EXPERIMENTAL TRANSFER OF MATERNALLY INHERITED ABNORMAL SEX-RATIO IN DROSOPHILA WILLISTONI

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In previous communications (MALOGOLOWKIN and POULSON 1957; MALOGOLOWKIN 1958) a cytoplasmically inherited sex-ratio condition has been described in *Drosophila willistoni* and in *D. paulistorum*. In both species, certain females produce progenies consisting mostly or only of daughters. This capacity for the production of unisexual broods is inherited by their entire female progenies and is not transmitted at all by the few exceptional male descendants that appear in some cultures. Many of the eggs deposited by sex-ratio females of *D. willistoni* are recognizably abnormal within two to four hours of oviposition; and there are reasons to suppose that these dying eggs represent the male zygotes, i.e. eggs fertilized by Y-bearing spermatozoa (MALOGOLOWKIN and POULSON 1957). Although the sex-ratio condition is due to the nonchromosomal transmission of a causative agent, through the cytoplasm of the egg, it is not wholly independent of the influence of chromosomal genes. Crossing of sex-ratio females to males from strains of certain geographic origins results, after a few generations, in an irreversible loss of the sex-ratio condition (MALOGOLOWKIN 1958). These strains evidently contain genes which are less favorable for the multiplication of the causative agent in the cytoplasm than are the genes of many other strains.

The present article reports in detail the results of experiments which demonstrate that the causative agent of the sex-ratio condition may be mechanically introduced into the germinal materials of females of previously normal strains. Thus a female of *D. willistoni* may deposit the sex-ratio type of abnormal, inviable eggs within about ten days after the injection into her abdomen of ooplasm from an abnormal egg of a sex-ratio female. Moreover, at least a portion of the female progenies of such females carry the causative agent of the sex-ratio condition in their eggs, and the condition is inherited through the female lines from there on with same persistency and regularity as in the original sex-ratio lines of females.

MATERIALS AND METHODS

The sex-ratio, or unisexual, strains used in the present investigation are those reported on by MALOGOLOWKIN and POULSON (1957) and genetically analyzed by MALOGOLOWKIN (1958). They are derived from a fly collection at Bath, Jamaica, by DRS. W. B. HEED and M. WASSERMAN of the University of Texas.

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This fly, a female, was observed by MR. B. SPASSKY to yield an unisexual progeny. The resulting strain was back crossed repeatedly to a laboratory strain containing the sex-linked mutants eosin (w^e), singed (sn), yellow (y), Incomplete (Inc), and roughoid (ro), and their daughters in turn repeatedly back crossed to males of this strain. Except for a few males which appeared in the tenth generation of this backcrossing, the strain produced exclusively females. Other series of crosses and backcrosses were made to the autosomal recessive mutant pink (p) and to a wild strain derived from flies collected on the island of Barbados. Aside from a very few occasional males these progenies consisted of females only. These strains served as sources of eggs containing the sex-ratio causative agent.

A study was made of the proportion of the eggs of sex-ratio and of normal strains which survive and give rise to adults. For this purpose three pairs of flies from a given strain were introduced into sterilized vials and given paper spoons with fresh Drosophila nutrient medium. Ovipositing females, about three days old, of the sex-ratio strains were used; and for controls, females of similar age from various strains of diverse geographic origins giving normal progenies. The flies were allowed to oviposit for about 12 hours after which the spoons with eggs were replaced by fresh spoons. The eggs on the spoons were examined every three to six hours and the larvae that hatched were transferred to creamers containing culture medium. Counts of eggs, pupae, and adult flies were recorded.

For the injection experiments eggs were collected on spoons which were changed every four hours. The eggs were transferred into watch glasses containing WADDINGTON's modified Drosophila Ringer's solution. The eggs were washed in this with the aid of a pipette to clear them of adherent bits of yeast and medium. Most of the solution was then pipetted away and a few drops of a diluted Chlorox solution (equal parts Chlorox and Ringer's solution) added to dechorionate the eggs. This action was watched under a binocular microscope; and as soon as the chorions became transparent Ringer's solution was added and the eggs were washed repeatedly and thoroughly to remove all traces of hypochlorite.

