EFFECTS OF SR FACTOR AND DA GENE ON THE VIABILITY OF THE HYBRID BETWEEN *DROSOPHILA MELANOGASTER* AND *D. SIMULANS*¹⁾

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Received August 30, 1976

The unisexuality of the progeny in the hybrid between *Drosophila melanogaster* and *D. simulans* has been known since the two species were distinguished. Sturtevant (1929) reported that the hybrid offspring between *melanogaster* females and *simulans* males were usually females with a few exceptional males and that the offspring in the reciprocal mating were mostly males with a few females. In case of the cross between attached-X *melanogaster* females and *simulans* males, the offspring were only males. As a rule, the hybrids do not survive unless they carry a *simulans* X chromosome. All hybrids are sterile, and the hybrid males from *melanogaster* ♀ × *simulans* ♂ seem to die in the larval stage.

The maternally inherited infectious 'sex ratio' (SR) agent in *Drosophila* causes the production of unisexual (female) progenies (review by Poulson 1963). The so-called SR spirochetes observed in the hemolymph of SR mothers, if injected into normal females of the same species or even of more distantly related species, multiply in the recipient and establish the abnormal sex-ratio condition.

The present paper describes that the expression of the male-specific lethality by the SR spirochetes is alleviated in the *melanogaster-simulans* hybrid males. The effect of the *daughterless* gene (*da*; Bell 1954) on the hybrid is also examined.

MATERIALS AND METHODS

Stocks:

1. One *Drosophila simulans* strain which had been collected in Mishima in 1975 and maintained by mass culture in our laboratory was used.
2. Oregon-R (OR) and ORNSR stocks of *D. melanogaster* were provided by Dr. K. Oishi. The latter had been established by injection with the SR-spirochete-carrying hemolymph from *D. nebulosa* to OR (Oishi 1971).
3. An attached-X stock with *yellow* and *forked* genes (*yf*: =) kept in our laboratory was used for making an *yf*: =-NSR stock. The hemolymph from ORNSR females was injected to the *yf*: =-females and the sex-ratio condition has been established.
4. A stock of *D. melanogaster* carrying *daughterless* (*da*), an autosomal maternal effect male-killing gene (2-39; Bell 1954) was provided by Dr. I. I. Oster.

The hybrid crosses were made at 25°C and the F₁ larvae reared at 18°C. 10×3 cm

1) Contribution No. 1,109 from the National Institute of Genetics

glass vials with about 10 ml of cornmeal, agar, molasses and yeast medium were used for all experiments.

RESULTS AND DISCUSSION

Infection of SR to D. simulans. Since *D. simulans* has not been examined with respect to its susceptibility to the male-killing effect of the SR spirochetes, results of SR infection will be presented first. The hemolymph from ORNSR females was injected to 3-day-old *simulans* females which had already been inseminated by males of the same species. Sixteen injected *simulans* females were individually placed in vials each with two *simulans* males. They were transferred to new vials every two days until the 5th brood. The number of the F_1 progeny from each vial was counted and the average sex ratio ($\frac{\text{♀}}{\text{♀} + \text{♂}}$) determined. Control experiments were done by injecting Oregon-R females with the same volume of ORNSR hemolymph. Appearance of SR condition in the *simulans* and its establishment by transmission through mother to daughter are shown in Fig. 1. In the first generation (F_1), males were present in the first two broods, but from the third brood on only female progeny appeared. When this was compared with OR, the sex-ratio condition for *simulans* was about one brood (two days) later to appear. Females from the 4th brood were taken as mothers for the following generations and the *simulans* strain became the complete sex-ratio by the F_3 generation. Thus it is apparent that *D. simulans* is a good host species for the SR agent. The lethal effect appears to be expressed in the embryonic stage, since no died larvae and pupae were observed.

Effect of SR on progeny of OR ♀ × simulans ♂. OR females with or without SR-

Fig. 1. Changes of sex ratio in the brood of SR-injected (F_1) and SR-established (F_2 , F_3) *Drosophila simulans* females. *D. melanogaster* (OR F_1) is presented as a control.

spirochetes were crossed in groups of 5 flies each with 5 *simulans* males at 25°C. They were transferred to new vials every three days until the third brood. Immediately after the transfer, old vials were placed at 18°C and kept there until the hybrid adults emerged, since the viability of hybrids is greatly reduced at higher temperatures such as 25°C (Sturtevant 1929). Table 1 shows the results. If the larvae were detected in the culture vial it was regarded as an indication of a successful mating or a fertile cross. The percent of the successful matings was nearly equal in the SR carrying females and in normal females. The average number of emerged flies per fertile cross (each with five female parents) was not significantly different in both kinds of crosses and all progeny were females. The hybrid females from ORNSR mothers were shown, upon the hemolymph examination under the dark-field microscope, to carry the SR spirochetes at a similar density as in the ORNSR stock. Thus, hybrid females were not affected by the SR spirochetes.

