INTERSPECIFIC TRANSFER OF THE "SEX-RATIO" CONDITION FROM DROSOPHILA WILLISTONI TO D. MELANOGASTER[1]

BUNGO SAKAGUCHI[2] AND D. F. POULSON

Department of Zoology,[3] Yale University, New Haven, Connecticut

Received February 18, 1963

THE infectious nature of the maternally transmitted "sex-ratio" condition, SR, in *Drosophila willistoni* has been clearly established (MALOGOLOWKIN and POULSON 1957; MALOGOLOWKIN, POULSON and WRIGHT 1959) and it has been demonstrated that the infectious SR agent is present at high levels in the hemolymph of adult SR females of that species (SAKAGUCHI and POULSON 1961a). The latter finding suggested that the otherwise tedious procedure of experimental transfer of SR to other species (MALOGOLOWKIN, CARVALHO and DA PAZ 1960; MALOGOLOWKIN, and CARVALHO 1961) would be considerably facilitated by the use of SR hemolymph, rather than whole-fly extracts, as the source of the infectious agent in such experiments. The previous successes of MALOGOLOWKIN and her associates in transfer between members of the *willistoni* group of species, and earlier limited success in the transfer to *D. melanogaster* (POULSON and MALOGOLOWKIN 1959) led us to undertake the experiments described in detail here. Some of the results have been reported in preliminary form (SAKAGUCHI and POULSON 1960; POULSON and SAKAGUCHI 1961).

Persisting complexities of the biology of *D. willistoni* as well as the rather limited possibilities for genetic analysis in that form make it highly desirable to be able to maintain and analyze the SR condition in a species such as *D. melanogaster* for which highly sophisticated genetic techniques and extensive developmental genetic information are available. That genotype is of fundamental importance for the persistence of the SR condition was demonstrated in *D. willistoni* by MALOGOLOWKIN (1958) and in *D. prosaltans* by CAVALCANTI, FALCÃO and CASTRO (1957, 1958). Success in the transfer of SR to *D. melanogaster* and its maintenance there has contributed toward the answering of a number of questions of long standing concerning the SR condition: What are the SR agents? What is the basis for the differential action on zygotes of the two sexes? What genetic and environmental conditions influence the stability and persistence of the

[1] This paper is dedicated with respect and admiration to PROFESSOR A. H. STURTEVANT whose researches and insights have illuminated the biology as well as the genetics of Drosophila for over fifty years. *D. willistoni* was described by STURTEVANT in 1916. The work reported here has received generous support from the National Science Foundation through G-6017 and G-14747.

[2] Present address: National Institute of Genetics, Misima, Japan.

[3] Since July 1, 1962 united with the Department of Botany to form the new Department of Biology.

SR condition? To what extent do SR conditions represent merely special cases of widespread unrecognized hereditary infections?

During the course of these experiments clues concerning the nature of the SR agents of *D. willistoni* and related species were obtained and these studies contributed to the recognition of these SR agents as spirochetes of the genus Treponema (POULSON and SAKAGUCHI 1961). Partial answers to some of the other questions have also been obtained.

MATERIALS AND METHODS

The source of the materials for the transfers were mature adult females of the SR strain of *D. willistoni* known as SR.B-3 maintained by mating to males from the normal strain, Barbados-3. Recipient strains of *D. melanogaster* consisted of two inbred wild-type lines, Oregon-R and Sevelen, directly derived from those used by DOANE (1960a, b) in studies on egg production; a triploid strain carrying attached-X chromosomes, y^2 *sc* w^a *ec*/FM4, y^{31d} sc^8 *dm B*, obtained from PROF. E. B. LEWIS at the California Institute of Technology; an attached-X strain derived from this, y^2 *sc* w^a *ec* × FM4, y^{31d} sc^8 *dm B*; and a strain resulting from the detachment of the attached-X, y^2 *sc* w^a *ec* (det.-X)/FM4, y^{31d} sc^8 *dm B*, maintained by selecting heterozygous females and mating to FM4 males. A strain carrying the transformer gene, *tra*, (*tra/Ubx*; attached-XY ♀ ♀ × *tra/tra*; $w^a v$, XY ♂ ♂) was kindly provided by PROF. LEWIS.

Methods of transfer have been described previously (SAKAGUCHI and POULSON 1961a). Except in the earlier experiments where hemolymph was transferred by micropipet from individual to individual, the procedure developed here was to pool the hemolymph from many donors in a moist chamber and thoroughly mix before introducing constant volumes of inoculum into the recipients. This procedure insured uniformity of inocula by eliminating the variability of individual donors. Uniformly high levels of SR agent were assured by choosing as donors females 15 to 20 days of age, at which period a high level of infectious SR agent is present in the hemolymph (SAKAGUCHI and POULSON 1961a). As in the previous work recipients were young virgin females less than three days of age, but usually not more than 24 hours old. After introduction of the SR hemolymph into the abdominal cavity by micropipet the females were mated singly with males of their own strain and placed in individual creamers containing standard corn-meal-molasses-agar food seeded with live yeast. The flies were transferred to new food every second day and the numbers of the sexes in each two-day brood were determined for each injected female. Usually controls consisted of females injected with hemolymph of mature females of the maintainer strain, Barbados-3, of *D. willistoni*. In certain experiments in which hemolymph from SR lines in *D. melanogaster* was employed, the controls consisted of females injected with hemolymph of the normal *melanogaster* strain. In certain other series (the triploids and attached-X) uninjected controls were also employed to establish norms for phenotypic ratios. Except in the cases indicated the experiments were carried through at 25°C.

Egg counts were made for many of the early broods in most series since egg mortality accounts for the majority of missing males in SR strains of *D. willistoni*. Those data are not presented here since the main features of the establishment of the SR condition are demonstrated by the data on adults.

Examination of the hemolymph of SR donors, recipients, and SR offspring became a routine procedure during the course of these experiments. With care small volumes of hemolymph can be removed from flies without serious injury and the flies, after feeding, can be used for further breeding. Micro-drops of hemolymph placed on a microscope slide beneath a small drop of Crown immersion oil and protected by a No. 0 coverglass were examined by phase contrast microscopy using Zeiss-Winkel Ph 3 oil immersion objective 100/1.30 and the appropriate phase condenser. Observations of these led to the recognition of treponemata in the hemolymph of SR females and their absence from normal strains of *D. willistoni* and *D. melanogaster* (POULSON and SAKAGUCHI 1960, 1961a).

RESULTS

Transfers to wild-type strains: The full data from one series of experiments in which transfer was made into wild-type strains are given in Table 1. Summaries of these and other series of transfers are presented in condensed form in Table 2 and in Figures 1, 2, and 3. The form of Table 1 allows direct comparison with the intraspecific transfers by MALOGOLOWKIN, POULSON and WRIGHT (1959) and SAKAGUCHI and POULSON (1961). In Table 1 it is seen that the eight females of Oregon-R infected with hemolymph of SR.B-3 produced very few sons and these, with one exception, Brood 7f, appeared within the first three broods. In the cases of SR-1 and SR-8 no males were produced at all. Only SR-5 and SR-7 gave 1:1 ratios in the first brood. Taking as a measure of the incubation time the first brood in which a significant departure from 1:1 is observed, the mean incubation time for the appearance of SR in this series is 2.5 days (Table 2). The egg mortalities (not presented here) followed a pattern presaging the disappearance of males in the particular broods. A further notable effect was the reduction in survival time of the SR injected series (a difference of 11.7 days) as compared with the controls which went on producing males in proportions remarkably close to 1:1 right up to the ends of their reproductive lives. While producing large numbers of total progeny per female the control series produced slightly fewer daughters per female (Table 2). Although there was variation among the individual Oregon-R females, and female SR-4 was lost after the eggs for Brood d had been deposited, only two females produced fewer than 100 offspring. Among the controls no female produced fewer than 165 offspring.