As previously reported (MALOGOLOWKIN and POULSON 1957) the abnormal eggs are characterized by increasing translucent areas at the time of blastoderm formation and so are readily distinguishable from normally developing zygotes and from unfertilized eggs. The microinjection apparatus used was that of RIZKI (1953) which is most convenient and allows for rapid changing of micropipettes without danger of contamination. Abnormal eggs (three to six hours after oviposition) were punctured with a micropipette and the ooplasm sucked out. The ooplasm was then mixed with a small amount of the Ringer solution in the watch glass and a portion taken back into the pipette for injection into the host female. The recipient females were kept lightly etherized. They consisted of one to two day old virgin females of the Recife-3 strain of *D. willistoni* which has always yielded normal sex-ratios. The suspension of ooplasm was injected into the abdomen at about the level of the ovaries taking care not to pierce the gut. In each

instance about one third the content of an egg was injected. While an effort was made to keep the quantities as uniform as possible, there was obvious variability in some instances which were noted in the records.

The injected females were left for several hours to recover in separate sterile vials, provided each with two males of the Recife-3 strain, and fed with honey-yeast mixture on spoons of Drosophila medium. The spoons were replaced every other day, the bit of food with the two day collection of eggs being transferred into a creamer containing fresh medium. This prccedure was continued throughout the life of each female so that her entire progeny was obtained as a series of successive two-day broods. The creamers were kept under close observation and in some instances unhatched eggs were removed for examination. The numbers of females and males hatching on successive days in each of the creamers was recorded.

Control experiments were carried through in precisely the same manner, except that ooplasm from unfertilized eggs laid by virgin females of the normal Recife-3 strain was utilized to inject other young virgin Recife-3 females. The injected controls all survived and all laid eggs.

All experiments were carried out at 25°C and with humidity never less than 50 percent in the incubator.

Mortality among eggs of sex-ratio females

To establish whether male zygotes are usually not formed, or die at some stage of development, it was necessary to examine mortality in normal and sex-ratio strains. Table I is a summary of certain data on the survival of eggs from several

TABLE 1

The survival of eggs in "sex-ratio" and in normal strains

Strain	Total eggs	Eggs hatched	Percent eggs hatched	Pupae	Adult		Percent total survival
					Females	Males	
Control	1,141	987	86.5	962	431	359	69.2
Sex-ratio	1,487	418	28.1	349	332	3	22.5

normal and sex-ratio strains of *D. willistoni* obtained early in the course of this study. It is seen that a total of 1141 eggs from several normal strains yielded 790 adult flies with a slight but significant preponderance of females. The percentage of eggs giving rise to adults under the conditions of experiment (which may not have been the most ideal for *D. willistoni*) was 69.2. However, the hatchability of eggs was 86.5 percent and the largest part of the mortality was clearly post-embryonic, chiefly in the pupal stage. In the case of the sex-ratio females mortality is much higher and is confined almost completely to egg and early larval stages. From 1487 eggs only 335 adults, or 22.5 percent, came through and only three of these adults were males. The hatchability of eggs (about 28 percent) is strikingly below that of any normal strains.

At the time these counts were done no attempt was made to analyze the low hatchability in developmental terms. Subsequent examination of eggs laid by sex-ratio females showed that considerable numbers of unfertilized, or unfertilizable, eggs are almost always present in the collected sample. When these eggs are taken into account the proportions of normally developing to abnormal, dying eggs is usually close to 1:1. Examination of the ovaries of mature, actively laying sex-ratio females shows that numerous large ovarian eggs are clearly aberrant and presumably incapable of being fertilized. These appear to be the source of the numerous unfertilized eggs observed among the samples collected from many sex-ratio females.

The developmental disturbance in the abnormal, dying eggs is very striking and led to the conclusion (MALOGOLOWKIN and POULSON 1957) that they represent the missing male zygotes. If so, then these eggs ought to be a potent source of the sex-ratio causative agent. Accordingly the injection experiments were planned to test this possibility.

An account of the developmental disturbances will be published separately.

Progenies of injected females

Table 2 presents the composition of the progenies of the fourteen Recife-3 females (SR) which were injected with the sex-ratio ooplasm, survived the injection, and were fertile. Table 3 presents the comparable data for the fifteen control females (UN) injected with the ooplasm of unfertilized eggs from other Recife-3 females.

It will be seen at once that the control females produced both daughters and sons as long as they remained alive and fertile. Even among the last eggs deposited by these females the proportions of the two sexes remain close to 1:1. There is considerable range in the productivity among these females. Whether this be the result of the injection of the ooplasm or normal variability cannot be told in the absence of comparable data for an uninjected series of Recife-3 females. There is no significant deviation from the normal sex-ratio here.

