SEX-LIMITED INHERITANCE AND ABNORMAL SEX RATIO IN STRAINS OF THE HOUSEFLY[1]

TOSHIKI HIROYOSHI

Department of Genetics, Osaka University Medical School, Osaka, Japan

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MALES of the housefly (*Musca domestica* L.) causing sex-limited inheritance of the mutants on the second chromosome have frequently been observed in strains of North American origin (SULLIVAN 1958, 1961; MILANI and FRANCO 1959; HIROYOSHI 1960, 1961). TSUKAMOTO, BABA and HIRAGA (1961) also found similar males in a strain from Japan. When those males were crossed to females carrying recessive mutant markers on the second chromosome, the mutant characters failed to be recovered in males of the F_2 generation, and when heterozygous males derived from crosses between those abnormal males and the mutant females were backcrossed to the homozygous mutant females, only mutant-type females and wild-type males were segregated in the backcross progenies, but no mutant male nor wild-type female appeared. According to genetical analyses done by the above authors, the segregational abnormalities or sex-limited inheritance seemed to be caused by translocations of part or all of the Y chromosome to an autosome, that is to the second chromosome.

KERR (1960, 1961) reported a case of holandric inheritance of DDT-resistance in an Australian strain of the housefly. Assuming that the DDT-resistance gene was originally located on the second chromosome to which the Y chromosome was translocated, KERR's observation might be essentially the same as the findings mentioned above. Thus, the probable translocation of the Y chromosome to an autosome may be of world-wide occurrence in the housefly, and investigation of this subject seemed important even in connection with the study of insecticide resistance of the insect.

The present paper deals with further genetical analyses and cytogenetical observations on these peculiar housefly males, and also on abnormal sex-ratio characters occasionally associated with the sex-limited inheritance. Some aspects of the mechanism of sex-determination in the housefly brought up through this study are also discussed.

MATERIALS AND METHODS

Adult houseflies of stock and experimental cultures were fed with powdered milk, cube sugar and water given separately. Rearing cages were 16 × 16 × 16 cm for stock cultures and 10 × 10 × 12 cm for experimental cultures. Larvae were reared with a medium made from wheat bran, powdered fish meat, powdered mouse biscuit (product of Oriental Yeast Co., Ltd., Japan), water, and dry yeast (in the proportion 2:2:1:2: trace). Eggs were raised in open-mouth glass

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jars (13 cm high and 9 cm in diameter), in which up to 800 larvae grew satisfactorily. Breedings through whole developmental stages were carried out in a room regulated at 25°C.

Multi-recessive mutants *w bwb* (white-eyed and brown-body), *bwb ge ro* (brown-body, green- and rough-eyed), and *bwb ge ro pcv* (brown-body, green- and rough-eyed, posterior-cross-veinless) were frequently utilized as markers of the second chromosome. Descriptions of these mutant markers have already been made by HIROYOSHI (1960) or by TSUKAMOTO *et al.* (1961).

Various phenotypically wild-type strains were studied for sex-limited inheritance and also for abnormal sex ratios. Some of these strains had been maintained in the laboratory for many years, but others for only one or two generations after being collected from the field. Their origins are listed in Table 2, together with experimental results. It should be noted here that the two terms *wild* and *normal* are used in different meanings in this paper; that is, the term wild signifies the wild phenotype or the wild-type allele of mutant genes, for which the symbol + is used, but the term normal is used only to designate chromosomal normality.

Cytological studies reported in this paper were based on observations of mitotic nuclei of larval ganglion cells. A larva was operated upon in distilled water, and the whole cerebral ganglion, both dorsal and ventral portions, was taken out. After steeping in distilled water for a while, the ganglion was placed on a slide and stained with aceto-orcein (45 percent acetic acid); barely squashed materials under a cover-glass were used for observation. Preserved samples sealed with paraffin for several days were the best for observation, with dark stained chromosomes.

RESULTS

Occurrence of sex-limited inheritance and abnormal sex ratio: During the long course of our crossing experiments aimed to establish linkage relationships and to prepare linkage maps of the housefly, there occasionally occurred cases that deviated markedly from the 3:1 F_2 and 1:1 testcross ratios expected with autosomal recessive genes. The whole study reported here was initiated from such occasional findings.