The ten injected Sevelen females (Table 1) all gave SR progenies after a slightly longer incubation period (3.6 days). The proportion of males was about double that in the Oregon-R series (Figure 1). Also in Sevelen there was a marked reduction (10.9 days) in the mean survival time as well as a shortening of the mean reproductive life (Table 2). Thus while the presence of the injected SR agent shortens the lives of females its effect on reproductive span is less marked.

TABLE 1

Progenies of females of the Oregon-R and Sevelen inbred strains of D. melanogaster *injected with hemolymph from adult females of the SR. B-3 strain of* D. willistoni, *and progenies of control females injected with hemolymph from normal females of the B-3 strain of* D. willistoni

Broods Days		a 0–2 ♀	♂	b 3–4 ♀	♂	c 5–6 ♀	♂	d 7–8 ♀	♂	e 9–10 ♀	♂	f 11–12 ♀	♂	g 13–14 ♀	♂	h 15–16 ♀	♂	i 17–18 ♀	♂	j 19–20 ♀	♂	k 21–22 ♀	♂	l 23–24 ♀	♂	m 25–26 ♀	♂	n 27–28 ♀	♂	o 29–30 ♀	♂	p 31–32 ♀	♂	q 33–34 ♀	♂	Totals ♀♀	♂♂
Oregon-R	1	12	0	44	0	20	0	35	0	26	0	21	0	26	0	44	0	19	0	30	0	3	0	1	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	281	0
injected	2	2	1	20	0	21	0	20	0	26	0	32	0	33	0	35	0	17	0	17	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	221	1
with	3	15	4	38	0	0	0	0	0	5	0	16	0	2	0	0	0	3	0	0	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	79	4
SR	4	9	3	56	0	27	0	27	0	(♀ parent lost)		. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	119	3
	5	27	31	27	16	12	6	32	0	45	0	60	0	51	0	22	0	14	0	8	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	298	53
	6	12	2	60	0	24	0	36	0	32	0	28	0	12	0	0	0	3	0	2	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	209	2
	7	12	11	25	19	16	11	26	0	16	0	36	1	21	0	46	0	15	0	3	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	216	42
	8	4	0	13	0	3	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	20	0
Totals		93	52	283	35	123	17	176	0	150	0	193	1	146	0	147	0	71	0	60	0	3	0	1	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	1443	105
Oregon-R	1	13	14	22	31	15	21	37	33	20	27	29	20	28	26	3	6	3	2	1	2	2	6	0	0	0	0	0	0	0	0	0	0	0	0	173	188
controls	2	8	7	17	16	16	17	18	15	31	34	36	31	36	39	38	42	35	33	27	28	39	30	25	19	23	22	3	5	0	0	0	0	0	0	352	338
injected	3	4	7	35	25	15	17	40	28	27	20	27	33	26	36	21	14	22	17	36	30	14	31	8	10	17	11	17	25	7	7	0	0	. .	. .	316	311
with	4	12	8	12	14	23	11	21	31	11	16	20	26	27	21	1	2	7	8	0	0	9	19	0	2	0	3	1	3	1	1	. .	. .	. .	. .	145	165
normal	5	2	6	23	20	6	6	4	7	12	17	6	12	15	10	0	2	2	1	0	0	1	2	3	3	0	4	0	1	0	0	0	0	0	0	74	91
B-3	6	17	21	21	21	17	23	12	5	28	21	30	18	38	26	3	4	31	25	20	32	23	12	0	4	0	0	0	0	0	0	. .	. .	. .	. .	240	212
	7	12	18	22	20	18	18	31	27	25	27	20	19	38	24	14	16	24	22	11	13	19	16	8	8	1	2	0	0	0	0	. .	. .	. .	. .	243	230
	8	18	15	4	2	29	27	21	34	8	18	21	12	18	16	29	38	4	5	0	0	7	7	0	0	0	0	0	0	0	0	0	0	0	0	159	174
Totals		86	96	156	149	139	150	184	180	162	180	189	171	226	198	109	124	128	113	95	105	114	113	44	46	41	42	21	34	8	8	0	0	0	0	1702	1709
Sevelen	1	27	13	28	5	6	4	24	3	38	4	25	6	23	8	6	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	177	43
injected	2	31	34	45	3	34	2	38	0	30	0	11	0	37	3	36	0	11	0	17	0	8	0	4	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	302	42
with	3	32	22	22	20	40	10	40	7	22	15	47	5	54	0	39	0	43	0	25	0	25	0	17	0	5	0	3	0	. .	. .	. .	. .	. .	. .	414	79
SR	4	17	15	40	7	32	6	22	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	111	28
	5	27	14	29	0	13	5	12	0	18	0	10	4	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	109	23
	6	36	25	48	3	18	6	33	0	26	3	32	7	23	0	19	0	11	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	246	44
	7	48	24	34	23	29	20	27	1	29	0	25	0	49	0	36	0	44	0	18	0	11	0	26	0	15	0	4	0	0	0	. .	. .	. .	. .	395	68
	8	23	21	38	28	42	2	47	4	33	4	35	10	38	12	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	256	81
	9	17	2	30	0	50	0	49	1	43	2	26	5	2	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	217	10
	10	13	6	38	4	44	0	46	1	39	33	40	12	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	220	56
Totals		251	176	332	93	306	55	338	17	278	61	250	49	216	23	136	0	109	0	60	0	42	0	47	0	20	0	7	0	0	0	. .	. .	. .	. .	2447	474
Sevelen	1	25	21	1	0	0	0	14	11	0	1	2	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	42	33
controls	2	0	1	25	24	18	14	21	36	49	58	32	29	33	32	49	36	33	56	30	26	26	14	8	3	1	5	0	0	0	0	. .	. .	. .	. .	325	334
injected	3	12	10	20	21	13	17	3	16	9	29	14	16	7	8	8	6	4	4	8	4	4	6	3	6	6	2	3	2	5	3	1	0	. .	. .	115	150
with	4	23	12	37	27	28	25	15	28	37	51	16	39	14	12	24	33	16	14	2	4	0	4	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	212	249
normal	5	20	8	21	14	0	0	0	1	0	0	0	0	1	3	0	0	0	1	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	42	27
B-3	6	25	14	27	43	29	34	4	17	31	37	21	12	32	40	18	17	17	16	7	3	0	1	6	5	2	2	0	0	0	1	0	0	0	0	219	242
	7	14	16	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	14	16
	8	27	22	30	26	35	23	24	27	2	9	10	8	1	2	14	4	7	10	0	0	1	1	0	0	0	0	0	0	0	0	0	0	. .	. .	151	132
Totals		146	104	161	155	123	113	81	136	128	155	95	104	81	97	113	96	73	101	47	37	31	26	17	14	9	9	3	2	5	4	1	0	0	0	1120	1183

TABLE 2

Summaries of transfer of SR to wild-type strains of D. melanogaster *by injections of hemolymph from adult females of SR strains*

Donor strain	Host strain	No. females tested	No. SR	Incub. time (days)	Total in progenies ♀♀	Total in progenies ♂♂	Percent males	Mean no. daughters per female	Mean surv. time (days)	Mean repro. life (days)	Mean no. ♀♀ per ♀ per day repro. life
SR.B-3	Ore-R	8*	8	2.5	1304	102	7.2	217.3	20.8	18.2	11.9
B-3	Ore-R	8	0	. .	1702	1709	50.1	212.7	32.2	23.7	8.9
SR.B-3	Sevelen	10	10	3.6	2447	474	15.5	243.7	18.8	17.7	13.8
B-3	Sevelen	8	0	. .	1120	1183	50.4	160.0	24.0	23.7	6.8
SR.B-3	Ore-R	6	5†	4.0	722	196	21.3	144.4	24.0	14.6	9.8
SR.B-3	Sevelen	6	5†	4.0	1241	220	15.0	248.2	13.3	13.6	18.2
SR.*mel*	Ore-R	4	3	6.6	424	171	28.7	141.3	25.0	16.0	8.8
SR.*mel*	Sevelen	6	(6)‡	4.3	2630	2270	45.8	438.3	24.0	21.6	22.9

* In these computations females Nos. 4 and 8 are omitted.
† Tests of subsequent generations are summarized in Table 4. Only SR females used in computations in this table.
‡ No transmission to subsequent generations.