A very different situation is observed in some of the progenies of the females injected with ooplasm from sex-ratio eggs. For some days following the injections, females, SR-1, SR-103, and SR-109 deposited eggs from which daughters and sons developed with the expected frequencies of about 1:1. However, between the 13th and the 17th days after injection SR-1 produced 72 daughters and 19 sons, and after the 17th day 127 daughters and only one son. SR-103 gave 206 daughters and 201 sons from the eggs deposited up to the 13th day following injection, and 49 daughters and no sons thereafter. In the case of SR-109 there were 169 daughters and 182 sons from eggs laid up to the 15th day followed by 51 daughters and only four sons thereafter. The most striking case is that of SR-2 who from the beginning of egg laying on the third day gave in each brood a ratio of two daughters to each son for a total of 186 daughters and 75 sons through the 13th day, and thereafter to the 31st day 101 daughters and no sons.

Thus it appears that at least four out of the 14 females injected with the

TABLE 2

The progenies of the females injected with the sex-ratio ooplasm. The numbers of daughters and sons which developed from the eggs deposited on different days after the injections are shown. The zeros indicate that the mother was alive but deposited no eggs

Days	1–3		3–5		5–7		7–9		9–11		11–13		13–15		15–17		17–19		19–21		21–23		23–25		25–27		27–29		29–31	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
SR–1	2	3	84	59	43	47	55	60	4	4	34	30	27	14	45	5	19	0	20	0	12	0	19	1	28	0	7	0	22	0
SR–2	0	0	33	15	47	15	45	17	32	15	29	16	14	0	25	0	27	0	11	0	2	0	0	0	0	0	0	0	22	0
SR–3	2	1	34	36	35	37	47	53	41	37	29	37	30	20	7	0	0	0	0	0	0	0	0	0	0	0	..	..	..	..
SR–5	10	13	74	42	46	32	27	17	23	26	23	20	2	4	0	1	0	0	..	..	..	..	..	..	..	..	..	..	..	..
SR–8	0	0	33	24	77	66	79	76	55	59	50	58	11	11	10	14	2	3	0	0	..	..	..	..	..	..	..	..	..	..
SR–9	18	13	64	69	51	37	41	34	18	17	16	24	0	0	1	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..
SR–11	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3	2	10	10	7	3	4	1	2	2
SR–12	16	20	61	32	46	43	50	40	45	52	29	53	17	20	43	52	0	0	0	0	1	0	0	1	0	0	0	0	0	0
SR–103	0	0	30	23	41	41	68	59	30	42	37	36	13	0	36	0	0	0	0	0	0	0	..	..	..	..	..	..	..	..
SR–105	0	0	43	37	56	48	69	66	23	24	33	36	0	0	0	1	0	0	..	..	..	..	..	..	..	..	..	..	..	..
SR–107	0	0	23	31	20	28	60	46	15	30	10	9	0	3	2	3	0	0	0	0	0	0	..	..	..	..	..	..	..	..
SR–108	0	0	8	7	0	0	18	21	0	0	0	0	0	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
SR–109	0	0	24	21	44	40	56	60	26	45	15	25	4	11	28	4	15	0	8	0	0	0	..	..	..	..	..	..	..	..
SR–111	0	0	12	10	16	30	0	0	0	0	0	0	1	2	2	3	5	5	4	2	2	0	0	0	..	..	..	..	..	..
SR–112	0	0	28	31	30	38	52	42	41	38	18	19	28	16	19	15	25	42	21	21	8	9	13	12	28	39	19	14	15	19

TABLE 3

Number of daughters and sons developed from eggs deposited by the control female. These females were injected with the ooplasm of unfertilized eggs of normal females

Days	1–3		3–5		5–7		7–9		9–11		11–13		13–15		15–17		17–19		19–21		21–23		23–25		25–27		27–29		29–31	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
UN–1	0	1	33	36	26	35	50	40	35	27	27	35	24	24	24	33	8	7	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–2	2	2	47	25	26	34	2	3	0	0	0	0	0	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–3	8	13	20	23	59	63	60	53	34	27	39	34	21	26	22	19	32	26	4	1	0	0	4	1	. .	. .	. .	. .	. .	. .
UN–4	5	3	49	34	44	46	60	55	46	28	45	46	14	21	32	38	19	22	1	4	0	0	1	0	0	0	. .	. .	. .	. .
UN–5	18	17	41	26	49	50	32	39	11	14	28	33	7	7	3	0	1	1	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–6	2	1	51	42	30	50	40	42	10	14	39	34	25	29	26	14	20	22	1	2	0	1	0	0	. .	. .	. .	. .	. .	. .
UN–7	11	11	34	20	30	31	41	44	26	38	43	48	7	5	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
UN–8	6	3	38	33	55	82	94	89	50	43	68	76	62	43	25	35	46	34	5	5	30	32	19	13	29	21	6	12	2	2
UN–9	0	0	56	46	16	20	25	27	37	25	6	8	9	3	1	5	1	3	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–10	3	0	49	24	4	5	0	2	11	11	0	0	2	3	3	1	3	2	1	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .
UN–11	3	5	7	5	0	0	0	0	30	20	0	0	0	0	0	1	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–12	1	6	13	14	0	0	0	0	16	11	0	0	0	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–13	21	13	27	21	65	58	77	76	27	36	49	47	32	37	33	37	28	34	30	37	2	2	19	21	3	2	. .	. .	. .	. .
UN–14	1	1	12	20	10	6	20	22	2	3	16	22	0	0	0	0	0	0	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .
UN–15	0	0	14	11	5	8	1	1	0	0	26	17	28	19	6	8	1	2	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .

ooplasm from sex-ratio eggs have become infected with the sex-ratio agent and, after a period of some days begin themselves to deposit sex-ratio eggs. This interpretation was immediately confirmed by examination of samples of eggs laid by these females during the later days of oviposition. Numerous eggs of the type characteristic of the original sex-ratio strain were found; some were photographed and fixed for embryological and cytological study. The latter were from eggs deposited by female SR-1 during the period from the 25th to the 27th day following injection.

Second generation progenies of the injected females

It is now logical to inquire whether the females in the progeny of the injected mothers will themselves manifest any deviation from the normal proportions of the two sexes in their offspring.

The number of females in the progeny of the injected mothers was obviously too great for them to be tested as fully as might be desired in the following generations. Tests had to be made selectively, but even so the records are far too bulky for full publication. Table 4 presents a summary of the data for the progenies of the daughters of injected females (this is the second generation, the in-

TABLE 4

Second generation progenies of normal females injected with "sex-ratio" ooplasm. The numbers of daughters tested from different broods and the members producing unisexual progenies are shown. The letters in parentheses indicate the broods which were used from the third generation tests reported in Table 5

Injected female	Days after injection	Daughter tested	Unisexual progenies	Injected female	Days after injection	Daughter tested	Unisexual progenies
SR–1	3–5	14(A)	0	SR–3	11–13	5(L)	0
SR–1	5–7	6	0	SR–3	15–17	4(M)	0
SR–1	7–9	6	0	SR–5	3–5	2	0
SR–1	11–13	7(B)	0	SR–5	7–9	11	0
SR–1	13–15	4(C)	0	SR–5	9–11	4(N)	0
SR–1	17–19	12	6(D)	SR–5	11–13	7(O)	0
SR–1	19–21	13	4	SR–9	7–9	5	0
SR–1	23–25	11	4	SR–9	15–17	1	0
SR–1	25–27	9	1	SR–12	3–5	6	0
SR–1	29–31	4	2	SR–12	7–9	3	0
SR–2	3–5	3(E)	0	SR–12	9–11	15(P)	0
SR–2	5–7	11(F)	0	SR–12	29–31	2	0
SR–2	7–9	1	0	SR–103	7–9	6(Q)	0
SR–2	11–13	9(G)	0	SR–103	11–13	14(R)	1(S)
SR–2	13–15	6(H)	0	SR–103	15–17	25(T)	21(U)
SR–2	15–17	16(I)	8(J)	SR–105	7–9	7	0
SR–2	17–19	2	2	SR–109	15–17	6(V)	0
SR–2	21–23	1	0	SR–109	17–19	5(W)	1
SR–2	27–29	2	1	SR–111	27–29	2	0
SR–3	3–5	1	0	SR–112	9–11	4	0
SR–3	9–11	2(K)	1	SR–112	13–15	10(X)	0

jected females being the parental and their daughters the first generation). The test progenies were obtained by mating single females from the respective cultures with two brothers where available, or with two males from other cultures of the same experimental series, or with males from the Recife-3 stock.

As shown in Table 2, the injected female SR-1 produced normal progenies, containing both males and females, for about 15 days from the beginning of egg laying. Table 4 shows that we tested 14 females from the brood produced between the 3rd and 5th days after injection, six females from the brood of the 5th to 7th days etc. Examination of the progenies of the 37 females tested through the 15th day showed normal proportions of daughters and sons. However, in the broods which the injected female SR-1 produced between the 17th and 31st days after injection there were 127 daughters and one son (Table 2). Of these daughters, 49 were tested (Table 4). These 49 progenies fall into two rather clearcut groups: 32 yielded essentially normal proportions of the sexes for totals of 1706 ♀ ♀ and 1406 ♂ ♂ ; 17 yielded unisexual progenies which totalled 1160 ♀ ♀ and 12 ♂ ♂ ; and only two cultures which might be doubtful yielding, respectively, 113 ♀ ♀ and 31 ♂ ♂, and 141 ♀ ♀ : 77 ♂ ♂.

