ANALYSIS OF GYNANDROMORPH SURVIVALS IN
DROSOPHILA MELANOGASTER INFECTED WITH THE
MALE-KILLING SR ORGANISMS¹

SUMI TSUCHIYAMA AND BUNGO SAKAGUCHI²

*Laboratory of Sericultural Sciences, Faculty of Agriculture,
Kyushu University, Fukuoka 812, Japan*

AND

KUGAO OISHI

*Department of Biology, Faculty of Science, Kobe University,
Nada, Kobe 657, Japan*

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ABSTRACT

The effects of SRO (Sex-ratio organism; previously called SR spirochetes) infection on the viability of gynandromorphs of *D. melanogaster* were examined. A fraction of gynandromorphs, most of them with small areas of XO tissue, survived, but the majority were not viable when XO nuclei or cells are affected by the infecting SRO. Apparently the replacement of lethal XO nuclei or cells by adjacent XX ones does not take place. Some possibilities are discussed as to the time and the primary site of the lethal action of SRO.

THE maternally inherited "sex-ratio" agent, previously called the SR spirochete, in *Drosophila* is characterized by its selective lethality for male zygotes in early embryonic stages (earlier studies reviewed by POULSON 1963). Several strains of SR spirochetes have been described, each derived from a single female recovered from natural populations of *D. willistoni*, *D. equinoxialis*, *D. nebulosa* and *D. paulistorum* (POULSON 1963; POULSON and OISHI 1973). They can be successfully transferred into normal females of the same species or of different species, where they establish persistent infection and exhibit male-specific lethal action. Two strains of SR spirochetes, WSR from *D. willistoni* and NSR from *D. nebulosa*, proved to be quite stable in *D. melanogaster*, and these have been most frequently used in studies to elucidate the basis for the differential action on zygotes of the two sexes (SAKAGUCHI and POULSON 1963; COUNCE and POULSON 1966; OISHI 1971; MIYAMOTO and OISHI 1975).

It has been shown using *D. melanogaster* hosts with various chromosomal or genotypic constitutions that flies with single X chromosome are killed regardless of their phenotypic sex. Whether cells with a single X chromosome when present

¹ This paper is dedicated with respect and admiration to DONALD F. POULSON who has made many significant contributions on the genetics and development of *Drosophila*.

² To whom correspondence should be addressed.

in association with cells with two *X* chromosomes in the same host fly can escape the lethal action of infecting SR spirochetes, however, has not been known. In the present study we have examined this point using gynandromorphs of *D. melanogaster* in an attempt to localize the primary site of the male-lethal action. The SR spirochetes are now recognized as Mycoplasma-related (WILLIAMSON, OISHI and POULSON 1978), and thus will be simply referred to as SRO, SR organisms, in this report.

MATERIALS AND METHODS

The NSR strain of SRO was used throughout the present study. It was maintained in an Oregon-R strain of *D. melanogaster* in which it had been established by injection with SRO-carrying hemolymph from *D. nebulosa*. General procedures and other particulars were described previously (OISHI 1971).

The unstable ring-*X* chromosome, *In(1)w^{vo}* (HINTON 1955), was used to generate gynandromorphs. Stocks carrying this chromosome were provided by Y. HORTA of the University of Tokyo and by H. J. BECKER of the Zoologisches Institut der Universität München.

In Experiment 1, $\gamma w spl/In(1)w^{vo}$ females were infected with NSR, mated to $\gamma w spl/B^sY$ males, and their F_1 progeny examined according to the brood. The infection with NSR was done as follows. Oregon-R females carrying NSR were first injected with as much volume as possible of 0.15 M NaCl solution and left for a few minutes. Hemolymph was then taken out, using a glass microinjection pipet, and kept in a glass capillary tube. This diluted hemolymph was then injected 0.1 μ l each, into $\gamma w spl/In(1)w^{vo}$ females 2–3 days old. Oregon-R females carrying no SRO were processed as above for the control experiment. Flies were reared at $24 \pm 1^\circ$ on a sucrose-yeast-agar medium (MIYAMOTO and OISHI 1975).