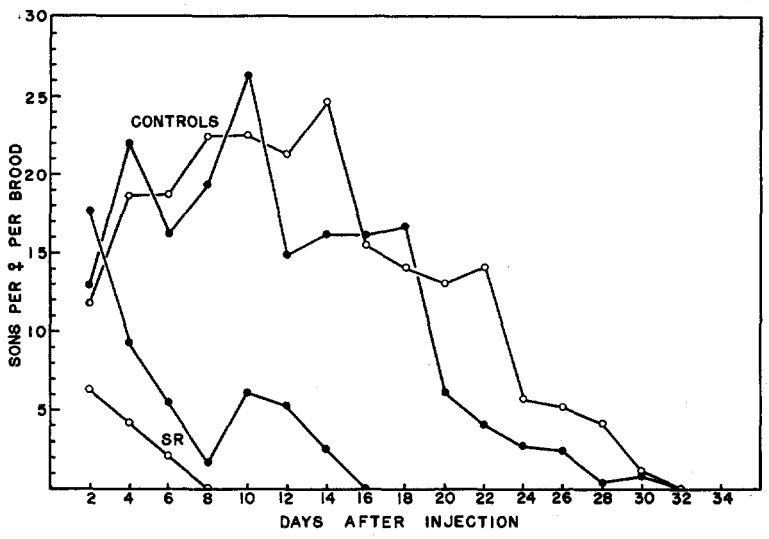

FIGURE 1.—Mean numbers of sons per female per brood from eggs laid in successive two-day periods by SR-injected and by control females, individual data for which are given in Tables 1 and 2. Circles for Oregon-R inbred; solid points for Sevelen inbred strain.

Variation among the Sevelen recipients of SR was slighter and productivity was higher than among B-3 injected control series of which one female (No. 7) lived only four days, giving but 30 offspring, all in the first brood. This female was omitted from the totals and means given in Table 2.

Condensed data for another series of SR transfers into Oregon-R and Sevelen are given in lines 5 and 6 of Table 2. These, which had been carried out at an earlier date, without controls, in each case showed a longer incubation period

before the appearance of SR in the progenies and gave more males in the early broods. The reduced survival time was also apparent in these and was marked for Sevelen females. The cause of the extreme reduction is not known but may have been a consequence of concomitant infection with microsporidia which have since proved troublesome in the B-3 and SR.B-3 strains.

When the mean numbers of daughters per female per brood (♀ ♀ / ♀ /brood) during reproductive life are plotted (Figures 2 and 3) it is seen in the first instance that SR infection raises the daily productivity of Oregon-R females as compared to controls and even more markedly affects productivity among Sevelen females. In the second and the third series (described below) the mean daily productivity of SR injected Sevelen females was extraordinarily high compared with the first series. The Oregon-R females in the latter series did not differ significantly in this respect from the controls of the first series. Lines 7 and 8 of Table 2 summarize data concerning the transfer into Oregon-R and Sevelen females of SR which had previously been passed from SR.B-3 into another strain of *D. melanogaster* in one of the experiments summarized in Table 4. These transfers of hemolymph with SR which had persisted in *D. melanogaster* for four generations gave results differing in degree from those in other series. There was an increased incubation time for the appearance of the SR effect and much larger percentages of males were found. Mean survival time and mean repro-

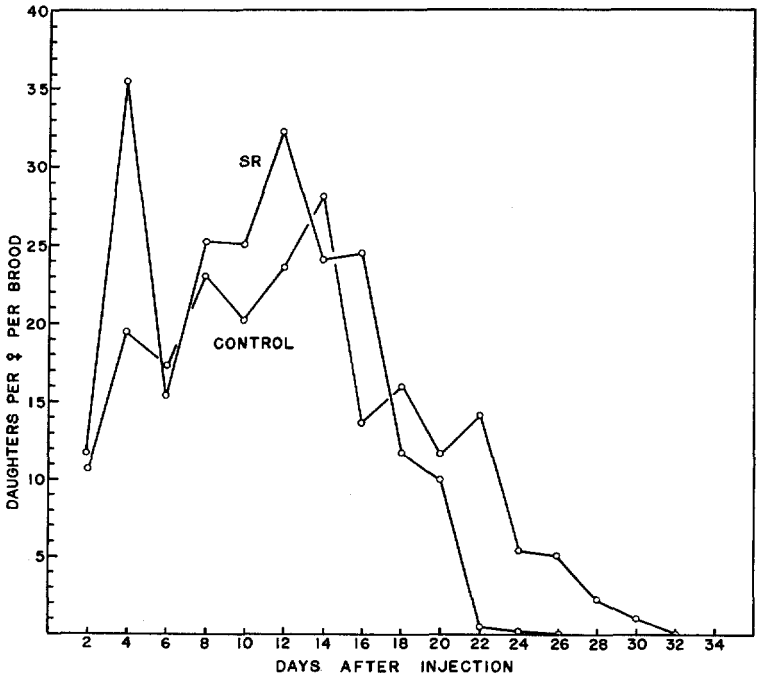

FIGURE 2.—Mean numbers of daughters per female per brood from eggs laid in successive two-day periods by SR-injected and control females of the Oregon-R inbred strain.

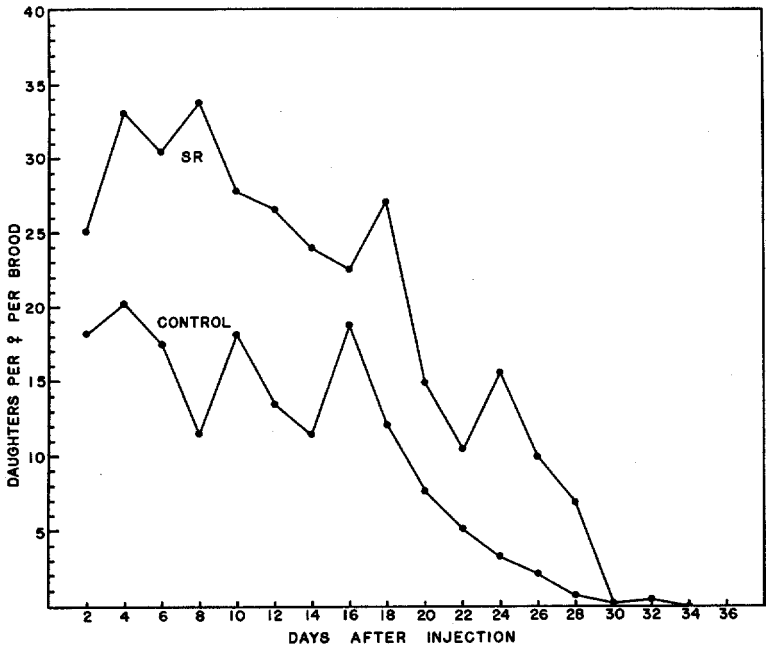

FIGURE 3.—Mean numbers of daughters per female per brood from eggs laid in successive two-day periods by SR-injected and control females of the Sevelen inbred strain.

ductive life were less affected than in the direct transfers. The most striking feature was the response of the Sevelen recipient females. These showed a sharp drop in male production in the first three broods followed by restoration of the 1:1 ratio in subsequent broods. Furthermore these Sevelen females produced excessively large total progenies and the mean numbers of daughters per female per day of reproductive life was 22.9 or about three times the number of the Sevelen controls of the first series.