The injected female SR-2 produced progenies showing a ratio of about 2♀♀ : 1♂ for the first 13 days following the injection and only unisexual progenies thereafter (Table 2). A total of 24 of her daughters produced before the 13th day, and 26 daughters produced thereafter were tested. Table 4 shows that the former yielded bisexual progenies, while 11 of the latter gave only daughters. Among the 16 tested females from the 15th - 17th brood, eight yielded no sons, two gave about equal numbers of females and males, and six produced twice or more times as many daughters as sons.

The injected females SR-3, SR-5, SR-9, SR-12, SR-105, SR-111, and SR-112 continued to produce apparently normal broods for as long as they remained alive and fertile (Table 2). Of a total of 91 of their daughters tested all but one yielded approximately normal proportions of the sexes (Table 4). The exceptional female, a daughter of SR-3, produced 16 daughters and no sons. The females SR-103 and SR-109 produced some, although small, unisexual broods toward the end of their reproductive lives. Table 4 shows that we were able to test 56 of their daughters and that 22 of these, all of them daughters of SR-103, yielded unisexual progenies. More precisely, the six females from the brood produced by SR-103 on the 7-9th days all gave normal progenies; of the 14 females from the brood on the 11–13th days, 11 gave normal progenies, one gave 69 ♀ ♀ and no ♂ ♂, another gave 134 ♀ ♀ : 14 ♂ ♂ and a third 92 ♀ ♀ : 9 ♂ ♂ ; of the 25 tested females from the brood on the 15–17th days, 21 gave few or no males and four gave ratios of 105 ♀ ♀ : 15 ♂ ♂, 194 ♀ ♀ : 38 ♂ ♂, 116 ♀ ♀ : 40 ♂ ♂, and 74 ♀ ♀ : 37 ♂ ♂ respectively.

The number of tested daughters from the control series was far fewer as it seemed more important to test for transmission in the injected series. We have records of tests of 37 females of the first generation of the control experiment (reported in Table 3) and have carried certain of these for a number of further

experiments. As expected all of these yielded progenies in which the proportions of the sexes closely approached 1:1.

Third and fourth generation progenies of the injected females

The data which have been presented show that the absence of males in the last broods produced by some of the injected females (Table 2) was not accidental. A part, but only a part, of the daughters in these unisexual broods yielded in turn unisexual, or near unisexual, progenies. The rule here is not as consistent as that for the original sex-ratio strain which says that daughters from unisexual broods always produce unisexual or near unisexual broods. In the present case some of the daughters from unisexual broods produce unisexual broods, others produce bisexual broods. Usually the females from bisexual broods of the injected females give bisexual progenies and their daughters continue to do so. There are some exceptions to this; for example, a single female from the bisexual brood produced by SR-3 between the 9th and 11th days following injection. This daughter (Table 4) produced a progeny consisting of 16 ♀♀ and no ♂♂. In most cultures there is no doubt as to whether a given progeny is unisexual or not: either no or very few sons are produced, or the ratio of the sexes approaches equality. Only a small minority of cultures show an intermediate situation and these have been included in Table 4 among the nonunisexual progenies.

It was clearly important to establish whether the proportions of the sexes became finally stabilized in further generations (third, fourth, and later) descended from the injected females. For this purpose some females were tested from the third generation broods marked by capital letters in the "Daughters Tested" columns in Table 4. The results of these tests for the third generation are presented in Table 5. The situation can be summarized as follows. The

TABLE 5

Third generation progenies of normal females injected with "sex-ratio" ooplasm. Numbers of females tested and numbers yielding unisexual progenies are shown. The letters designating the broods refer to the data reported in Table 4

