EFFECTS OF SR-SPIROCHETE INFECTION ON *DROSOPHILA MELANOGASTER* CARRYING INTERSEX GENES[1]

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ABSTRACT

Three sex-transforming genes (*ix*, tra^D, and *dsx*) in *D. melanogaster* were examined with respect to possible interactions with the NSR strain of SR-spirochetes. The SR-spirochetes exerted a lethal effect on *XY* but not on *XX* individuals, regardless of whether they were phenotypically males, intersexes, or females. These results, taken together with those reported by SAKAGUCHI and POULSON (1963) on *tra* and *2X3A* intersexes, both of which are resistant to the androcidal action of SR-spirochetes, support the interpretation that male susceptibility is a consequence of the single *X* condition.

THE transovarially transmitted so-called SR-spirochetes that infect Drosophila cause male-specific death in early embryonic stages, thus producing the abnormal sex-ratio condition (see review by POULSON 1963). Microscopically, the SR-spirochetes are most easily observed in hemolymph of adult females. They can be taken into a micropipet and injected into normal females of the same species or even of more distantly related species where they multiply and establish the abnormal sex-ratio condition. When two populations of the SR-spirochetes are mixed and they form clumps, they are considered to be different strains (SAKAGUCHI, OISHI and KOBAYASHI 1965). All strains of the SR-spirochetes so far examined carry their own associated viruses which can attack some other strains of SR-spirochetes (OISHI and POULSON 1970; POULSON and OISHI 1973). Certain strains of SR-spirochetes, even after lysis by a virus, maintain the abnormal sex-ratio condition-producing effect for one fly generation. It has been proposed that the SR-spirochetes produce an exotoxin-like substance, *androcidin*, the potency of which is different in different strains of SR-spirochetes and which is responsible for the death of male fly zygotes (OISHI 1971).

Several genes have been reported in *D. melanogaster* that cause phenotypic transformation of females to males (transformer, *tra*), females to intersexes (intersex, *ix*; transformer-dominant, tra^D), or both females and males to intersexes (doublesex, *dsx*) (cf. LINDSLEY and GRELL 1968). Further, genes with sex-specific or sex-differential lethal action with maternal effect are known both in the *X* chromosome (sonless, *snl*, COLAIANNE and BELL 1970, 1972) and in an autosome (daughterless, *da*, BELL 1954). Interactions between sex-transforming

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genes and sex-specific lethal genes have been reported (COLAIANNE and BELL 1972).

SR-spirochete infections have been examined with regard to their effects in the presence of the gene *tra* and in the triploid intersexes ($2X3A$). Neither the transformed flies ($2X$; *tra/tra*) nor triploid intersexes were killed by the infecting SR-spirochetes (SAKAGUCHI and POULSON 1963). It thus appeared important to determine whether this might also be the case in strains carrying other intersex-producing genomes. This report presents the results of SR-spirochete infections in flies carrying the sex-transforming genes, *ix*, tra^D, or *dsx*.

MATERIALS AND METHODS

Mutant stocks used:

1. intersex, *ix* 2–60.5: Females are transformed to intersexes. Stocks maintained as follows: *pr cn ix/In(2LR)SM5, al*^{2} *Cy lt*^{V} *sp*^{2}.
2. transformer-dominant, tra^D 3- : Females transformed to intersexes. Maintained by crossing *T(1;3)OR60/X; In(3LR)* Ubx^{130}, Ubx^{130} e^s females with *X/Y*; tra^D *Sb e/In(3LR)*Ubx^{130} males. This gene was originally called Hermaphrodite, *Hr*, and has been considered to be an allele of *tra* (cf. LINDSLEY and GRELL 1968). Recently, however, a claim appeared in DIS that tra^D is not an allele of *tra* but is rather of *dsx* (DENELL and JACKSON 1972).
3. doublesex, *dsx* 3–45: Both females and males transformed to intersexes. Maintained by crossing *C(1)M3*, y^2*/Y*; p^p *dsx/In(3LR)*Ubx^{130} females with *X/Y*; p^p *dsx/In(3LR)*Ubx^{130} males.