The extent to which the SR condition persisted into subsequent generations was tested in each series. Although lines derived from each of the series of tests were followed, more complete data on the immediately following generations are available for the Oregon-R and Sevelen females of the second series (lines 5 and 6 of Table 2) and are presented in Table 3. There it is seen that the condition persisted in a high proportion of the tested daughters of Oregon-R females, but declined in subsequent generations of Sevelen females, being lost before the fifth generation in this series. From the first series certain single female lines of SR in Oregon-R and Sevelen have been maintained up to the time of writing. Maintenance in Oregon-R has proved relatively easy, but stringent selection and larger numbers of test females per generation have been necessary to keep SR in Sevelen flies. In the series in which SR.*mel.* was introduced into Sevelen females (line 8, Table 2) none of the tested daughters from early or late broods gave any signs of transmission of SR.

TABLE 3

Summary of tests of later generations from females of Oregon-R and Sevelen strains injected with hemolymph from the SR.B-3 strain of D. willistoni

Generation tested	Oregon-R			Sevelen		
	No. ♀♀ tested	No. SR progenies	Percent SR progenies	No. ♀♀ tested	No. SR progenies	Percent SR progenies
F_1	61	55	90.2	82	46	56.1
F_2	23	19	82.6	22	12	54.5
F_3	16	5	31.1	22	11	50.0
F_4	13	13	100.0	4	0	0

Thus the striking differences in the response of these two wild-type strains to infection with SR, in the level of SR expression, and in the degree of stability and persistence of SR in subsequent generations, indicate basic genotypic differences with regard to susceptibility and resistance to the SR agents in the two strains.

Transfers into 3n and attached-XY strains: Among the reasons for attempting the transfer of SR into *D. melanogaster* was to test the specificity of the killing effect on male zygotes and to determine whether it is the presence of the Y chromosome or the X-autosomal balance which determines the susceptibility to the action of the SR agent. Since it is known that occasional female zygotes die in SR strains of *D. willistoni* (COUNCE and POULSON 1962) it was desirable to know whether such deaths might result from the presence of a Y chromosome in a female or other individual variations in genotype. For these purposes it was decided to introduce SR.B-3 hemolymph into attached-X females and triploid females of *D. melanogaster* rather than attempt obtaining nondisjunctional lines of *D. willistoni*. Accordingly a series of presumptive 3n and attached-XY females derived from a stock bottle of the 3n strain were injected with SR.B-3 hemolymph and mated with sib males carrying y^{91d} sc^{8} *dm B*, FM4 in the X-chromosome. The injected females were kept at 20°C and transferred with males onto fresh food every other day. It turned out, however, that detachment of the attached-X had occurred in the particular line from which these had been derived and that the presumed 3n females were really heterozygous for a detached-X and the FM4 chromosome while the presumed attached-X females were really homozygous for the detached-X. The data on these are presented in Table 4 in summary form. Eight out of ten females (heterozygous detached-X as proved by their progenies) showed marked reductions in numbers of males produced after the earliest broods. In extensive tests in subsequent generations the SR condition was found to be remarkably stable and lines derived from these have been maintained. In these experiments two types of control were used: those injected with B-3 normal hemolymph, and uninjected controls to provide a reference for the proportions of the different classes of flies. In comparison with controls the mean survival times was much reduced. However, the B-3 injected controls showed a reduction of survival as compared with the uninjected controls. The homozygous detached-X females also made good SR hosts as seen in the lower part of Table 4.

TABLE 4

Transfer of SR into D. melanogaster *using host females from a detached-X strain,* y² sc w^a ec/ *FM4,* y^{31d} sc^8 dm B × *FM4* ♂ ♂, *upper half of table; and with females homozygous for the detached-X,* y² sc w^a ec/y² sc w^a ec × *FM4,* y^{31d} sc^8 dm B ♂ ♂ *in the lower portion of the table. Injected and uninjected controls are included, as well as data for second and third generations. 20°C*

Treatment	No. females tested	No. SR progenies	Mean incubation time	Mean survival time	F_1 progeny			
					y² sc w^a ec/FM4 females	FM/FM4 females	*y² sc w^a ec* males	FM4 males
SR.B-3 blood	10	8*	6.7 days	28.3 days	732 (60.6%)	302 (24.9%)	25 (2.0%)	151 (12.5%)
B-3 blood control	8	0	. .	46.2 days	581 (37.5%)	154 (9.9%)	203 (13.1%)	610 (39.4%)
Uninjected control	6	0	. .	52.0 days	408 (34.4%)	184 (15.5%)	211 (17.8%)	382 (32.2%)
*Second gen.	419	331						
Third gen.	298	236						
SR.B-3 blood	7	7	5.8 days	24.4 days	376 (83.9%)	. . .	72 (16.1%)	. . .
B-3 blood control	9	0	. .	26.3 days	242 (52.6%)	. . .	218 (47.4%)	. . .

* Second generation is from the starred progenies.

A repetition of these experiments with true 3n and attached-X females as hosts was carried out, again at 20°C. However, since the SR lines derived from the detached-X strain were very stable, third generation descendents were used as the source of the SR hemolymph (SR.*mel.*) introduced into these hosts. The results are summarized in Table 5, the upper portion of which presents the data for the 3n hosts, the lower portion the data for the attached-X females. The reduction of numbers of males among the progeny of 3n females was particularly marked, but intersexes continued to be produced in the proper proportions as were 3n and 2n females. Triploid SR lines were maintained for a number of subsequent generations with similar results and at no time were any supermales observed.

The lower part of Table 5 gives the data for transfer into attached-XY females. The results are exceptionally clear, the progenies consisting solely of females following the incubation period which was distinctly longer as compared with that for the 3n females. A small but regular amount of detachment was characteristic of this strain, hence the heterozygous detached-X females and the detached-X males making up about 4 percent of the controls. As already seen in Table 4, it is clear that the *y² w^a sc ec* detached-X males are markedly affected by the SR agent. SR strains descended from these attached-XY females have proved remarkably stable and continue to be maintained at the time of writing by matings to *y^{31d} sc^8 dm B*, FM4, males from the maintainer strain of normal

TABLE 5

Transfer of SR into a triploid strain, 3n, y^2 sc w^a ec/*FM4,* y^{31d} sc^8 dm B × *FM4* ♂♂, *and into a diploid attached-X strain,* y^2 sc w^a ec × *FM4* ♂♂, *in* D. melanogaster

Treatment	No. females tested	No. SR pro-genies	Mean incubation time	Mean survival time	F_1 progeny: 3n females	y^2 *sc* w^a *ec* females	FM4 females	Intersexes	FM4 males
3n Series									
SR.*mel* blood (detached-X)	7	6*	2.6 days	26.5 days	66 (36.6%)	66 (36.6%)	32 (17.8%)	11 (6.1%)	5 (2.8%)
Normal blood (detached-X)	8	0	. .	31.0 days	39 (22.2%)	49 (27.8%)	40 (22.7%)	11 (6.5%)	37 (21.0%)
Uninjected control	10	0	. .	38.6 days	75 (29.1%)	52 (20.2%)	40 (15.5%)	9 (3.5%)	82 (31.8%)
*Second gen.	21	11							
Third gen.	18	14							
Fourth gen.	12	12							
2n Series						y^2 *sc* w^a *ec* females	FM4 y^2 *sc* w^a *ec*	y^2 *sc* w^a *ec* males	FM4 males
SR.*mel* blood (detached-X)	10	10†	6.6 days	21.0 days		337 (89.4%)	15 (3.9%)	0 (0)	25 (6.6%)
Normal blood (detached-X)	7	0	. .	30.2 days		231 (38.1%)	12 (2.0%)	12 (2.0%)	351 (57.9%)
Uninjected control	10	0	. .	36.4 days		314 (38.1%)	19 (2.3%)	13 (1.5%)	473 (57.9%)
†Second gen.	84	62							
Third gen.	12	12							

Injected and uninjected controls for both series as well as data on subsequent generations are included. The SR-carrying hemolymph was derived from third generation females of the detached-X series of Table 5. 20°C.
*† Second generation is from the progenies bearing the same symbol.

y^2 *sc* w^a *ec* ♀ × y^{31d} sc^8 *dm B*, FM4♂, which has been regularly selected for low frequency of detachment of the X's.