Injected female	Second generation brood	Females tested	Unisexual progenies	Injected female	Second generation brood	Females tested	Unisexual progenies
SR–1	A	3	0	SR–3	M	3	0
SR–1	B	16	0	SR–5	N	15	0
SR–1	C	18	0	SR–5	0	24	0
SR–1	D	3	2	SR–12	P	27	0
SR–2	E	1	0	SR–103	Q	1	0
SR–2	F	24	0	SR–103	R	14	6
SR–2	G	46	3?	SR–103	S	1	1
SR–2	H	33	1?	SR–103	T	1	1
SR–2	I	26	1	SR–103	U	39	11
SR–2	J	30	9	SR–109	V	6	0
SR–3	K	5	4	SR–109	W	1	0
SR–3	L	32	3	SR–112	X	4	3

daughters from the normal-sexed broods (A,B,C,E,F,G,H,I,K,L,M,N,O,P,Q,R,-T,V,W, and X, see Table 4) produced in the third generation mostly, but not exclusively, broods with normal ratios of the sexes (Table 5). The exceptions, some of them marked in Table 5 by question marks, deserve careful consideration. Thus, females of the brood G derived from SR-2 produced three cultures with 13 ♀♀:2 ♂♂, 27 ♀♀:5 ♂♂, and 30 ♀♀:3 ♂♂. respectively. One female from brood H produced a culture with 41 ♀ ♀:4 ♂ ♂, and a female from brood I one with 84 ♀♀:0 ♂♂. Females from brood K produced cultures with 216 ♀♀:0 ♂♂, 247 ♀♀:0 ♂♂, and 194 ♀♀:11 ♂♂. Three of the females of brood L produced cultures consisting of 123 ♀♀:0 ♂♂, 119 ♀♀:0 ♂♂ and 205 ♀♀:0 ♂♂.

The daughters from the unisexual broods tested (D,J,S, and U in Table 4) produced in the third generation either unisexual or bisexual progenies (Table 5). In the cases of the larger numbers tested (J and U) the unisexual progenies amounted to nine out of 30 and 11 out of 39 respectively. Clearly the proportion of unisexual broods is much higher than in the majority of progenies derived from the bisexual broods, although in the case of brood X four out of five third generation progenies were unisexual.

The fourth generation, although tested on a smaller scale, seemed to show a better stabilization of the recently acquired "sex-ratio" condition. For instance, a culture of the third generation (of SR-2 provenience, a culture of brood J, Table 5) produced 61 females and no males. From among these three were crossed to males of the normal strain Recife-3. They produced a total of 469 females and no males. A strain was thus established which in each generation is maintained by crossing with males of the Recife-3 strain. At the time of writing this strain is in the fourteenth generation, counting from the injected female. While no exact counts of flies produced have been kept, it is pretty certain that the progenies have been unisexual or nearly so, for in no generation have any males (except those used as parents) been observed.

[As of March 1959, four transferred strains are still maintained with strong degrees of unisexuality (SR-2, SR-5, SR-12, and SR-103) on a Recife-3 background.]

From the brood U of SR-103 origin (see Table 5) 15 females from unisexual progenies were outcrossed to Recife-3 males. Their progenies (=fourth generation) aggregated 2507 females and no males. From another culture of brood U which yielded a third generation of 149 ♀♀:11 ♂♂ (a case of an intermediate ratio) three females were tested and yielded a fourth generation which was unisexual and totalled 465 ♀♀. From another culture with an intermediate ratio (125 ♀♀:25 ♂♂) four females were tested. These produced respectively the following progenies: 128 ♀♀:0 ♂♂, 128 ♀♀:1 ♂, 118 ♀♀:118 ♂♂, and 98 ♀♀:95 ♂♂.

Injections into adult males

It seemed desirable for several reasons to test the effects of the sex-ratio agent on adult males. Accordingly a small but adequate series of injections of ooplasm from abnormal eggs of the original strain of sex-ratio females into young males

of Recife-3 were made. The experiments were carried through with controls in precisely the same manner as those with females except that no progeny tests were made. The results which are presented in Table 6 demonstrate that the sex-ratio agent is lethal to adult as well as to embryonic male zygotes.

The conclusion is evident that the "sex-ratio" condition (=unisexual progenies) in *Drosophila willistoni* can be introduced by mechanical means into a strain of flies normally free from it, passed to further generations, and subsequently incorporated as a regular feature of the hereditary system of this strain. The normal method of transmission in the "sex-ratio" strains is through an agent which has so far proved to be limited to the female germ line and is transmitted through the egg from mother to daughters. This agent produces a cataclysmic disturbance of development in XY zygotes which is thus responsible for the appearance of unisexual progenies. Ooplasm from the degenerating XY eggs of females of the original "sex-ratio" strains provides a source of the agent which on introduction by injection into the abdomens of normal females becomes incorporated through "infection" into a germ line previously free from it.