For complete information, see LINDSLEY and GRELL (1968).

Flies were cultured on a sucrose-yeast-agar medium (per liter of water: sucrose 55 g, dry powdered yeast 35 g, agar 19 g, with 4 ml of propionic acid added), and reared at 24 ± 0.5°.

SR-spirochete strain: The NSR strain of SR-spirochetes was used. It was maintained in Oregon-R (Ore-R) strain of *D. melanogaster* in which it had been established by injection with SR-spirochete-carrying hemolymph from *D. nebulosa*. The sources of the SR-spirochetes and their maintenance in Ore-R flies were described previously (OISHI 1971). Ore-R females carrying NSR, ORNSR females, were mated with normal males, placed in culture bottles, and transferred to new bottles every four days. Flies from the third brood provided the material for the present study.

Injection of flies: For injection of the SR-spirochetes, relatively young flies (less than 10 days old) of ORNSR (brood 3) were homogenized in a buffered sucrose solution (OISHI and POULSON 1970) using an all-glass homogenizer (0.1 g flies with 1 ml solution), and centrifuged at 3,000 rpm for 10 min. The resulting supernatant (0.2 μl each) was injected into test flies (24–48-hr-old) using the injection apparatus described by RIZKI (1953) with a modification.

For controls, relatively young normal Ore-R females (less than 10 days old) were processed as above and injected.

RESULTS

1. Intersex, *ix*, gene and SR-spirochetes.

Table 1 shows the effect of SR-spirochete (NSR) infection on the F_1 progeny of the cross between *ix/SM5* females and males. Injected flies were placed singly in small culture bottles each with two males, transferred to new culture bottles every two days, and progenies were examined according to the brood. The F_1 progeny are composed of four phenotypic classes as shown in the first column in

TABLE 1

Effect of SR-spirochete (NSR strain) infection on X/X; ix/ix *intersexes*

X/X; pr cn ix/SM5, al² Cy lt^V sp² females were infected with NSR by injecting the homogenate of NSR-carrying Ore-R flies (ORNSR), mated to *X/Y; pr cn ix/SM5* males, and F_1 progeny examined according to the brood. In parentheses are shown *per cent* flies of each phenotypic class of F_1 progeny in each brood, taking the number of *pr cn ix/SM5* females to be 100%.

Brood		Days after injection 3–4	5–6	7–8	9–10	11–12
X/X; ix/SM5 females ← NSR (ORNSR)						
No. flies injected and examined: 18						
F_1 progeny:						
(1) *X/X; ix/SM5*	females	153(100)	210(100)	172(100)	195(100)	154(100)
(2) *X/Y; ix/SM5*	males	142(93)	189(90)	100(58)	8(4)	0(0)
(3) *X/X; ix/ix*	intersexes	75(49)	102(49)	92(54)	86(44)	61(40)
(4) *X/Y; ix/ix*	males	38(25)	35(17)	14(8)	0(0)	0(0)
X/X; ix/SM5 females ← Control (Ore-R)						
No. flies injected and examined: 10						
F_1 progeny:						
(1)		82(100)	118(100)	96(100)	107(100)	132(100)
(2)		84(102)	106(90)	79(82)	124(116)	104(79)
(3)		43(52)	57(48)	36(38)	55(51)	63(48)
(4)		19(23)	30(25)	34(35)	42(39)	35(27)

the table. To simplify the comparison between each phenotypic class, actual fly numbers in each brood were expressed in *per cent* of the class (1), *X/X; ix/SM5*, females as shown in parentheses. Although the segregation ratio in each phenotypic class was not quite as good as mendelian predictions, it was constant throughout the broods in the control. In the experimental, the ratio of males in both *X/Y; ix/SM5* and *X/Y; ix/ix* began to decrease in the 7–8-day brood and became zero by the last brood examined, while that of *X/X; ix/ix* phenotypic intersexes was constant and similar to the control. Thus, it is clear that the *X/X; ix/ix* phenotypic intersex individuals were not affected by the androcidal effect of the SR-spirochetes.