These findings clearly show that the presence of a Y chromoosme *per se* is not the genotypic factor predisposing to SR mortality but rather the single-X condition. The survival of intersexes of the 2X + 3A type, often with markedly male phenotype, supports this interpretation, as do other findings described below.

Transfers into other strains: A further test of the specificity of the action of the SR agent was made by introducing hemolymph from SR.B-3 females of *D. willistoni* into females of *D. melanogaster* heterozygous for the gene (*tra*) found by STURTEVANT (1945). When homozygous this gene transforms females into phenotypic but sterile males. The stock used has the transformer gene balanced with Ubx^{130} in the third chromosome, and females carry attached-X's homozygous for FMA3 of LEWIS while males are either homozygous or heterozygous for *tra* and carry an X marked by apricot and vermilion. Injected young virgin females, *tra/In(3LR), Ubx*^{130}; FMA3, attached-XY were mated to males, *tra/tra;* w^a *v*, XY, and transferred to new creamers every three days at 25°C. The findings are presented in abbreviated form in Table 6, from which it is clear that the

TABLE 6

Summary of transfers of SR.B-3 from D. willistoni *into females of* D. melanogaster *carrying the transformer gene,* tra, *and controls. All recipients,* tra/Ubx; *FMA3, attached-XY females, were mated to* tra/tra; w^a v, *XY males and kept at 25°C*

Donor strain	No. females tested	No. SR progenies	Mean incub. time	F_1 progeny				
				tra/Ubx females	*tra/tra* ♀♀ ⟶ ♂♂	*tra/Ubx* males	*tra/tra* males	Total flies
SR.B-3	10	10	6.2 days	437 (43.8%)	182 (18.2%)	205 (20.4%)	177 (17.6%)	1001
B-3	10	0	. .	142 (32.7%)	65 (14.9%)	113 (26.1%)	114 (26.3%)	434

only class in which reduction occurred was that of the XY males. The *tra/tra;* attached-XY phenotypic males survived very well and like their heterozygous sisters (*tra/Ubx*^{130}; attached-XY) possessed numerous spirochetes in their hemolymph. Control females injected with hemolymph from normal B-3 females produced 52.4 percent w^a *v*, XY males, 14.9 percent *tra/tra;* attached-XY phenotypic males, and 32.7 percent *tra/Ubx*^{130}, attached-XY females. The SR-injected females gave 38.0 percent w^a *v*, XY males, 18.2 percent *tra/tra;* attached-XY phenotypic males, and 43.8 percent *tra/Ubx*^{130}; attached-XY females. These findings lend support to the interpretation advanced on the basis of the triploid and attached-X data above, for the differences between the SR-injected and the controls are highly significant ($X^2 = 81.27$, $P \ll 0.001$ for 3 degrees of freedom). Thus it appears that it is the single-X condition rather than the presence of a Y chromosome *per se* that increases the susceptibility of zygotes to the action of the SR agents.

Tests of a number of other strains as possible hosts for *willistoni*-SR were also carried through. Of the mutants tested a strain of adipose derived from a Kaduna stock by W. W. DOANE, and being studied by her with regard to its reproductive physiology, proved to be the best host yet found for the expression and persistence of *willistoni*-SR in *D. melanogaster*. Study of this strain is continuing.

Stability of transferred SR in relation to host genotype and to temperature: That the stability and persistence of the transferred SR condition are functions of the host genotype in *D. melanogaster* is fully evident in the data presented in Tables 1 to 3. The Sevelen strain is resistant both to establishment and persistence of introduced SR infections while Oregon-R-4 and the other special strains tested (Tables 4 to 6) favor their expression and transmission in subsequent generations. One of the most stable SR strains proved to be that established in attached-X females (*y*^{2} *sc* w^a *ec*/Y) mated to FM4 (*y*^{31d} *sc*^{8} *dm B*) males. Lines of this strain have been continuously maintained with a high level of expression and stability for more than two years.

Crosses to test the effects of Sevelen and Oregon-R-4 chromosome complements when introduced into the eggs of such SR females were carried out. Single females, all sisters, from a culture of the attached-XY line SR-6e8 were mated to males

from the F_{21} generations of the inbred Sevelen and Oregon-R-4 strains and to males (FM4, *y*^{31d} *sc*^{8} *dm B*) from the normal attached-XY (*y*^{2} *sc w*^{a} *ec*) strain used as the source of maintainer males. The cultures were begun at 25°C, a temperature which was maintained for approximately one week, after which the temperature in the laboratory rose to 28°C and remained at this level for another week. Two counts of progenies were made, the first ten days after the cultures were begun, the second ten days later. The data are summarized in Table 7. With a single exception, the females mated to the maintainer FM4 males gave only daughters through the first count; likewise those mated to Oregon-R-4 males gave only daughters. However, those mated to Sevelen males all gave sons, as well as daughters, and in most instances more sons than daughters. In the second counts, which were of progeny that had developed at 28°C, three of the females mated to FM4 and all of those mated to Oregon-R-4 males gave sons. Among the latter,

TABLE 7

Effects of outcrossing and temperature on SR expression in the attached-XY SR6e8 strain in D. melanogaster. *Progenies of single females, all sisters*

Source of male parents used in crosses	First count 25°C		Second count 28°C		Totals	
	Females	Males	Females	Males	Females	Males
FM4, *y*^{31} *sc*^{8} *dm B*	19	12	13	6	32	18
FM4, *y*^{31} *sc*^{8} *dm B*	31	0	22	0	53	0
FM4, *y*^{31} *sc*^{8} *dm B*	15	0	20	17	35	17
FM4, *y*^{31} *sc*^{8} *dm B*	7	0	18	6	25	6
FM4, *y*^{31} *sc*^{8} *dm B*	6	0	3	0	9	0
FM4, *y*^{31} *sc*^{8} *dm B*	2	0	1	0	3	0
FM4, *y*^{31} *sc*^{8} *dm B*	1	0	4	0	5	0
Totals	81	12	81	29	162	41
Oregon-R-4, inbred	11	0	20	14	31	14
Oregon-R-4, inbred	19	0	8	14	27	14
Oregon-R-4, inbred	19	0	3	11	22	11
Oregon-R-4, inbred	5	0	9	12	14	12
Oregon-R-4, inbred	36	0	14	10	50	10
Oregon-R-4, inbred	6	0	4	7	10	7
Oregon-R-4, inbred	10	0	31	28	41	28
Oregon-R-4, inbred	7	0	7	16	14	16
Oregon-R-4, inbred	4	0	5	29	9	29
Oregon-R-4, inbred	3	0	16	35	19	35
Totals	120	0	117	176	237	176
Sevelen, F_{21} inbred	19	12	10	14	29	26
Sevelen, F_{21} inbred	16	21	5	10	21	31
Sevelen, F_{21} inbred	8	20	7	8	15	28
Sevelen, F_{21} inbred	17	30	6	9	23	39
Sevelen, F_{21} inbred	13	8	12	11	25	19
Sevelen, F_{21} inbred	7	11	2	3	9	14
Sevelen, F_{21} inbred	1	9	9	19	10	28
Sevelen, F_{21} inbred	2	1	10	8	12	9
Totals	83	112	61	82	144	194

seven produced more sons than daughters. In the case of the matings to Sevelen males, the results were essentially the same at 28° as at 25°, with a preponderance of males in five instances.

To verify the effect of the Sevelen chromosome complement in the F_1 crosses to attached-XY SR females, another set of sisters was mated with Sevelen males and control crosses of normal attached-XY females to Sevelen males were carried out. These crosses were made two generations after the original crosses, the Sevelen males being derived from the F_{23} of the inbred strain. The attached-XY SR females were second generation descendents of the same line used in the first crosses. The single female cultures were set up at 25°C. After the first week the temperature in the laboratory dropped to 20° and remained there throughout the lives of the cultures. As in the earlier tests the first counts were made on the tenth day, but the second counts were a full fourteen days later. Table 8 shows that the effects of introduction of Sevelen chromosomes were repeated in the progeny developing at 25°, but were markedly reduced when development took place at 20°. In the control crosses there was a slight excess of males at 25° but the reverse was true at 20°. In the inbred Sevelen line there is a tendency toward excess males compared with other wild-type inbred lines.

