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Interference between "Sex-Ratio" Agents of Drosophila willistoni and Drosophila nebulosa

Abstract. *Interference between two "sex-ratio" agents—that is, treponema-like spirochetes—of different origin, one from* Drosophila willistoni *and the other from* D. nebulosa, *was demonstrated by experiments in vivo and in vitro. When the two sex-ratio agents were combined in females of the Oregon-R strain of* D. melanogaster, *expression of the sex-ratio condition was temporarily interrupted. Several lines of evidence indicate that a substance produced by the sex-ratio agent of* D. nebulosa *may kill or incapacitate the sex-ratio agent of* D. willistoni.

A maternally transmitted condition known as "sex-ratio" (SR) has been demonstrated in a number of species of *Drosophila* (*1*). The condition is characterized by an extreme departure from the normal 1 : 1 sex ratio to give all or nearly all female offspring, the consequence of differential mortality of males, usually at an early stage in their development (*2*). The characteristics of the SR condition and the nature of the causative agents were reviewed recently in detail (*3*).

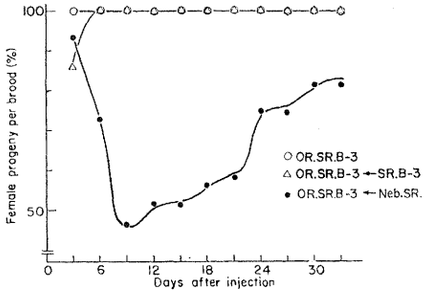

Fig. 1. Frequencies of female progeny per female per brood from eggs laid in successive 3-day periods by females injected with hemolymph of SR.B-3 or Neb.SR and by noninjected females of the OR.SR.B-3 strain.

The SR agents of *D. nebulosa* and *D. willistoni* are small treponema-like spirochetes, 10 μ or less in length and 0.1 μ wide, occurring in high concentration in the hemolymph of adult SR females (*4*). Differences between the SR agents of the two species have been demonstrated by their behavior when transferred into an inbred Oregon-R strain of *D. melanogaster*: the SR agent of *D. nebulosa* is easily transferred and the SR condition produced is very stable and persistent; that of *D. willistoni* is also readily transferrable but the SR condition produced is somewhat less stable (*5*).

We have studied the phenomenon of interference which occurs between SR spirochetes of *D. willistoni* and *D. nebulosa* when mixed in vivo and in vitro.

To test the effects of mixed infections of the two SR spirochetes in vivo, hemolymph of the SR strains PV-45 of *D. nebulosa* or of the SR.B-3 strain of *D. willistoni* was introduced into young, newly emerged adult females. The females were members of the second generation of artificially established SR strains of *D. melanogaster* derived from transfer of SR.B-3 or SR.PV-45 spirochetes into an inbred Oregon-R strain. These artificially established strains will be referred to as OR.SR.B-3 and OR.Neb.SR, respectively.

Since the SR condition in the *D. melanogaster* strains is not uniformly expressed in the first progeny, females used as hosts in these studies of mixed infections were always taken from broods produced by their mothers on days 9 to 12 or later. Methods of injection and examination of progenies from inoculated females are described elsewhere (*6*).

Results of the experiments in vivo, which are summarized in Table 1, showed that mixed infections of SR agents of different origin, SR.B-3 and Neb.SR, in the same SR host female, result in the production of male progeny, presumably as a result of some kind of interference between the two SR agents.

The proportions of female offspring in successive broods in these experiments and in the original *D. melanogaster* SR strains are shown in Figs. 1 and 2. The proportion of females in progenies of OR.SR.B-3 females injected with Neb.SR hemolymph was reduced from 93 percent in the first brood to 45 percent in the third brood

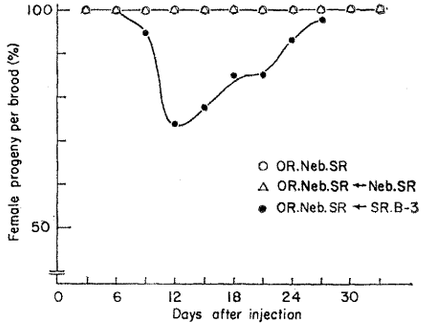

Fig. 2. Frequencies of female progeny per female per brood from eggs laid in successive 3-day periods by females injected with hemolymph of SR.B-3 or Neb.SR and by noninjected females of the OR.Neb.SR strain.

