both SR and ST strains. The mean number of daughters per normal female is significantly higher than that of the SR females. In general, low productivity is less advantageous for the maintenance of a population of a species. However, as shown in the results on larval competition, SR females are more sensitive to crowding effects. It must be noted that higher productivity would not always be profitable for the persistence of a character. In contrast, SAKAGUCHI and POULSON (1963) found that the daily productivity (the number of daughters) of Sevelen females of *D. melanogaster* which had been infected with the [SR] agents of *D. willistoni* was markedly higher (about three times) than the control, normal sex ratio, females. They considered that the stimulatory effects of the [SR] agents on egg production resulted in an increase in the production of infected females. Such extreme difference between SAKAGUCHI and POULSON's data and the present results may be due to the differences in species and the characteristics of [SR] agents.

According to the data on crowding effects, there is no significant difference in preadult viability between SR and normal strains at any density where the larvae of each strain were reared separately. However, when they compete with each other, the proportion of SR females emerging from a mixed population is significantly lower than the proportion initially introduced. These results demonstrate that SR females are inferior to normal females under competitive conditions.

In addition, differences in wing length between SR and ST females are found under the higher larval crowding at 15°C and 20°C and also under the lower larval density at 15°C. Both strains are similar to each other with respect to genetic background, since the SR_{ST} strain has been backcrossed to the ST strain for more than 90 generations. It is likely that the inferiority of SR flies shown in the present results is probably brought about by the characteristics of the cyto-

plasm. In other words, females carrying the [SR] condition appear to have a disadvantage and its expression may be enhanced under competitive conditions. This suggestive finding has been reported by OHBA (1961), who pointed out that the size of an adult fly is more sensitive to larval crowding than the preadult viability.

The wing length of females sampled from cage populations is very similar to the value for females on 10% yeast medium at D400 (the highest density) in spite of the fact that caged flies were reared on 20% yeast medium which is nutritionally superior to 10% yeast medium. The shift in the frequencies of SR females in cage populations appeared to be slower than expected from the results obtained in the competition tests and the comparisons of wing length. This inconsistency might be due to the following cause. In the competition tests, as shown in Table 8, the proportions of pupae buried in the food increased with increase in larval densities. These inviable pupae are responsible for the reduction in percentage emergence and in turn appear to influence the decrease in SR females. On the other hand, in population cages, some of the third instar larvae escaped from the food cups, pupated and emerged outside of cups. Moreover, some pupae in cages are able to emerge even though pupation occurred in the medium, because conditions in the cages are drier than in vials—the food is almost parched by the time the pupae begin to emerge.

The results of both the productivity experiments and those on larval competition are not enough to determine the mechanisms for the maintenance of SR females at an equilibrium frequency either in experimental or in natural populations because the present data do not provide any evidence as to the forces which might increase the proportions of SR females. If the detection of intermediate females in nature means that the change from the normal to SR occurs spontaneously, it is probable that newly arisen SR females contribute to maintenance of the SR condition at a given frequency in a population. It seems most unlikely that this change could occur since the infection experiments show that the [SR] condition in *D. bifasciata* is not transferred to normal females by the injection with ooplasm from abnormal [SR] eggs (MALOGOLOWKIN and POULSON 1957; MALOGOLOWKIN, CALVALHO and DA PAZ 1960), and is hardly transferred even with crude homogenates of ovaries of SR females (LEVENTHAL 1968).

Nuclear genes that are necessary for the multiplication of [SR] agents have been detected in *D. prosaltans* (CAVALCANTI, FALCÃO and CASTRO 1957, 1958), *D. willistoni* (MALOGOLOWKIN 1958; POULSON *et al* 1960b) and *D. paulistorum* (MALOGOLOWKIN 1958). Moreover, on the basis of these data, a statistical study of the behavior of these genes in populations had been developed by WATSON (1960). As WATSON (1960) has pointed out, gene-cytoplasm interaction seems to be one of the important factors in the explanation of the fact that SR females are maintained in natural populations. Unfortunately, in *D. bifasciata* direct evidence for gene-cytoplasm interaction has not been obtained in any test in which SR females were crossed with males either from several wild-type and mutant strains or from the reestablished normal strain originating from an intermediate strain.