In Experiment 2, females of a previously established SR stock, $\gamma w f/\gamma w f$ carrying NSR, were crossed to *In(1)w^{vo}/ $\gamma+Y$* males, and the gynandromorphs in the F_1 progeny examined. Non-SR $\gamma w f/\gamma w f$ females were mated in the control experiment. Since the pattern of transmission of NSR to the progeny is not usually fully expressed in the first or second brood (SAKAGUCHI, OISHI and KOBAYASHI 1965), the examination of gynandromorphs in the SR stock was made from the time of disappearance of males in the successive broods. Flies were reared at $22 \pm 0.5^\circ$ on a malted rice-yeast-agar medium (per liter of water: malted rice 140 g, sucrose 100 g, fresh yeast 25 g, dry powdered yeast 5 g, agar 12–15 g, with 7 ml of propionic acid; a drop of fresh yeast suspension added to each bottle before use).

In both experiments, gynandromorphs were examined under a dissecting microscope at a magnification of 40 $\times$ or more.

These experiments were undertaken quite independently, Experiment 1 at Kobe and 2 at Kyushu, and are presented here jointly.

RESULTS

Experiment 1: The F_1 progeny of females of the genotype $\gamma w spl/In(1)w^{vo}$ injected with the SRO (NSR strain) and mated to $\gamma w spl/B^sY$ males, as described above, were examined according to brood (Table 1). To simplify the comparison, actual fly numbers in each phenotypic class in the F_1 progeny were expressed as a percent of the class (1), $\gamma w spl/\gamma w spl$ females, in parentheses. Gynandromorphs did appear from the parental females injected with the SRO, but their frequency (8%) was much lower than that in the control (23%). Since the frequency of the $\gamma w spl/In(1)w^{vo}$ females was the same (11%) as in the control (12%), it may be concluded that the much lower frequency of recovered

TABLE 1
Effects of SRO (NSR strain) infection on gynandromorphs

		Brood: Days after injection					8-14 total	
		2-3	4-5	6-7	8-9	10-11	12-24	
Control								
F ₁ progeny								
(1)	<i>γ w spl/γ w spl</i>	395(100)	640(100)	422(100)	419(100)	358(100)	355(100)	1132(100)
(2)	<i>γ w spl/BSY</i>	362(92)	495(77)	435(103)	350(84)	301(84)	326(92)	977(86)
(3)	<i>γ w spl/In(1)^{w^{sc}}</i>	—*	—	—	43(10)	37(10)	51(14)	131(12)
(4)	<i>In(1)^{w^{sc}}/BSY</i>	0	0	0	0	0	0	0
(5)	<i>γ w spl/O</i>	—	—	—	43(10)	35(10)	62(17)	140(12)
(6)	gynandromorphs	—	—	—	97(23)	76(21)	84(24)	257(23)
Experimental								
F ₁ progeny								
(1)		467(100)	656(100)	535(100)	459(100)	311(100)	384(100)	1154(100)
(2)		260(56)	19(3)	0	0	0	0	0
(3)		—	—	—	55(12)	28(9)	43(11)	126(11)
(4)		0	0	0	0	0	0	0
(5)		—	—	0	0	0	0	0
(6)		—	—	—	48(10)	22(7)	24(6)	94(8)

Females carrying $\gamma w spl/In(1)w^{sc}$ were injected with a diluted hemolymph of Oregon-R females carrying NSR, mated to $\gamma w spl/BSY$ males, and F₁ progeny were examined according to the brood. For controls, a diluted hemolymph of non-SR Oregon-R females was injected.

* Not counted.

gynandromorphs compared with the control is a consequence of the death of a majority of those generated by the loss of the ring-X chromosome in the presence of SRO.