2. Transformer-dominant, tra^D, gene and SR-spirochetes.

The effect of SR-spirochete infection on the *X/X*; $tra^D/+$ phenotypic intersexes was next examined (Table 2).

T(1;3)OR60/X; In(3LR)Ubx^130 females were injected with SR-spirochetes (NSR) and mated to *X/Y*; $tra^D/In(3LR)Ubx^{130}$ males. Since the characteristics of *T(1;3)OR60* are such that males carrying this chromosome die, the F_1 progeny consist of four phenotypic classes as shown in Table 2. Again, intersex flies (*X/X*; tra^D/Ubx and *T(1;3)OR60/X*; tra^D phenotypic intersexes) were not affected at all.

3. Doublesex, *dsx*, gene and SR-spirochetes.

Both *ix* and tra^D genes examined above were sex-specific in their action, acting

TABLE 2

Effect of the NSR infection on X/X; tra^D/+ *intersexes*

T(1;3)OR60/X; *In(3LR)Ubx*^{130}, *Ubx*^{130} *e*^{8} females were injected with NSR, mated to *X/Y*; *tra*^{D} *Sb e/In(3LR)Ubx*^{130} males. Other details are as in Table 1.

Brood	Days after injection				
	3–4	5–6	7–8	9–10	11–12
Experimental (17 flies)					
F_1 progeny:					
(1) *T(1;3)OR60/X; Ubx* females	33(100)	50(100)	64(100)	56(100)	45(100)
(2) *X/Y; tra*^{D}*/Ubx* males	36(109)	58(116)	45(70)	2(4)	0(0)
(3) *X/X; tra*^{D}*/Ubx* intersexes	37(112)	65(130)	57(89)	52(93)	52(116)
(4) *T(1;3)OR60/X; tra*^{D} intersexes	60(182)	91(182)	123(192)	75(134)	64(142)
Control (19 flies)					
F_1 progeny:					
(1)	61(100)	107(100)	83(100)	62(100)	61(100)
(2)	83(136)	117(109)	71(86)	58(94)	58(95)
(3)	60(98)	82(77)	90(108)	75(121)	46(75)
(4)	97(159)	151(141)	143(172)	102(165)	78(128)

only on female individuals and transforming them into intersexes. Doublesex gene, however, is unique in its action: both females and males carrying *dsx* in homozygous condition are transformed into phenotypic intersexes.

C(1)M3, y^2*/Y; dsx/In(3LR)Ubx*^{130} females were injected with SR-spirochetes (NSR), mated with *X/Y; dsx/In(3LR)Ubx*^{130} males, and their F_1 progeny examined (Table 3). No metafemales (*3X2A*) were found in the F_1 progeny in the present study, thus there were only four phenotypic classes as shown in the

TABLE 3

*Effect of the NSR infection on attached-*XX/Y; *and* X/Y; dsx/dsx *intersexes*

C(1)M3, y^2*/Y; p*^{p} *dsx/In(3LR)Ubx*^{130} females were injected with NSR, mated to *X/Y*; *p*^{p} *dsx/In(3LR)Ubx*^{130} males. Other details are as in Table 1.

