INTERSPECIFIC TRANSFER OF THE "SEX-RATIO" CONDITION IN DROSOPHILA

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THE first case of abnormal sex ratio probably due to a cytoplasmic effect in Drosophila, was observed by BARROS (1949) in *D. mercatorum pararepleta* from Brazil. However, the author did not advance any explanation for it. From then on, cytoplasmically inherited "sex-ratio" conditions have been discovered and genetically studied by MAGNI (1952), CAVALCANTI and FALCÃO (1954), CARSON (1956), and MALOGOLOWKIN (1958, 1959). Later on MAGNI (1954) and MALOGOLOWKIN (1959) were able to "cure" the "sex-ratio" condition by exposing the flies to high temperature, and finally MALOGOLOWKIN and POULSON (1957) showed that the inherited abnormal "sex-ratio" condition can be transferred by injecting the ooplasm from abnormal eggs from "sex-ratio" females of *D. willistoni* to young females from a normal strain of the same species.

The present article reports experiments where the "sex-ratio" condition was transferred: (a) from "sex-ratio" females of *D. equinoxialis* to a normal strain of the same species; (b) from "sex-ratio" females of *D. equinoxialis* into a normal strain of *D. willistoni*. Also, it was observed that the incubation period for the same agent is different in different species.

MATERIAL AND METHODS

The "sex-ratio" strains used in the present investigation are those reported on by MALOGOLOWKIN and POULSON (1957) and MALOGOLOWKIN (1959).

One of the experiments (the transfer of the "sex-ratio" condition from *D. equinoxialis* into *D. willistoni*) was performed by ooplasm injection as reported by MALOGOLOWKIN and POULSON (1957), except that, in this case the control injection was also made with ooplasm from fertilized eggs 3–6-hours old. All the other experiments were performed with L'HERITIER's technique (L'HERITIER 1958) of injecting the supernatant of a macerate of flies. This method proved to be easier and more efficient, as reported by MALOGOLOWKIN (1960).

L'HERITIER's method consists in obtaining a macerate of 100 abnormal "sex-ratio" virgin females 3–20-days old in 1 cc of Waddington's modified Drosophila Ringer's solution, centrifuged for 15 minutes at 3,000–4,500 rpm. The supernatant, kept in ice, is injected in virgin females 3–7-days old, from normal "sex-ratio" strains lightly etherized. Control experiments were performed in the same way, except that the supernatant injected was from virgin females from the same normal strains used as recipients.

The recipient females were: (1) Females of *D. equinoxialis* from a normal

strain derived from a "sex-ratio" strain which originated from one female collected in 1957 in Puerto Rico by DR. W. B. HEED and sent to the laboratory of PROFESSOR TH. DOBZHANSKY at Columbia University, New York. This strain has been yielding normal broods for more than 50 generations. (2) Females of *D. willistoni* from a mutant strain containing a genetic marker ebony known to be located in the third chromosome, which came from the laboratory of PROFESSOR DOBZHANSKY. This strain has always yielded normal ratio of sexes.

The injected females were left overnight to recover in separate sterile 1/4 liter bottles, provided with a spoon containing fresh banana-agar-yeast mixture. Each injected female was then crossed to one or two males and kept in separate vials, containing fresh media, for 3–5 days. Thereafter they were transferred, without etherizing, to new vials, every second day. This procedure was continued throughout the life of each female, so that her entire progeny was obtained as a series of successive two-day broods starting 4–5 days after the injection.

Samples of daughters from each brood of injected females were taken in order to test the next generation.

Results of the second generation of injected females are given in Tables 2, 4, and 6. Each of the females tested was crossed either to her brothers, or to males from a normal strain, or to any other descendant from any other of the injected females.

Third and fourth generations of the "infected" females were made but are not described here. All unisexual progenies continued to yield unisexual broods while all the normal progenies yielded only normal broods.

Transfer of the "sex-ratio" condition in Drosophila equinoxialis: As shown in Table 1, from the 14 females of the normal strain of *D. equinoxialis* injected with the supernatant of the "sex-ratio" of the same species, eight stopped producing descendants before the 17th day after injection without having produced abnormal progenies. The six remaining females yielded progenies among which "sex-ratio" females were found (Table 2).