Table 2 shows the adult external structures scored in the gynandromorphs and the frequency of each of these structures being XO. In examining the gynandromorphs, it was noted that most of those in the experimental series showed only very small areas of XO tissues, and similar small areas were also found in the controls. Out of 257 gynandromorphs in the control, there were 51 in which only a single structure was mosaic or XO, all other tissues being XX. Similarly, 45 out of 94 in the experimental were such gynandromorphs. Of the 51 gynandromorphs in the control, 44 were XO in an abdominal half-tergite or

TABLE 2

External structures scored and frequencies of each structure being XO in gynandromorphs produced by the cross of y w spl/In(1)w^{vc} × y w spl/B^{SY}

Structures	Control (C: non-SR) 257 gynandromorphs (514 sides)	Experimental (E: SR) 94 gynandromorphs (188 sides)	E/C%
Head:	0.364	0.016	4.3
antenna, palpus, ocellar bristle, orbital bristle (a and p)*, vertical bristle (inner and outer), post vertical bristle, vibrissa, occiput	— 0.380	— 0.027	— 7.4
Proboscis:	0.427	0.061	14.3
Humeral bristles:	0.427	0.059	13.8
Thorax:	0.391	0.021	5.2
notopleural bristle (a and p), presutural bristle, dorsocentral bristle (a and p), supraalar bristle (a and p), postalar bristle (a and p), scutellar bristles	— 0.413	— 0.035	— 8.0
Wing:	0.386	0.024	7.0
Legs:	0.396	0.021	5.3
first, second, third	— 0.410	— 0.037	— 9.0
Sternopleural bristles:	0.411	0.032	7.8
Sternal bristles:	0.395	0.027	6.8
Abdominal tergites—Second:	0.404	0.069	17.1
Third:	0.423	0.133	31.4
Fourth:	0.408	0.128	31.4
Fifth:	0.412	0.120	29.1
Sixth:	0.386	0.125	32.4
Abdominal sternites—Second:	0.371	0.056	15.1
Third:	0.384	0.066	17.2
Fourth:	0.352	0.061	17.3
Fifth:	0.349	0.059	16.9
Sixth:	0.332	0.069	20.8
Genitalia:	0.327	0.048	14.4

Right and left structures counted separately except for the genital structures; mosaics counted as 0.5.

* Anterior and posterior.

sternite, and seven were in other structures, for example, a single outer vertical bristle being *XO*, etc. Of 45 in the experimental group, 35 were in a tergite or sternite and ten in others. In most of those with a mosaic in the tergite or sternite, only a few yellow (*XO*) bristles were observed.

The distribution of *XO* tissue sizes among the gynandromorphs was determined by counting 77 sites (Table 2, right and left structures counted separately except for the genital structure) assigning one point each to the structures that were *XO* and 0.5 to those which were themselves mosaic in each gynandromorph. Figure 1 shows the results for 257 gynandromorphs in the control and 97 in the experimental group. The frequencies in the experimental were expressed as percentages of the total expected gynandromorphs (1154×0.23) to facilitate the direct comparison. The distribution pattern thus obtained is rather unusual. There are two peaks in the distribution of *XO* sizes in the control, one being with very small *XO* sizes. The reason for this unusual distribution pattern is not clear, but two most likely probabilities are first, that the gynandromorph-producing stock used in the present study has, in the course of maintenance in our laboratory, acquired a tendency to lose the unstable ring-*X* chromosome very late in development at some sites detected most frequently in the abdominal sternites and tergites; the other is that the stock has a high somatic mutation rate under the present conditions.

Nevertheless, it is clear from the figure that a fraction of gynandromorphs with very small *XO* sites (assuming late loss) is not affected by the lethal action of the SRO. There were 84 gynandromorphs with less than 10% *XO* tissue: 45 with a single *XO* structure and 39 with more than two. Ten gynandromorphs with relatively large areas of *XO* tissue (more than 10% in the figure) were also recovered. It may be mentioned here that when the blastoderm fate-mapping procedure (GARCIA-BELLIDO and MERRIAM 1969; HOTTA and BENZER 1973; HALL, GELBART and KANKEL 1976) was followed using 97 gynandromorphs obtained from the eight to nine day brood in the control, a reasonably good agreement with published results was obtained.