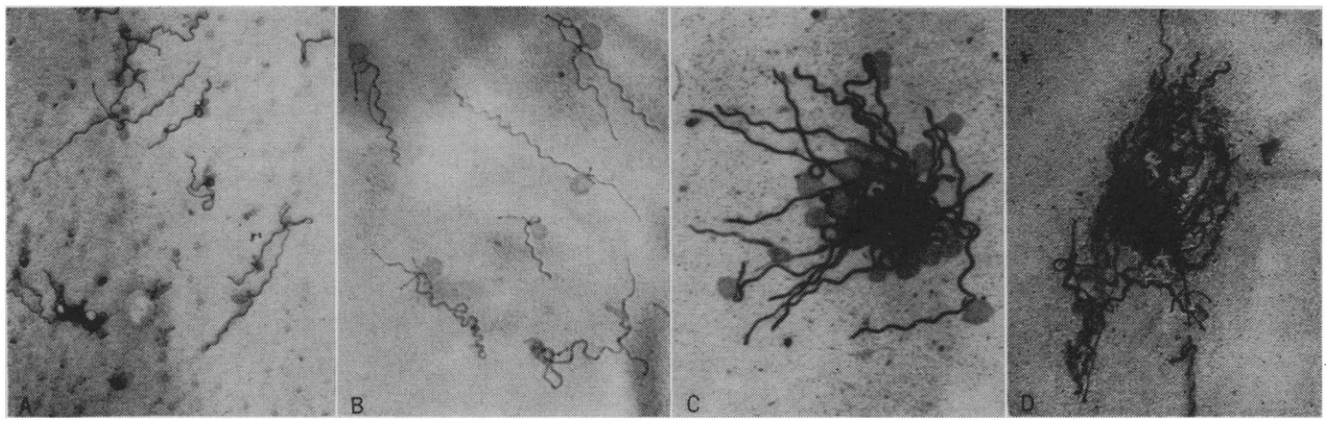

Fig. 3. Electron micrographs of SR spirochetes, fixed in osmium tetroxide vapor, in the hemolymph of adult females of *D. willistoni* strain SR.B-3 and *D. nebulosa* strain Neb.SR. (*A*) SR spirochetes of strain SR.B-3 (about × 4000). (*B*) SR spirochetes of strain Neb.SR (about × 6000). (*C*) Clump of SR spirochetes in mixed hemolymph 1 hour after mixing SR.B-3 with Neb.SR (about × 7500). (*D*) The same situation as shown in (*C*) but 6 hours after mixing. Knoblike bodies and parts of filaments already melted away (about × 6000).

(6 to 9 days after injection) but then gradually increased, reaching 80 percent by the tenth or eleventh brood. Among the controls, no males were produced except in the first brood of OR.SR.B-3 females injected with SR.B-3 hemolymph.

Females of strain OR.Neb.SR (Fig. 2) injected with SR.B-3 hemolymph produced males from the fourth to ninth broods. The percentage of females in the fourth brood was 74 and it gradually increased in subsequent broods, finally reaching 100 percent. No males were produced in the control series.

The effects of mixed infections in the original *D. willistoni* and *D. nebulosa* SR strains were also studied. Hemolymph from Neb.SR was injected into 23 young SR.B-3 females who were then mated singly with males of the normal B-3 strain of *D. willistoni*. Sex ratios in the progenies of the injected females were then followed. Nine of the injected females proved to be fertile, and all produced both male and female progeny, males first appearing in the second brood after 3 to 6 days. As a control, hemolymph of a non-SR strain of *D. nebulosa*, PV-59, was also injected into 13 SR.B-3 females, and was without effect on the SR condition.

In the reciprocal experiment, 21 Neb.SR females were injected with SR.B-3 hemolymph; the five fertile females in this series produced all female progenies. In the control series (hemolymph from normal B-3 females injected into 13 Neb.SR females) no effect on the SR condition was observed.