Table 1. Mating success, number of progeny and the sex ratio in the cross of ORNSR ♀ × *simulans* ♂

	Female parent	
	OR (Control)	ORNSR
No. of crosses	26	26
No. of fertile crosses	11	9
% of mating success	42.3	34.6
Average No. of progeny per fertile cross	168.2	220.4
Sex ratio (% ♀)	100	100

Effect of SR on progeny of simulans ♀ × *melanogaster* ♂. The reciprocal matings between *melanogaster* and *simulans* are not equally successful. As the *simulans* ♀ × *melanogaster* ♂ matings are more difficult than the reciprocal (Sturtevant 1929), groups of 10 *simulans* females were mated each with 10 Oregon-R males. Table 2 shows the results. Although the frequency of successful matings was not different between normal and SR females, the average number of F₁ progenies per fertile cross (each with

Table 2. Mating success, number of progeny and the sex ratio in the cross of *simulans*-NSR ♀ × *melanogaster* ♂.

	Female parent	
	<i>simulans</i> (Control)	<i>simulans</i> -NSR
No. of crosses	18	18
No. of fertile crosses	8	10
% of mating success	44.4	55.5
Average No. of progeny per fertile cross	37.0	2.0
Sex ratio (% ♂)	99.7	35.0

10 female parents) was quite different. Hybrid progenies from normal *simulans* mothers were mostly males as expected, while those from *simulans*-NSR mothers, though the number was very small (average 2.0 per cross), were 35% males and 65% females. All these males and females carried SR-spirochetes when their hemolymph was examined. Since NSR spirochetes usually killed males in the embryonic stages in *melanogaster* as well as in *simulans*, the appearance of adult males suggests that the hybrid males are somewhat resistant to the male-killing effect of SR agent. The reason that female hybrids appeared is unknown but something of SR agent might cure the hybrid females being destined to die.

Effect of SR on progeny of $yf: = \text{♀} \times \text{simulans} \text{ ♂}$. The yellow females of *melanogaster*, regardless of the X chromosome constitution (XX or XXY), showed superior receptivity to *simulans* males (Watanabe *et al.* 1977). Groups of five $yf: =$ -NSR females were mated each with five *simulans* males. The results are shown in Table 3. The frequency of successful matings was 100% in both normal and SR females. Normal ($yf: =$) females produced 112 hybrid sons on the average, but SR ($yf: =$ -NSR) females did not have any offspring. Many larvae and pupae died on the wall of vials in the latter cross, while few died in the former. The average number of died larvae was 39.5 and that of died pupae was 62.1. The total number of died offspring (101.6) was close to that of control (112.0) emerged as sons. Thus, the NSR apparently killed hybrid sons at larval and pupal stages. Further confirmation comes from the fact that out of 14 pupae died in the eye pigmentation stage 13 were proved to be males from their sex combs and genitalia. Here again, a slight resistance to NSR killing effect on the hybrid males was recognized.

Table 3. Mating success, number of progeny and the sex ratio in the cross of $yf: =$ -NSR $\text{♀} \times \text{simulans} \text{ ♂}$.

	Female parent	
	$yf: =$ (Control)	$yf: =$ -NSR
No. of crosses	24	23
No. of fertile crosses	24	23
% of mating success	100	100
Average No. of progeny per cross	112.0	0
Sex ratio (% ♂)	99.9	—

Effect of da gene on the hybrid progeny. The *daughterless* gene is a maternal effect mutant located on the second chromosome of *D. melanogaster*. The homozygous *da* females produce sons but not daughters regardless of the genotype of the father. The female embryos die before the eggs hatch (Counce, see Sandler 1972). Groups of five *da/da* females were crossed each with five *simulans* males. The results are shown in Table 4. In the Table are also shown the results of cross between *da/da* females and $+/+$ *melanogaster* males as a control. The frequency of successful matings in the

Table 4. Mating success, number of progeny and the sex ratio in the cross of *da/da* ♀ × *simulans* ♂.

	Male parent	
	<i>melanogaster</i> (Control)	<i>simulans</i>
No. of crosses	6	7
No. of fertile crosses	6	7
% of mating success	100	100
Average No. of progeny per cross	122.0	0
Sex ratio (% ♂)	100	—

hybrid cross was 100%, but all hybrid progeny died as larvae. Since it was difficult to examine the sex by observing the gonad of dying larvae, these larvae were dissected and their salivary gland chromosomes examined. They were all males. These results suggest that *da* gene killed the hybrid females just as the *melanogaster* females in the embryonic stage.

SUMMARY

1. The NSR-spirochetes injected to *Drosophila simulans* killed sons in the embryonic stage and the SR condition was established in this species by transmission of the spirochetes from mother to daughter through generations.
2. Inter-species hybrid males between *D. melanogaster* and *D. simulans* were killed by the NSR agent mostly at the stages of larvae and pupae, while the hybrid females were not affected.
3. *Daughterless* gene (*da*) killed the hybrid females in the embryonic stage.

ACKNOWLEDGMENTS

We thank Dr. Kugao Oishi for valuable discussions and for reading the manuscript.

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