Two examples of such abnormal segregations are shown in Table 1, together with results of control experiments. When flies from a normal strain named $Lab_{em\text{-}7\text{-}em}$ were crossed to the second-chromosome mutants, as the control experiment, their F_2- and testcross segregations were approximately as expected. Slight deviations were merely due to the reductions in the number of flies in the mutant classes. Since multiple mutants *bwb ge ro pcv* or *bwb ge ro* were used for the actual crosses, most of the offspring classified as *bwb* in the table were also associated with other mutant characters, *ge*, *ro* and/or *pcv*, and the low viability of mutant individuals was anticipated.

In contrast to these control experiments, when males from Furen and ND wild-type strains were crossed with mutant females, the segregations in the F_2 and the testcrosses departed absolutely from expectations. That is, no *bwb* male was recovered in the F_2's from the experiment with Furen males, and almost no *bwb* male appeared from the experiment with ND males (two exceptions were detected out of 15,107 offspring). Neither *bwb* males nor wild-type females appeared in the testcross progenies with Furen and ND males. On the other hand, the reciprocal crosses of these, that is, the crosses between the mutant males and Furen or ND females, did not deviate seriously from the normal 3:3:1:1 (+♀: +♂: mutant ♀: mutant ♂) or 1:1:1:1.

These results indicate that transmission of the abnormal segregation, or the

TABLE 1

F_2 tests and testcrosses between bwb* *mutant strain (second chromosomal recessive) and three wild-type strains*

Type of cross	F_2 segregation				Total	Expected segregation ratio	Sex ratio (♂/♀)
	+ ♀	+ ♂	*bwb* ♀	*bwb* ♂			
F_2 (*bwb* ♀♀ × $Lab_{em\text{-}7\text{-}em}$ ♂♂)	2590	2290	773	738	6391	3:3:1:1	0.90
F_2 (*bwb* ♀♀ × ND ♂♂)	3755	8168	3182	2	15107	3:3:1:1	1.18
F_2 (*bwb* ♀♀ × Furen ♂♂)	921	4079	748	0	5748	3:3:1:1	2.44
F_2 ($Lab_{em\text{-}7\text{-}em}$ ♀♀ × *bwb* ♂♂)	1999	1967	587	600	5153	3:3:1:1	0.99
F_2 (ND ♀♀ × *bwb* ♂♂)	3136	2972	727	672	7507	3:3:1:1	0.94
F_2 (Furen ♀♀ × *bwb* ♂♂)	2120	1990	699	689	5507	3:3:1:1	0.95
bwb ♀♀ × F_1 ♂♂ (*bwb* ♀♀ × $Lab_{em\text{-}7\text{-}em}$ ♂♂)†	380	327	353	350	1410	1:1:1:1	0.92
bwb ♀♀ × F_1 ♂♂ (*bwb* ♀♀ × ND ♂♂)	0	1224	889	0	2113	1:1:1:1	1.38
bwb ♀♀ × F_1 ♂♂ (*bwb* ♀♀ × Furen ♂♂)	0	2856	839	0	3695	1:1:1:1	3.40
bwb ♀♀ × F_1 ♂♂ ($Lab_{em\text{-}7\text{-}em}$ ♀♀ × *bwb* ♂♂)	1307	1245	1045	1120	4717	1:1:1:1	1.01
bwb ♀♀ × F_1 ♂♂ (ND ♀♀ × *bwb* ♂♂)	788	640	464	360	2252	1:1:1:1	0.80
bwb ♀♀ × F_1 ♂♂ (Furen ♀♀ × *bwb* ♂♂)	530	495	448	459	1932	1:1:1:1	0.98

* Multigenic mutant strains *bwb ge ro* or *bwb ge ro pcv* were used for the actual crossing experiments, but only the *bwb* character was traced and all the other markers were neglected.
† Several gynandromorph individuals appeared from this cross, but they are neglected in this table.

sex-limited inheritance of the second chromosomal mutants, was due to the males of Furen and ND strains, but not to the females. Neither males nor females of the mutant strains were responsible.