The presence of a marked temperature effect on the expression of SR in the crosses to Sevelen and Oregon-R-4 males, and even in the attached-XY SR ♀♀ × FM4 ♂♂ line, led to the setting up of a series of crosses in which temperature was closely controlled. This experiment was carried through several months after the first series, utilizing flies seven generations removed. To obtain a wider comparison of the effects of wild-type chromosomes, males from inbred lines of Canton-S and Swedish-b as well as the inbred Sevelen and Oregon-R-4 strains were crossed to attached-XY SR females. Five single-female matings to males from each of these wild-type inbreds as well as to FM4 males from the maintainer strains were

TABLE 8

Effects of outcrossing and temperature on SR expression in the attached-XY SR 6e8 strain and on the control attached-XY strain in D. melanogaster

Female parent	Male parent	First count 25°C		Second count 20°C		Totals	
		Females	Males	Females	Males	Females	Males
SR, *y² sc w^a ec*	Sevelen, F_{23} inbred	16	14	19	4	35	18
SR, *y² sc w^a ec*	Sevelen, F_{23} inbred	11	20	21	2	32	22
SR, *y² sc w^a ec*	Sevelen, F_{23} inbred	9	16	8	6	17	22
SR, *y² sc w^a ec*	Sevelen, F_{23} inbred	8	6	21	7	29	13
SR, *y² sc w^a ec*	Sevelen, F_{23} inbred	19	4	26	0	45	4
Totals		63	60	95	19	158	79
Normal, *y² sc w^a ec*	Sevelen, F_{23} inbred	9	12	15	14	24	26
Normal, *y² sc w^a ec*	Sevelen, F_{23} inbred	16	6	7	10	23	16
Normal, *y² sc w^a ec*	Sevelen, F_{23} inbred	7	16	22	18	29	34
Normal, *y² sc w^a ec*	Sevelen, F_{23} inbred	6	13	23	20	29	33
Normal, *y² sc w^a ec*	Sevelen, F_{23} inbred	13	17	26	21	39	38
Totals		51	64	93	83	144	147

set up at 25°C and 20°C and maintained at those temperatures throughout in Precision Incubators. In some cases sterility or mold resulted in failure of the cultures. The data presented in Table 9 represent totals of counts over a two-week period in the case of cultures at 25° and a three-week period at 20°.

The effects of Sevelen chromosomes were again evident but were less marked than in the earlier crosses. The effects of Canton-S chromosomes were similar to those of Oregon-R-4. The most striking effects were in the crosses to Swedish-b where there was a preponderance of males at 25°. Although one female mated to Swedish-b males failed to produce any males at 20° and one gave a 2:1, the others showed the same effect as the 25° series. F_1 daughters from the different

TABLE 9

Effects of outcrossing at different temperatures on SR expression in the attached-XY SR 6e8 strain in D. melanogaster

Source of males used in crosses	F_1 progenies raised at 25°C		F_1 progenies raised at 20°C	
	Females	Males	Females	Males
Attached-XY × FM4, *y*^{31d} *sc*^{8} *dm B*	13	1	45	0
Attached-XY × FM4, *y*^{31d} *sc*^{8} *dm B*	58	0	52	0
Attached-XY × FM4, *y*^{31d} *sc*^{8} *dm B*	31	0	30	0
Attached-XY × FM4, *y*^{31d} *sc*^{8} *dm B*	33	0	. .	. .
Attached-XY × FM4, *y*^{31d} *sc*^{8} *dm B*	71	0	. .	. .
Totals	206	1	127	0
Sevelen, F_{28} inbred	13	0	32	0
Sevelen, F_{28} inbred	65	13	17	2
Sevelen, F_{28} inbred	46	10	26	0
Sevelen, F_{28} inbred	73	4	20	1
Totals	197	27	95	3
Oregon-R-4, F_{28} inbred	30	6	22	0
Oregon-R-4, F_{28} inbred	6	0	17	0
Oregon-R-4, F_{28} inbred	. .	. .	50	0
Oregon-R-4, F_{28} inbred	. .	. .	11	0
Oregon-R-4, F_{28} inbred	. .	. .	4	0
Totals	36	6	104	0
Canton-S, F_{28} inbred	78	18	22	0
Canton-S, F_{28} inbred	35	5	35	0
Canton-S, F_{28} inbred	22	7	36	0
Canton-S, F_{28} inbred	20	0	36	0
Canton-S, F_{28} inbred	4	0	. .	. .
Totals	159	30	129	0
Swedish-b, F_{28} inbred	31	42	28	0
Swedish-b, F_{28} inbred	33	33	30	29
Swedish-b, F_{28} inbred	9	16	22	24
Swedish-b, F_{28} inbred	27	44	17	45
Swedish-b, F_{28} inbred	. .	. .	22	12
Totals	100	135	119	110

Progenies of single females, all sisters in the cases of the matings to FM4 and Sevelen males, cousins in the matings to Oregon-R-4, Canton-S, and Swedish-b males.

crosses at each temperature were backcrossed to males of the paternal types. In the backcross generation, a considerable proportion of the Oregon-R-4 and Canton-S mated females kept at 25° gave few or no males, whereas most of the Sevelen and all of the Swedish-b mated females produced many males and the SR condition completely disappeared in subsequently tested generations. Among the 20°. backcrosses, SR was predominant and males rare in all except the case of Swedish-b where SR was wholly disrupted in each instance where it was followed.

Attempts to isolate individual chromosomes for tests of their effects by crossing attached-XY SR females to the multiple stock H-40 (M-5; *In SM1, al Cy* sp^2/ *dp Pm* ds^{33k}; *C Sb/Ubx*^{130} e^s) resulting in every instance in the appearance of a full complement of males in the F_1 generation. No further crosses to H-40 were tried.

The picture that emerges is one of SR stability and relative insensitivity to temperature in the background of the original attached-XY × FM4 strain, increased temperature sensitivity in the F_1 and later generations in crosses to Oregon-R-4 and Canton-S, strong disruption of the SR effect in the F_1 and later generations in crosses to Sevelen, and essentially complete suppression in the F_1 and subsequent generations of crosses to the Swedish-b strain or the H-40 tester stock under the conditions of these experiments.

To determine the extent to which the suppression of the SR effect in the F_1 of the original attached-XY SR × Sevelen crosses (Table 7) was accompanied by reduction in infectious SR agent in the F_1 females, infection tests were carried through by injecting hemolymph from each of eight females (all sisters from a female giving excess sons at 25°C) into young virgin Oregon-R-4 females. The injected females were mated to Oregon-R-4 males and successive broods of progeny were observed for the appearance of males. The results (first line of Table 10) showed the appearance of males in 1:1 ratio throughout the lives of seven of the eight tested females. Only one female gave no sons. Thus the heterozygous complement of Sevelen autosomes suppressed the multiplication of infectious SR agent in seven out of eight female zygotes. This suggests that the survival of males is attributable to suppression of the multiplication of the agent rather than to resistance to its action.

Infection tests following the same procedure were carried out using hemolymph of flies of both sexes from regular lines of SR established in Oregon-R-4 and Sevelen which had reverted from SR to the normal 1:1 condition. Two different reverted lines of each of these were tested. The flies used in the tests came from the second generation following the reversion to normal. The results, lines 2 to 9 in Table 10, show that in no case did males carry any detectable infectious agent. In the Sevelen strains, four out of six tested females contained SR agent in their hemolymph as shown by the short incubation times for the appearance of SR in the progenies of the test hosts.