Experiment 2: Females of the genotype $\gamma w f/\gamma w f$ were infected with the NSR strain of SRO and the SR stock established. The SR females were crossed to $In(1)w^{vo}/\gamma+Y$ males, and their F_1 progeny examined in the second experiment (Table 3). Only those gynandromorphs that appeared from later broods (later than the second brood, with no males) were scored in the experimental cross. In the control cross, gynandromorphs from the third and fourth broods only were scored. The number of gynandromorphs was 7% of the number of $\gamma w f/In(1)w^{vo}$ females. In the control, in which non-SR $\gamma w f/\gamma w f$ females were mated, the number of gynandromorphs was 77% of the number of $\gamma w f/In(1)w^{vo}$ females. Again it is apparent that most gynandromorphs cannot survive in the presence of the infecting SRO. The difference between the frequencies of gynandromorphs in the control and in the experimental in Experiments 1 and 2 may be a reflection of the different systems and the characteristics of $In(1)w^{vo}$ stocks used.

FIGURE 1.—Frequency of gynandromorphs with different XO tissue sizes in F_1 progeny of $\gamma w spl/ln(1)w^{v0} \times \gamma w spl/B^sY$. White bar: Control non-SR; Black bar: SR. Frequency in the SR is expressed as percent of the number of total expected gynandromorphs.

TABLE 3

Effects of SRO (NSR strain) infection on F_1 progeny of cross $y w f/y w f \times In(1)w^{vC}/y+Y$

F_1 progeny	Control (non-SR)	Experimental (SR)
$y w f/In(1)w^{vC}$	162 (100)	1433 (100)
gynandromorphs	125 (77)	102 (7)
$y w f/O$	358 (221)	0
$y w f/y+Y$	1556 (960)	0

TABLE 4

External structures scored and frequencies of each structure being XO in gynandromorphs from the cross, $y w f/y w f \times In(1)w^{vC}/y+Y$

Structures	Control (C: non-SR) 125 gynandromorphs (250 sides)	Experimental (E: SR) 102 gynandromorphs (204 sides)	E/C%
Head:	0.392	0.005	0.0
antenna, palpus, ocellar bristle, orbital bristle (a and p)*, vertical bristle (inner and outer), post- vertical bristle, vibrissa, occiput	— 0.448	— 0.010	— 2.5
Proboscis:	0.436	0.005	1.1
Humeral bristles:	0.472	0.017	3.6
Thorax:	0.480	0.039	8.1
notopleural bristle (a and p), presutural bristle, dorsocentral bristle (a and p), supraalar bristle (a and p), postalar bristle (a and p), scutellar bristles	— 0.488	— 0.056	—11.6
Wing:	0.484	0.059	12.2
Legs:	0.446	0.005	1.1
first, second, third	— 0.476	— 0.022	— 4.6
Abdominal tergites—First:	0.476	0.105	22.1
Second:	0.470	0.105	22.3
Third:	0.478	0.145	30.3
Fourth:	0.476	0.150	31.5
Fifth:	0.510	0.292	57.3
Sixth:	0.480	0.228	47.5
Abdominal sternites—First:	0.436	0.025	5.7
Second:	0.432	0.025	5.8
Third:	0.416	0.049	11.8
Fourth:	0.404	0.049	12.1
Fifth:	0.404	0.064	15.8
Sixth:	0.416	0.074	17.8
Genital tergite:	0.504	0.069	13.7
Genital sternite:	0.488	0.025	5.1

Mosaics counted as 0.5.

* Anterior and posterior.