TABLE 6

Effects of injection of "sex-ratio" ooplasm into adult males of Recife-3; and controls

Series	Ooplasmic donor	Host male	No. injected	No. surviving to:			
				24 hr.	48 hr.	72 hr.	96 hr.
Sex-ratio	3–6 hr. abnormal egg	Recife-3	11	4	1	0	..
Control	3–6 hr. unfertilized egg of Recife-3	Recife-3	10	10	10	10	10

DISCUSSION

That the infection is not 100 percent effective in all oogonia of both ovaries in all of the injected females is scarcely surprising. The remarkable thing is the comparative success of infection. The apparent inefficiency of artificial transfer compared with natural transmission in the original strains is perhaps to be expected. It may be due to any, or a combination, of several factors. In the first place, the phenotypic expression (with the possible exception of SR-2 in Table 2) of "sex-ratio" makes its appearance among the progeny of the injected females only after a latent or incubation period. This period of from 10–14 days represents about one half the maximum reproductive life of a female, and some females (SR-5, SR-9, and SR-108 in Table 2) ceased reproduction close to this time. Furthermore, although an attempt was made to control the quantity of ooplasm injected, and the site of the injection, there is no assurance that in each female the oogonia were exposed at the same time, or to the same quantity of the infective agent.

As another expression of inefficiency might be taken the fact that only a fraction of the unisexual broods of females continue to transmit the capacity for pro-

ducing such unisexual broods. In fact, a majority of females relapsed to the production in subsequent generations of normal bisexual progenies.

The extent to which the agent may be transmitted without phenotypic expression, as indicated by certain instances of females from bisexual broods (see Table 5) who subsequently produced unisexual progenies, remains to be fully examined. This is a point of considerable interest in relation to a feature of these experiments to be discussed below.

The decisive fact remains that in at least some of the lines of descent from the injected females the unisexual progenies become a trait which is inherited as faithfully, from the mother to her daughters, as it is in the original "sex-ratio" strain from which the transfers were made.

It is necessary now to discuss in some detail a feature of the above experiments which is due solely to chance. In a preliminary experiment (not presented here) we injected "sex-ratio" ooplasm into females of a number of different normal wild strains with some indications of success. Most of the females who survived more than a few days produced large numbers of eggs, a relatively small proportion of which showed any normal development, the majority resembling the degenerating abnormal or "unfertilizable" eggs commonly found among the eggs of the original "sex-ratio" strains. With this favorable indication the more extensive series of experiments reported in this paper were begun. At this time the most favorable culture for obtaining large numbers of virgin wild females proved to be one of the Recife-3 strain (derived from wild progenitors collected near the city of Recife, Brazil). Now it happens, as was demonstrated by the genetic studies of MALOGOLOWKIN (1958), that this strain is the *least favorable* for the perpetuation of the "sex-ratio" condition via the normal mode of transmission. The Recife-3 strain contains genes which disrupt the "sex-ratio" when females from a "sex-ratio" strain are repeatedly outcrossed to males of this strain. Indeed, an excellent therapeutic for the "sex-ratio" condition is to cross with Recife-3 males! Thus it is probable that much of the inefficiency and irregularity in expression and transmission which were encountered represent a direct reflection of this genotypic resistance. It is therefore likely that injection of "sex-ratio" ooplasm into females of the strains which were found (MALOGOLOWKIN 1958) to be genotypically more favorable for the perpetuation of the causative agent would result in easier "infection" and more complete transmission of the unisexual condition to the progenies of injected females. It might also lead to such high infections as not to allow for the production of any normal eggs, resulting in the laying of nothing but "unfertilizable" eggs. The preliminary experiments mentioned briefly above provide some evidence in this direction.

The possession of an unfavorable genotype by the strain utilized in the experiments strongly suggests that other factors than these which were discussed above also came into play in the course of the establishment of the unisexual strains which we were able to derive in the later generations. While the introduction to and maintenance of the "sex-ratio" agent in a genetically favorable environment may be regarded largely as a problem of physical or mechanical

factors, an equivalent introduction to and maintenance in an unfavorable, or even hostile, genetic environment certainly involves problems of adaptation as well. In our case the "sex-ratio" agent has either produced changes in the host's cytoplasmically transmitted materials, or it has itself undergone mutation and subsequent selection. Little is yet known about the ultimate nature of the agent except that it is submicroscopic, and we cannot say whether it may be a single type of particle or a mixed population in the original "sex-ratio" strain. Which of these possibilities may prove correct can scarcely be decided until further information becomes available concerning the agent and its mode of multiplication. The situation here may have a good deal in common with the cyclic nucleocytoplasmic interactions reported by SONNEBORN (1954) in Paramecium.