Observations on hemolymph: Phase microscopic examination of hemolymph of SR females of *D. willistoni* and *D. nebulosa* (POULSON and SAKAGUCHI 1960, 1961) had demonstrated the regular presence of small spirochetes resembling in all characteristics those of the genus Treponema. Spirochetes were not found in

TABLE 10

Tests of infectivity of blood of reverted SR strains in D. melanogaster *by injection of adult hemolymph into young normal females of the inbred strain of Oregon-R. In most cases blood of both sexes was tested. Except in the first instance all are second generation reverted*

Strain tested	No. females tested	No. SR progenies	Percent infections	Incubation time
Attached-XY, SR 6e8–5 ♀ ×				
Sevelen ♂, F_1 females	8	1	12.5	3 days
Sevelen, SR 6e2 females	3	3	100	2 days
Sevelen, SR 6e2 males	5	0	0	.
Sevelen, SR 6e3 females	3	1	33.3	2 days
Sevelen, SR 6e3 males	2	0	0	.
Oregon-R, SR 4e5 females	4	2	50	2 days
Oregon-R, SR 4e5 males	5	0	0	.
Oregon-R, SR 6e5 females	4	4	100	3 days
Oregon-R, SR 6e5 males	6	0	0	.

the hemolymph of any individuals of the normal strains of those species. Furthermore we were able to show that transfer of the SR condition into normal strains of *D. willistoni* and into the strains of *D. melanogaster* described above is invariably correlated with the presence of the same Treponema-like spirochetes in the hemolymph (POULSON and SAKAGUCHI 1960, 1961). Direct confirmation of the relationships of the spirochetes to the SR condition comes from observations on the hemolymph of parents and offspring in the experiments summarized in Tables 7 and 8. Hemolymph of daughters from each of the progenies in which SR was expressed (males lacking or infrequent) contained large numbers of spirochetes while that of daughters from progenies including large numbers of males (attached-XY SR ♀ × S_{F21} ♂) lacked spirochetes. Since most of the parental females were still alive after the second counts, examination of their hemolymph was made. In every instance the hemolymph of these parental females was swarming with spirochetes. In progenies of the F_1 daughters backcrossed to Sevelen F_{22} males (1:1 restored), there was complete absence of spirochetes from the hemolymph of all individuals examined. Similar examinations of hemolymph were made in the case of the experiments summarized in Table 9. Again the parallels between SR expression and the presence of spirochetes were complete.

DISCUSSION

That *D. melanogaster* may serve as a host for the SR agent of *D. willistoni* is amply demonstrated by these data, and thus the way is open for a detailed genetic study of the relationship between SR and host genotype. The differences in response between the Oregon-R and the Sevelen strains with regard to establishment and persistence of SR are remarkably clear (Tables 1, 2, and 3 and Figure 1) and parallels between them and the Canton-S and Swedish-b strains are evident

in the outcrossing experiments (Table 9). The ease of establishment in the triploid and attached-X strains and in the heterozygous detached-X strains derived from them may be a reflection either of a genic background similar to that of the Oregon-R strain or the nature of the mutants used as markers. The former seems more probable since so many of the older laboratory strains of *D. melanogaster* have had their origin from Oregon-R. The similarities in behavior of the Oregon-R and the Canton-S strains on the one hand and of the Sevelen and Swedish-b strains on the other suggest the desirability of wider testing of wild strains of American and European origins as well as strains from other geographic regions for the presence of genes favoring or disrupting SR expression and transmission.

In each of the series of injected flies a constant feature was the reduction in the length of life of the SR-injected as compared with the control females. A further characteristic was the increase in the mean number of daughters per female among the SR-injected versus the controls. When computed as daughters per female per brood during reproductive life, this increase was particularly marked in females of the Sevelen strain (Table 2 and Figure 3). In the absence of uninjected controls in this series the effects of the control hemolymph in reducing the life span of the injected controls can only be inferred from the triploid and attached-X series (Tables 4, 5) in which uninjected controls were employed. No direct comparison can be made as the latter experiments were carried through at 20°C rather than 25° for reasons discussed below.

The increased production of daughters by the SR injected females suggests a stimulatory effect of the SR agent on egg production which may be the basis for the reproductive success of SR females in natural populations. This stimulatory effect was most pronounced in the series of Sevelen females injected with SR which had been in *D. melanogaster* for several generations (bottom line of Table 2). All the females of this group showed a marked drop in male production at four to five days, as though the SR condition were becoming established, and then all recovered. The mean number of daughters per female per day of reproductive life was about three times that in the controls for the earlier Sevelen series (line 4 of Table 2). These data indicate that the increased numbers of daughters per female in the other series are not a mere consequence of the absence of competition from males since the numbers of males in the last series were also high after the recovery.

The interpretation of any experiment in which whole hemolymph is transferred from one individual to another must recognize that any microfauna or -flora in the blood of the donor will be carried into the host. Thus, which of the effects (other than that on the males) may be strictly ascribable to the SR agent and which to mere concommittant transfer of other infectious agents may not always be clear. It had been found that the SR B-3 strain and the B-3 strain of *D. willistoni* carried an infection of a microsporidian (SAKAGUCHI and POULSON 1961a) which considerably complicated the SR studies in that species. These were present in the flies used for the initial transfers into *D. melanogaster*. Fortunately these microsporidians failed to become established in the SR strains of *D. melanogaster* and this complication was removed. Although it could be responsible for some of

the differences between injected and uninjected controls (Table 4), this is perhaps an effect of the operation itself (DOANE 1961).

An account of the microsporidian has been given by BURNETT and KING (1962) and further studies have been made by RICHARD P. MILLS (unpublished) of the mode of infection and transmission. The materials have been turned over to DR. JOHN P. KRAMER of the Illinois Natural History Survey, who will shortly publish a description of the species as a new member of the genus Nosema.

A striking characteristic of the first studied case of SR in Drosophila (that of *D. bifasciata*) was found by MAGNI (1954) to be its response to increased temperature. If eggs of SR females of that species develop at 25° to 26°C rather than at 20° to 22°, a full complement of males appears and the females derived from such eggs are "cured" of the SR condition. In the original SR strain of *D. willistoni* and the SR strain of *D. paulistorum*, MALOGOLOWKIN (1958) was unable to demonstrate any effect of temperature on the expression or transmission of SR although she later (MALOGOLOWKIN 1959) found the SR strain of *D. equinoxialis* to be temperature sensitive. The findings of temperature sensitivity in the transferred SR in *D. melanogaster* (Tables 7, 8, 9) is thus of considerable interest, indicating that the response is not a characteristic of the agent *per se* but rather of the host and more particularly of the host genotype.

Embryological studies on abnormal SR embryos in *D. willistoni* (COUNCE and POUISON 1962) show that the primary effects there are first recognizable in dividing nuclei, the nearly cleavage stages being markedly affected in certain of the strains examined. If the effect of increased temperature is the speeding up of the mitotic rate of zygotic nuclei relative to the multiplication of the SR agent, as seems probable from MAGNI's studies and from data on the early development of Drosophila (SONNENBLICK 1950; POULSON 1950), then any genotypic influence on mitotic rate during development may be expected to modify the temperature response. Whether there is normally a differential in mitotic rate between male and female embryos is unknown in any of the species of Drosophila which have been studied.

In undertaking the introduction of SR into *D. melanogaster*, the fact that the developmental rate is faster in *D. melanogaster* than in *D. willistoni* was considered. In the latter, adults begin to emerge ten days after oviposition at 25°C while in the former emergence begins at about eight days. Thus the first experiments were carried through at 20° lest the SR agent fail to establish itself in the more rapidly developing species. Fortunately the relative stability at 25°C made it possible to conduct most other experiments at that temperature. Most often SR stocks are regularly kept at 18° to 20° for convenience of maintenance.