Table 4 shows the external structures scored on the gynandromorphs and the frequencies of each of these structures being *XO*. The size of *XO* tissues in each gynandromorph was estimated in the same way as in Experiment 1, except that 80 structures were counted instead of 77 (left and right sides of 40 structures in Table 4 were scored separately) and the distribution of *XO* sizes for the control (125) and for the experimental (102) were determined (Figure 2). As in Figure 1, the frequencies of gynandromorphs recovered in the experimental are expressed as percentages of the total expected (1433×0.77) to facilitate direct comparison. It is clear from Figure 1 that only those with very small *XO* sizes survived in any substantial numbers in the experimental. About one-half of the gynandromorphs with *XO* tissue sizes below 10% survived. There were 27 gynandromorphs in the experimental and six in the control in which only a single structure was *XO*. Twelve gynandromorphs with more than 10% *XO* tissues were also recovered.

FIGURE 2.—Frequency of gynandromorphs with different *XO* tissue sizes in F_1 progeny of cross $\gamma w f / \gamma w f \times In(1)w^{vc} / \gamma + Y$. White bar: Control non-SR; Black bar: SR. Arrows indicate the means, 46% in non-SR control and 5% in SR. Frequency in the SR is expressed as percent of the number of total expected gynandromorphs.

DISCUSSION

In both Experiment 1 and 2, we have observed probably two different populations of gynandromorphs, one in which the loss of the ring-X chromosome took place early in the cleavage divisions and the other in which either the loss occurred late in development or somatic mutations took place at high frequency resulting in very small XO tissues. In the former population, a few gynandromorphs survived which had up to 40% XO tissues (Figures 1 and 2), but the majority did not survive. Apparently the replacement of damaged XO nuclei or cells by adjacent XX did not take place (Table 1). Thus, the XO nuclei produced early in an embryo either survived or resulted in the death of the whole embryo. On the other hand, in the latter population, gynandromorphs received only a little damage from the infecting SRO.

These results might suggest that XO nuclei present in very early cleavage stage are conditionally lethal and eventually result in the death of the whole embryo, and that XO nuclei or cells produced sometime later (assuming they are the product of late loss) become progressively resistant to the effect of SRO, resulting in the increasing rate of survival of gynandromorphs with increasingly smaller XO tissue areas.

Another way of looking at the present results would be to assume a large primary site for the action of SRO so that early-loss gynandromorphs have little chance to survive. The gynandromorphs with small areas of XO tissues in the control of Experiment 2 are probably the mixture of two different populations mentioned above, and the survivors in the experimental series may simply represent the population in which the loss of the ring-X chromosome occurred late in development as in Experiment 1. However, since the distribution pattern in Figure 2 is more normal than that in Figure 1, it is possible that survivors are from the population in which the loss of ring-X chromosome took place in early cleavage divisions. If one applies each of the E/C (%) values in Table 4 to corresponding structures in the published blastoderm fate map of *D. melanogaster* (e.g., HOTTA and BENZER 1973; HALL, GELBART and KANKEL 1976) and draws contours by uniting the points having the same values (a contour method of HOTTA and ISHIKAWA 1975; HOTTA personal communication), one obtains a broad primary site of action for SRO including most of the primordial nervous and mesodermal tissues (Figure 3). That the E/C (%) values calculated in Table 2, if drawn, would produce a somewhat different figure in detail is probably because the survivors in Table 2 are almost exclusively of very small XO tissue sizes and are from the population in which the loss of ring-X chromosome apparently took place very late in development.

It has been suggested that an exotoxin-like substance, androcidin, produced by the SRO is actually responsible for the death of male fly zygotes and that this substance can be transmitted into the egg without accompanying SRO transmission (OISHI 1971). Whatever the nature and the mechanism of action of the male-killing substance, it is apparent that the eggs produced by SR females are in some way made abnormal by maternal effects and they cannot sustain

FIGURE 3.—A contour map based on the data given in Table 4. Frequencies of each of the external structures examined being XO among 102 gynandromorphs (E) were divided by those among 125 gynandromorphs (C). The E/C (%) values thus obtained (Table 4) were plotted on the corresponding structures in a pictorial fate map of Hotta and Benzer (1973), and contours are drawn along the structures showing similar values.

the development of XY zygotes. We are now carrying out detailed histological examinations focusing particular attention to the primordial nervous and mesodermal tissues.

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Corresponding editor: D. T. SUZUKI