The hemolymph of OR.SR.B-3 females which had been injected with Neb.SR spirochetes was examined at intervals by phase-contrast and electron microscopy. Within hours, some clumping of SR spirochetes occurred although many free spirochetes were still observed. Within 3 to 6 days after injection, all spirochetes appeared in clumps and no free SR spirochetes were observed in the hemolymph; by 9 to 12 days after injections, the clumps gradually disappeared while the number of free spirochetes increased. The same phenomena were observed in the hemolymph of Neb.SR and OR.Neb.SR females injected with SR.B-3 and OR.Neb.SR spirochetes, respectively. No clumping of spirochetes was observed in hemolymph of females of strains OR.SR.B-3, Neb.SR, or OR.Neb. SR strains when they were injected with hemolymph of their own SR or normal strains.

The phenomenon of interference between SR spirochetes from *D. willistoni* and *D. nebulosa* can also be observed in vitro. Hemolymph from SR.B-3 and Neb.SR females was mixed in a depression slide and examined by phase and electron microscopy. Clumps of spirochetes started to form about 1 hour

Table 1. Sex-ratio in the progenies of OR.SR.B-3* females and OR.Neb.SR† females injected with the hemolymph of adult females of SR strains of *D. willistoni* (SR.B-3) and *D. nebulosa* (Neb.SR).

Host	Donor	No. of host flies	No. of first generation flies	Males (%)
OR.SR.B-3	Neb.SR	14	1410	39.8
OR.SR.B-3	SR.B-3	13	839	1.9
OR.SR.B-3		8	347	0
OR.Neb.SR	SR.B-3	12	948	11.6
OR.Neb.SR	Neb.SR	18	895	0
OR.Neb.SR		10	579	0

* SR strain of *D. melanogaster*, Oregon-R, derived by transfer of SR agent from *D. willistoni* SR.B-3 strain. † SR strain of *D. melanogaster*, Oregon-R, derived by transfer of SR agent from *D. nebulosa* Neb.SR strain.

Table 2. Clump formation between SR spirochetes from doubly infected *D. melanogaster* and SR spirochetes from the original SR.B-3 and Neb.SR strains in five experiments. Symbols: +, clump formation; −, no clump formation; ±, some individuals with clumps, others without.

SR spirochetes from superinfection	SR spirochetes* from original SR strain	Days after injection									
		3	6	9	12	15	18	21	24	27	30
		Experiment 1									
Host OR.SR.B-3	SR.B-3			+	+	+	+	+	+		+
Donor Neb.SR	Neb.SR			−	−	−	−	−	−		−
		Experiment 2									
Host OR.Neb.SR	SR.B-3		+	+	+	+	+	+	+		+
Donor SR.B-3	Neb.SR		±	±	±	−	−	−	−		−
		Experiment 3									
Host OR.SR.B-3	SR.B-3		−	−	−	−	−	−	−		−
Donor SR.B-3	Neb.SR		+	+	+	+	+	+	+		+
		Experiment 4									
Host OR.Neb.SR	SR.B-3		+	+	+		+	+			
No donor	Neb.SR		−	−	−		−	−			
		Experiment 5									
Host OR.SR.B-3	SR.B-3		−	−	−	−	−	−	−		
No donor	Neb.SR		+	+	+	+	+	+	+		

* Tester spirochetes.

later and gradually increased in size. The clumps were usually irregular in shape and 20 to 50 μ in diameter.

In electron micrographs of the hemolymph of SR.B-3 and Neb.SR females and of the mixed hemolymphs of the two strains, typical SR spirochetes with knoblike granules along the filaments were found in the hemolymph of SR.B-3 and Neb.SR females (Fig. 3, *A* and *B*). In the mixed hemolymphs, however, clumps appeared approximately 1 hour after the hemolymphs were mixed. Individual spirochetes and the knoblike bodies could be distinguished within the clumps after 1 hour (Fig. 3*C*), but after 6 hours neither individual spirochetes nor knoblike bodies could be seen clearly (Fig. 3*D*). When hemolymphs of different SR.B-3 females, or of Neb.SR females, were combined, clumps did not form. Thus a means is provided whereby populations of SR.B-3 and Neb.SR spirochetes can be distinguished from one another by the formation of clumps in vivo and in vitro.