Available evidence indicates that in the original "sex-ratio" strain synchronization of multiplication of the agent with the other genetic materials of the host female is very close indeed. While occasional failure of the agent to become incorporated into an egg allows for development of exceptional males, the frequencies of these and of bisexual females is not influenced by temperature as shown by MALOGOLOWKIN (1958). It is quite possible that in the genotypically unfavorable strains the "sex-ratio" agent may be in strong competition with other particles normally present in the cytoplasm and in such an internal environment may be more sensitive to external agencies, such as temperature which MAGNI (1954) found to affect profoundly the "sex-ratio" condition in certain strains of *D. bifasciata* investigated by him.

It is not profitable at present to speculate extensively concerning the nature of the causative agent of "sex-ratio" in *D. willistoni*. While comparison with certain viruses suggests itself, it is more appropriate to limit our comparisons primarily to instances in Drosophila. The classical case of sensitivity to carbon dioxide discovered and studied by L'HERITIER and his collaborators (1946, 1947, 1951, 1955) provides some interesting analogies. L'HERITIER and DE SCOEUX (1947) demonstrated that the "genoid," or virus which causes the heightened sensitivity to CO_2 can be transmitted from generation via the egg (also male gametes) or, artificially by injection of hemolymph or certain other organs of sensitive strains into previously resistant ones. While the parallel with "sex-ratio" in *D. willistoni* is close in most respects there are some interesting differences e.g., CO_2 sensitivity can be transferred by injection of hemolymph and other organs, but indications are strongly that the "sex-ratio" agent is confined to the germ-line in females and is completely lethal in XY zygotes and adult males.

Another parallel, already discussed by MALOGOLOWKIN (1958) is the situation in *D. prosaltans* in which the "sex-ratio" strain discovered by CAVALCANTI and FALCÃO (1954) and analyzed by CAVALCANTI, FALCÃO and CASTRO (1957) is perpetuated, or disrupted, depending on the presence or absence of certain nuclear genes. The intriguing example of "sex-ratio" in *D. bifasciata* investigated by MAGNI (1953, 1954) while having some features in common with these differs from all of them, as mentioned above, in being profoundly affected by temperature. In turn these cases suggest resemblances to the well known "Killer"

trait in Paramecium worked out in the now classical studies of SONNEBORN (1943a, 1943b, 1951) and his collaborators (PREER 1948).

In the cases both of CO_2 sensitivity in *D. melanogaster* (L'HERITIER 1951, 1955) and the "Killer" character in *Paramecium aurelia* (DIPPEL 1950) mutant forms of the cytoplasmic particles are known and have been extensively studied. It seems not unlikely that mutant forms of the "sex-ratio" agent of *D. willistoni* may be distinguished sufficiently to employ them in the type of analysis which DIPPEL (1950) and HANSON (1956, 1957) have used in cases of mixed populations of the mutant forms of kappa.

The significance of these various cases, including that of "sex-ratio" in *D. willistoni*, lies in the contribution they make toward a clearer understanding of the interactions of the various elements of the genetic systems which are responsible for transmission and expression of the pattern of development from generation to generation.

SUMMARY

1. The "sex-ratio" condition, which has its origin in a single female of *D. willistoni* found in nature and subsequently maintained in a series of genetically derived strains, has been transferred by injection into adult females of a wild strain, Recife-3, of the same species previously free of this condition and producing normal proportions of the two sexes.

2. The unisexual progenies characterizing the "sex-ratio" strain arise through the action of an agent which is transmitted by the females to all their eggs and is lethal in all XY zygotes.

3. Ooplasm from the lethal XY zygotes provides the effective agent which when transferred into the abdomens of females of previously normal strains may give rise to intermediate or unisexual broods among their progeny.

4. The "sex-ratio" condition does not appear immediately in the progeny of injected females, but follows a latent period of 10–14 days (in those cases in which it is expressed) as demonstrated by analysis of successive two day broods.

5. The "sex-ratio" condition is not immediately stabilized in the host strain for many females from unisexual broods fail to transmit the condition and some produce intermediate progenies. In certain instances unisexual progenies made their appearance in subsequent generations from the daughters of bisexual progenies.

6. Stabilized unisexual strains derived from several of the injected females have been established and one has been maintained for 14 generations.

7. The wild strain, Recife-3, in which the artificially produced unisexual conditions have been established is genotypically unfavorable for the persistence of the "sex-ratio" agent indicating that adaptation, presumably involving mutation and selection of the agent, occurred during the course of stabilization.

8. The findings are discussed in relation to other cases of cytoplasmic inheritance.

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