The nature of the action of the SR agent on male zygotes remains to be clarified, i.e., whether competition for an essential metabolite or poduction of an inhibitor or toxin. Since it is known that occasional female zygotes may be affected, the specificity is not absolute. However, the findings with respect to sex genotype in the triploid series and in the case of the transformer gene make it clear that it is the single-X condition rather than the presence of the Y-chromosome which

sets the stage. The survival of attached-XY females in the triploid series and the attached-XY series and the subsequent high degree of stability of SR in the attached-XY line strongly support this interpretation. The data presented here and the developmental findings (COUNCE and POULSON 1962) are of interest in relation to the findings of FOX, MEAD and MUNYON (1959) and of CHEN and DIEM (1961) with respect to biochemical differences between the sexes. To what extent these particular differences may be related to the differential mortality remains to be seen.

The demonstration that the SR strains of *D. willistoni* and *D. nebulosa* are characterized by the presence of spirochetes in the hemolymph of adult females (POULSON and SAKAGUCHI 1961) and the further evidence presented here for a complete correlation between the transferred SR condition and the presence of spirochetes in *D. melanogaster* establishes them as the etiological agents of "sex-ratio" in this material. The suppression of the effects of the spirochetes by genotype in the crosses of attached-XY SR females to Sevelen or Swedish-b males (Tables 7, 8, 9) and the latent transmission of SR in the females of reverted lines (Table 10) all point to this same conclusion. However, one may ask whether the spirochetes are the actual agents or only the carriers of the effective agent. In the material which we have studied there are thus far no exceptions to the rule indicated and the filtration experiments reported separately (SAKAGUCHI and POULSON 1963), as well as the effects of penicillin G (SAKAGUCHI and POULSON 1961b) provide further support.

As pointed out in some detail elsewhere (POULSON 1963) all available information demonstrates that the "sex-ratio" condition of *D. willistoni* and its relatives is a type of host-parasite relationship not previously recognized in other organisms, in which the differential response of the male and female zygotes to the parasite produces the "sex-ratio" phenotype of unisexual progenies. But for the differential effects on the zygotes the hereditary transmission of these spirochetes would have gone unrecognized.

SUMMARY

The "sex-ratio" condition (SR) of *D. willistoni* is not species specific and is transferable into *D. melanogaster*. Stability of expression and persistence of SR in *D. melanogaster* is dependent on the genotype of the host flies.

When transferred into triploid females all classes of female offspring and intersexes survive and only males and supermales are lacking. When introduced into a strain heterozygous for the transformer gene only the true male offspring are affected. Thus the expression of SR is a consequence of the differential sensitivity of the single-X as compared with the diplo-X condition rather than of the presence of the Y chromosome in males.

Following transfer to *D. melanogaster* SR is temperature sensitive. The temperature sensitivity is more marked in some genotypic backgrounds than in others.

The persistence and expression of SR are completely correlated with the presence of treponema-like spirochetes in the hemolymph of adult females in the strains into which transfer was made. The "sex-ratio" condition of *D. willistoni* is a special type of host-parasite relationship in which the genotypes of the host flies and the parasitic spirochetes are of prime importance.

LITERATURE CITED

BURNETT, R. G., and R. C. KING, 1962 Observations on a microsporidian parasite of *Drosophila willistoni* Sturtevant. J. Insect Pathol. **4**: 104–112.

CAVALCANTI, A. G. L., D. N. FALCÃO, and L. E. CASTRO, 1957 Sex-ratio in *Drosophila prosaltans*, a character due to interaction between nuclear genes and a cytoplasmic factor. Am. Naturalist **91**: 321–325.

1958 The interaction of nuclear and cytoplasmic factors in the inheritance of the "sex-ratio" character in *Drosophila prosaltans*. Univ. Brasil Publ. Fac. Nacl. Fil., Ser. Cient., No. 1, 25–54.

CHEN, P. S., and C. DIEM, 1961 A sex-specific ninhydrin-positive substance found in the paragonia of adult males of *Drosophila melanogaster*. J. Insect Physiol. **7**: 289–298.

COUNCE, S. J., and D. F. POULSON, 1962 Developmental effects of the "sex-ratio" agent in embryos of *Drosophila willistoni*. J. Exptl. Zool. **151**: 17–32.

DOANE, W. W., 1960a Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. I. Adult morphology, longevity, egg production, and egg lethality. J. Exptl. Zool. **145**: 1–22.

1960b Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. II. Effects of altered environment and residual genome on its expression. J. Exptl. Zool. **145**: 23–42.

1961 Developmental physiology of the mutant female sterile (2) adipose of *Drosophila melanogaster*. III. Corpus allatum complex and ovarian transplantation. J. Exptl. Zool. **146**: 275–298.

FOX, A. S., C. G. MEAD, and I. F. MUNYON, 1959 Sex-peptide of *Drosophila melanogaster*. Science **129**: 1489–1490.

MAGNI, G. E., 1954 Thermic cure of cytoplasmic sex-ratio in *Drosophila bifasciata*. Proc. 8th Intern. Congr. Genet. Caryologia Suppl. Vol. 1213–1216.

MALOGOLOWKIN, C., 1958 Maternally inherited "sex-ratio" conditions in *Drosophila willistoni* and *Drosophila paulistorum*. Genetics **43**: 274–286.

1959 Temperature effects on maternally inherited "sex-ratio" conditions in *Drosophila willistoni* and *Drosophila equinoxialis*. Am. Naturalist **93**: 365–368.

MALOGOLOWKIN, C., and G. G. CARVALHO, 1961 Direct and indirect transfer of the "sex-ratio" condition in different species of Drosophila. Genetics **46**: 1009–1013.

MALOGOLOWKIN, C., G. G. CARVALHO, and M. C. DA PAZ, 1960 Interspecific transfer of the "sex-ratio" condition in Drosophila. Genetics **45**: 1553–1557.

MALOGOLOWKIN, C., and D. F. POULSON, 1957 Infective transfer of maternally inherited abnormal sex-ratio in *Drosophila willistoni*. Science **126**: 32.

MALOGOLOWKIN, C., D. F. POULSON, and E. Y. WRIGHT, 1959 Experimental transfer of maternally inherited abnormal sex-ratio in *Drosophila willistoni*. Genetics **44**: 59–74.

POULSON, D. F., 1950 Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meigen. Pp. 168–274. *Biology of Drosophila*. Edited by M. DEMEREC. Wiley and Sons, New York.

1963 Cytoplasmic inheritance and hereditary infection in Drosophila. Pp. 402–422. *Methodology in Basic Genetics.* Edited by W. J. BURDETTE. Holden-Day, San Francisco.

POULSON, D. F., and C. MALOGOLOWKIN, 1959 Transfer of "sex-ratio" condition in Drosophila and its genetic implication. Proc. XV Intern. Congr. Zool., London, 200–202.

POULSON, D. F., and B. SAKAGUCHI, 1960 Evidence concerning the nature of the "sex-ratio" agent in Drosophila. Anat. Rec. **138:** 376–377.

1961 Nature of the "sex-ratio" agent in Drosophila. Science **133:** 1489–1490.

SAKAGUCHI, B., and D. F. POULSON, 1960 Transfer of the "sex-ratio" condition from *Drosophila willistoni* to *D. melanogaster.* Anat. Rec. **138:** 381.

1961a Distribution of the "sex-ratio" agent in the tissues of *Drosophila willistoni.* Genetics **46:** 1665–1676.

1961b Some properties of the "sex-ratio" agent of *Drosophila willistoni.* Ann. Rep. Nat. Inst. Genetics (Japan) **11:** 22–23.

1963 Properties of the "sex-ratio" agent of *Drosophila willistoni:* dilution and filtration experiments. (In preparation.)

SONNENBLICK, B. P. 1950 The early embryology of *Drosophila melanogaster.* Pp. 62–167. *Biology of Drosophila.* Edited by M. DEMEREC. Wiley and Sons, New York.

STURTEVANT, A. H., 1945 A gene in *Drosophila melanogaster* which transforms females into males. Genetics **30:** 297–299.