Interspecific transfer: Table 3 shows the results of the injection of the super-

TABLE 1

Sex ratio of the progeny of 14 D. equinoxialis *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females (HSR), and of 11* D. equinoxialis *females injected with the supernatant from* D. equinoxialis *normal females (HUN-Control)*

Days of oviposition after injection	HSR		HUN	
	Number of first generation flies	Percent males	Number of first generation flies	Percent males
1–9	1,649	49.36	1,263	49.24
9–13	594	48.98	333	45.04
13–17	177	55.35	166	53.61
17–19	13	25.00	4	....
19–21	1	....	21	47.61
21–41	66	6.06	243	49.38

TABLE 2

Numbers of unisexual progenies from daughters of D. equinoxialis *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	44	..
9–13	54	..
13–17	9	..
17–19	6	1
19–21	..	..
21–37	39	37

TABLE 3

Sex ratios in the progeny of 24 D. willistoni *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females (HSR) and of 15* D. willistoni *females injected with the supernatant from* D. equinoxialis *normal females (HUN-Control)*

Days of oviposition after injection	HSR		HUN	
	Number of first generation flies	Percent males	Number of first generation flies	Percent males
1–9	1,099	46.75	618	48.86
9–13	685	33.13	413	43.34
13–17	186	12.36	376	46.80
17–19	112	3.57	182	47.78
19–21	223	10.76	443	47.40
21–43	414	0	1,377	49.01

TABLE 4

Number of unisexual progenies from daughters of D. willistoni *females injected with the supernatant from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	48	..
9–13	114	16
13–17	55	34
17–19	49	52
19–21	58	48
21–37	114	108

natant from *D. equinoxialis* "sex-ratio" females into females of *D. willistoni* from a normal strain. From 24 females injected, eight stopped producing descendants before the ninth day, without having produced abnormal progenies. The 16 remaining females yielded progenies among which "sex-ratio" females were found (Table 4).

Transfer of "sex-ratio" condition from *D. equinoxialis* to *D. willistoni* was also obtained by injection of the ooplasm (Table 6), as already reported by MALOGOLOWKIN (1960). Nineteen flies were injected, but only three yielded progenies

among which the "sex-ratio" condition was found. This shows that the ooplasm injection is not as efficient as the supernatant injection.

Differences in incubation periods: It can be seen in Table 1 that the broods obtained from eggs laid by the injected females up to the 17th day after the injection gave a normal ratio of sexes (51 percent of males), while from then on there was a decrease in males (10.35 percent of males from the 17th to the 41st day). From the 107 tested daughters born from eggs laid up to the 17th day (Table 2) there were no unisexual progenies. These began to appear from eggs laid after the 19th day of oviposition. Therefore, it can be said that the incubation period is 17–19 days for *D. equinoxialis* injected with the supernatant of a "sex-ratio" strain of the same species. The incubation period for *D. willistoni* injected with the same supernatant as the one in *D. equinoxialis* can be said to be 9–13 days (Table 3 and 4). The same incubation period was found when the ooplasm instead of the supernatant was used (Tables 5 and 6). This is also in agreement with the incubation period previously found for *D. willistoni* injected with the ooplasm of "sex-ratio" females of the same species (MALOGOLOWKIN and POULSON 1957).

These results show that the incubation period for injected females of *D. equinoxialis* is greater than that for injected females of *D. willistoni*, and that the

TABLE 5

Sex ratios of the progeny of 19 D. willistoni *females injected with the ooplasm of eggs from* D. equinoxialis *"sex-ratio" females (SR) and of 11* D. willistoni *females injected with ooplasm of eggs from* D. equinoxialis *normal females (UN-Control)*

Days of oviposition after injection	SR		UN	
	Number of first generation flies	Percent males	Number of first generation flies	Percent males
1–9	1,192	47.03	191	47.64
9–13	1,191	47.18	651	48.69
13–17	845	49.70	272	54.77
17–19	539	48.79	166	40.35
19–21	204	46.56	...	.....
21–31	587	48.04	115	59.12

TABLE 6

Number of unisexual progenies from daughters of D. willistoni *females injected with the ooplasm of eggs from* D. equinoxialis *"sex-ratio" females*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	15	..
9–13	37	1
13–17	36	..
17–19	15	..
19–21	6	..
21–29	25	7

incubation period for *D. willistoni* did not vary when the "infective" agent came from the same or from a different species, or when the supernatant was injected instead of ooplasm.

SUMMARY

A normal strain of *D. equinoxialis* was "contaminated" with the "sex-ratio" factor from a "sex-ratio" strain of the same species by injection of a supernatant. The same "sex-ratio" factor from *D. equinoxialis* was successfuly transferred to *D. willistoni*, a closely related species, both by supernatant and by ooplasm injection. The latter one proved to be less efficient than the former.

The incubation period in *D. equinoxialis* is longer than in *D. willistoni*.

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