As shown in Figs. 1 and 2, the proportion of males in progenies of SR females carrying both *D. willistoni* and *D. nebulosa* spirochetes increased in the third or fourth broods but gradually decreased in later broods, and the typical SR condition was restored. To clarify the mechanism of recovery of the SR condition in these later broods, hemolymph from doubly infected females was taken on different days following the initial injection and mixed with hemolymph containing SR.B-3 or Neb.SR spirochetes as testers. The results are summarized in Table 2 from which it is evident that the two kinds of spirochetes brought together artificially interfere with each other. Experiment 1 suggests that the SR.B-3 spirochete is the weaker of the two and was ultimately replaced in this experiment by the SR.Neb spirochete. Thus, when Neb.SR spirochetes are injected into an OR.SR.B-3 host, Neb.SR spirochetes interfere and finally replace SR.B-3 spirochetes in the host females. Similarly, when SR.B-3 spirochetes are introduced into an OR.Neb.SR host (experiment 2) there is interference, and SR.B-3 spirochetes are eliminated.

In an attempt to gain a better understanding of the mechanism of interference between SR.B-3 and Neb.SR spirochetes, approximately three hundred OR.Neb.SR females were homogenized in 0.24*M* sucrose and the homogenate then centrifuged at 30,000 rev/min for 1 hour. The supernatant, which was almost free of spirochetes, was injected into OR.SR.B-3 females and the proportion of males and females in successive broods from injected flies was tabulated. The proportion of females was 100 percent 6 to 9 days after injection but gradually decreased to approximately 50 percent in the fourth and subsequent broods; the SR condition was never restored. Moreover, no spirochetes were found in the hemolymph of injected OR.SR.B-3 females in the later periods following the injection. These results indicate that the supernatant contains some substance which can inactivate or destroy SR.B-3 spirochetes. The nature of this substance is not yet known.

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7. We thank Prof. D. F. Poulson for the generous supply of the original SR strains of *Drosophila* which made this work possible and for critical reading of the manuscript. We also thank Dr. Y. Tazima for helpful discussion and suggestions, and Miss T. Tokuda for excellent technical assistance. This work was supported by grant GM 10238 from NIH. Contribution No. 550 from the National Institute of Genetics, Misima, Japan.

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Activation Heat in Muscle: Method for Determination

Abstract. *By varying the interval between two stimuli it is possible to measure the activation heat in a skeletal muscle twitch. The method depends upon finding a range of stimulus intervals where complete mechanical fusion exists and where there is a plateau in the heat production. At 0°C, and when muscles are at normal body length, activation heat represents about 40 percent of the heat in an isometric twitch.*

The heat liberated in an isometric muscle twitch consists of the activation heat and the heat effects accompanying the internal shortening of the contractile element against the series elastic component. In the investigation described here, we measured the heat liberated in two twitches and studied the effect of varying the interval between the two stimuli. The results have shown a simple way of determining the activation heat separately and directly. The method depends on finding a stimulus interval such that the second contraction involves a complete activation, while the internal shortening events from the first stimulus still persist.

The experiments were conducted at 0°C in Ringer solution, *p*H 7.2, on the sartorius muscle of *Rana pipiens*. The thermopile itself was made up of 44 silver constantan junctions of which 29 were active and 15 were protective. The pile was 40 μ thick and its sensitivity was 732 μv/°C. The experimental records were corrected for heat loss and the total heat was generally read 3 seconds after the last stimulus.

The intervals between stimuli ranged from 10 msec to 2 seconds, and the total heat production in response to the two stimuli was plotted against the stimulus interval as shown in Fig. 1*A*. The dotted horizontal base line in Fig. 1*A* is the amount of heat produced in one single twitch. Heat production above this line rises in two stages separated either by a marked inflection point or by a small plateau. This inflection point or plateau occurred at a time when mechanical fusion was starting to occur as the stimulus interval was diminished. In fact, at stimulus intervals below 200 msec these two stimuli could represent the early part of an isometric tetanus. Hill (*1*) has made the suggestion that activation heats in a tetanus are summed to produce "maintenance heat" described by