Brood	Days after injection				
	3–4	5–6	7–8	9–10	11–12
Experimental (25 flies)					
F_1 progeny:					
(1) *XX/Y; dsx/Ubx* females	142(100)	162(100)	188(100)	154(100)	145(100)
(2) *X/Y; dsx/Ubx* males	163(115)	165(102)	98(52)	19(12)	0(0)
(3) *XX/Y; dsx/dsx* intersexes	80(56)	85(52)	95(51)	71(46)	60(41)
(4) *X/Y; dsx/dsx* intersexes	91(64)	85(52)	58(31)	5(3)	0(0)
Control (24 flies)					
F_1 progeny:					
(1)	149(100)	202(100)	154(100)	157(100)	155(100)
(2)	155(104)	160(79)	139(90)	171(109)	147(95)
(3)	89(60)	88(44)	78(51)	67(43)	68(44)
(4)	74(50)	84(42)	78(51)	70(45)	73(47)

table. Intersexes converted from genetic females, *C(1)M3/Y; dsx/dsx*, were apparently immune to the androcidal action of the infecting SR-spirochetes, while those transformed from genetic males, *X/Y; dsx/dsx*, were killed.

DISCUSSION

The results of the present study of the effects of SR-spirochete infection in strains carrying the sex-transforming genes, *ix*, *tra*^{D}, and *dsx* are very clear. Intersexes with two *X* chromosomes, female-to-intersex, were immune to the androcidal action of the SR-spirochetes, while those with single *X* chromosome, male-to-intersex, were killed. This is in agreement with the report of SAKAGUCHI and POULSON (1963) that females transformed to males by the gene *tra* were not killed by the infecting SR-spirochetes (WSR strain), nor were 2*X*3*A* intersexes. We have also examined the effect of NSR infection on *C(1)M3/Y; tra/tra* "males" and found that these "males" were not killed (data not presented here).

Thus, it appears that none of these sex-transforming genes are concerned with the processes which are interfered with by the androcidal action of the SR-spirochetes. HILDRETH (1965) has suggested that *dsx* acts before the normal sex determination mechanism sets in and establishes the intersexuality. It might have been expected, then, that either both *X/Y; dsx/dsx* and *X/X; dsx/dsx* intersexes would be killed or that both of them would survive the androcidal action of the infecting SR-spirochetes. This, however, did not happen.

The androcidal properties of the infecting SR-spirochetes are largely expressed before any of the imaginal discs are morphologically evident in the ORNSR strain of *D. melanogaster* (POULSON, personal communication), although this is not necessarily the case in the ORWSR (Ore-R flies carrying WSR) strain. Androcidal action as evidenced by abnormal development and subsequent mortality in ORWSR ranges through egg, larval, and pupal stages (COUNCE and POULSON 1966).

The lethal action of *snl* is exerted during the embryonic and the first 48 hr of larval development of *D. melanogaster* (COLAIANNE and BELL 1970). Since *snl* interacts with *tra* as well as with *dsx* (COLAIANNE and BELL 1972), it is apparent that these imaginal sex-transforming genes are already acting in these early developmental stages where each imaginal disc contains only a few dozens of cells or less. It is probable, however, that the primary lesion is in the larval tissues and not in the imaginal discs since flies with imaginal disc abnormalities may survive to the pupal stage (SHEARN *et al.* 1971).

It appears that the androcidal SR action, being in no way influenced by the "intersex" mutants as shown in these experiments, affects processes that are more basic or at a different level than those relating to imaginal sex differentiation. Effects of sex-transforming genes have been studied with regard to imaginal morphology and physiology, interactions with sex-differential lethals, and in relation to dosage compensation (e.g., BROWN and KING 1961; SMITH and LUCCHESI 1969), but nothing is known for their effects on embryonic and larval tissues.

So far only the number of *X* chromosomes and not the presence of a *Y* chromosome has been related to the male-specific lethal phenomenon in the SR-spirochete infections, and interactions with sex-transforming genes are not implicated. It is of importance, then, to see whether certain *X* chromosome segmental aneuploids are affected by the infecting SR-spirochetes. Females hypoploid for certain *X* chromosome segments may be killed and, conversely, hyperploid males may survive. Thus, the simplest explanation for the mechanism of androcidal action of the SR-spirochetes may be that there is a gene or genes on the *X* chromosome whose action is not related to the imaginal sex differentiation, but which is a dosage-uncompensated gene whose product, reacting with *androcidin*, happens to be critical to the normal early development of a fly.

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