It is of interest that in some areas such as Hokkaido, Shikoku and Kyushu, no

SR female has ever been collected, and in the southern part of Honshu, SR females were found rarely. There are two possible hypotheses to explain this observation. (1) The [SR] condition is produced by some agents that do not exist in Hokkaido, Shikoku or Kyushu, and the SR females in Honshu do not migrate to the other islands. (2) SR females were originally present on these islands but have been eliminated by some factor(s) unfavorable to them. After elimination, the immigration of SR females from Honshu to other islands did not occur. Hypothesis (1) is hardly probable, since in *D. bifasciata* the [SR] condition is almost impossible to transfer artificially or spontaneously in the laboratory. In addition, considering the fact that SR females are found in Italy in a frequency as high as that in Japan, it is unlikely that the [SR] agents are distributed in only one of the four islands in Japan. For hypothesis (2), the data presented to show that SR females are not at an advantage in either competition or population experiments suggest that SR females would be eliminated more readily than the normal ones in some natural populations. Competition with other Drosophila species or organisms may have an influence on the maintenance of SR females, since contamination with mites and *D. melanogaster* seemed to act as a cause for the rapid decrease of SR females in some cage populations.

Comparisons of wing length have been made between flies captured in nature and those obtained in the laboratory under various conditions of larval crowding and nutritional conditions. It is clear that flies from natural populations are similar to flies which have been reared under relatively uncrowded conditions in the laboratory. The coefficients of variation, however, are comparable to those of flies obtained under very crowded conditions. It seems likely that if the nutritive qualities of natural diets are comparable to those existing in laboratory situations, larval crowding in breeding sites in nature may not ordinarily be severe, but crowding is probably considerable in some breeding sites in a given area. This is suggested by SOKOLOFF's finding (1966) that "other organisms found at the slime fluxes are biotic factors which prevent Drosophila populations from reaching high densities. These organisms kill the adult flies or prevent them from reaching the breeding and feeding sites". Such a low larval density in breeding sites seems to contribute to the fact that SR females are present in relatively high frequencies in some natural populations.

D. bifasciata is usually the dominant species both in Hokkaido (MOMMA 1957; TAKADA 1957, 1958; IKEDA, unpublished) and in Honshu (IKEDA's observations over eight years) with the exception of Daisen. According to WAKAHAMA (1962), only 25 flies out of a total of 1150 Drosophilidae were *D. bifasciata* and the dominant species were *D. lutea*, *D. suzukii* and *D. immigrans* in Daisen. In Ishizuchi, according to the IKEDA's observations in 1966 and 1967, the dominant species was *D. curviceps* which is about 20 times as frequent as *D. bifasciata*. The Daisen and Ishizuchi populations may fall at the extremes of the distribution of *D. bifasciata* and these areas may be relatively unsuitable for this species. In such localities competition, whether inter- or intraspecific, should be taken into consideration as an important possible factor for the elimination of SR females.

Although it is still hard to prove the inability of SR females to emigrate from Honshu to other islands, it would be suggested by the following fact. *D. imaii*, a

sibling species of *D. bifasciata*, is sympatric with the latter Hokkaido, but has never been found in other islands. *D. imaii* was nearly always collected together with *D. bifasciata* on the same baits (MORIWAKI, KITAGAWA and OKADA 1967). Under laboratory conditions, *D. imaii* breeds as well as *D. bifasciata* (Dr. O. KITAGAWA personal communication). It seems that if the immigration of *D. imaii* is not inhibited by the channel between the islands, this species can flourish in a habitat like Honshu. The four main islands of Japan are separated from each other by channels whose widths are approximately 5 to 20 km, which rules out the possibility for a typical wild species such as *D. bifasciata* to migrate freely across the channels to the other islands.

Continuing investigations will examine the effects of genetic drift, ecological habits of *D. bifasciata*, and environmental conditions such as climatic and biotic factors on the [SR] condition. Such effects are necessary components of a truly critical study of this problem.

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SUMMARY

Females carrying the cytoplasmically-inherited "sex-ratio" condition in *D. bifasciata* are found with an average frequency of 6.0% in ten natural populations on Honshu, Japan. In eight other populations located on Hokkaido, Shikoku, or Kyushu, no SR female has been found.—The results from studies of experimental populations seem to demonstrate that SR females are slowly diminishing in number when in competition with normal flies, or with other species of Drosophila, or with other organisms.—A difference in the rate of development and fertility between SR and normal flies has not been observed.—Productivity (the number of daughters per female) is significantly higher in the normal strain than in the SR strain.—Wing length of SR females is significantly smaller than that of normal females under severe conditions.—When SR and normal flies compete with each other, the percent emergence of SR females in mixed populations is significantly lower than the expected value.—On the basis of these results, low larval competition in breeding sites in nature and elimination of SR females by either inter- or intraspecific competition under conditions unfavorable for *D. bifasciata* are suggested as possible mechanisms for the persistence of the SR condition.

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