Another remarkable point which appears in the table concerns the sex ratio. The sex ratios of the progeny from the control fit 1 : 1 very closely, whereas sex ratios of progeny from the crosses with Furen males greatly exceed 1; the excess of males is greater than of females. Although ND males caused the sex-limited inheritance, the sex-ratio of their progeny did not deviate so much from 1 : 1. The results of further studies to reveal the cause of this abnormal sex ratio are discussed in a later section.

In order to examine how widely such abnormal males which cause the sex-limited inheritance of the second chromosomal mutant are distributed in housefly populations, various wild-type strains available or obtained from other institutes, and male flies collected from the field in some districts in Japan, were tested. Several single males from each wild-type strain were crossed to second-chromosomal recessive mutant females, such as *w*, *bwb*, or *ge*, and the F_2 were examined for recovery of the mutant phenotype in males. To date, males from a total of 25 different strains or field-collected populations have been tested. As shown in Table 2, two Japanese and three North American strains have proved to include abnormal males which cause sex-limited inheritance. Among them, three strains, Akashi, NH and OL, contained both normal and abnormal males in mixed state, while two others, Furen and ND, consisted of only abnormal males. The existence of abnormal males in so high a frequency in housefly populations was rather unexpected, and this was the main reason why the study of this peculiar phenomenon has been continued further.

Establishment of TY-strains: From results of the preliminary studies described above, an assumption was drawn that the segregational abnormalities or the sex-limited inheritance might be due to the translocation of the Y chromosome (on which the male-determining factor(s) was located) to the second chromosome in abnormal males. If the assumption is correct, backcrossing hybrid males between these abnormal males and the second-chromosomal mutant females to the homozygous mutant females should lead to the establishment of a new type of strains in which the male progeny would always be wild-type and the female would be mutant.

For the purpose of synthesizing, abnormal males from OL, ND and Furen strains were crossed to *bwb ge ro pcv* females, and the F_1 males were backcrossed to the homozygous mutant females. In this way, three sex-limited strains were successfully established as was expected. Female progeny were always *bwb*, *ge*, *ro* and *pcv*, but males were all wild-type. Likewise, males from the Akashi strain were crossed to *w bwb* mutant females, and the backcross of the F_1-males to the homozygous *w bwb* mutant females resulted in a special strain in which females were always *w* and *bwb*, but males were wild-type. Since males of these special strains were believed to be carrying the translocated Y chromosome to the second chromosome, these strains were named TY-OL, TY-ND, TY-Furen and TY-Akashi.

TABLE 2

A list of wild-type strains examined for the presence of abnormal males causing sex-limited inheritance of the second-chromosome mutant

Strain examined	Origin	Presence of abnormal males
Akashi	Japan	Yes
Furen	Japan	Yes
Hikawa	Japan	No
Hikone	Japan	No
Kamikochi	Japan	No
Kirishima*	Japan	No
Nichinan*	Japan	No
Ogura	Japan	No
Sakurai*	Japan	No
Sora	Japan	No
Tanegashima	Japan	No
Delhi	India	No
BELL (Bellflower)	U.S.A.	No
D+++ (Illinois)	U.S.A.	No
ILL (Illinois)	U.S.A.	No
LDD (Orland)	U.S.A.	No
$Lab_{em\text{-}7\text{-}em}$	U.S.A.	No
L-R	U.S.A.	No
ND (NAIDM)	U.S.A.	Yes
NH (NAIDM)	U.S.A.	Yes
OL (Orland)	U.S.A.	Yes
Fr-R	France	No
P-9	Switzerland	No
OK_1	Italy	No
203d	Denmark	No

* Males collected from the field were directly used for the examination.

The phenotypes and sex ratios of progeny of these special TY-strains have been checked periodically. These strains have usually kept producing only mutant-type females and wild-type males. However, during many generations, exceptional mutant-type males and wild-type females have been found in very low frequency. Analysis of the cause of such exceptional individuals in low frequency has not yet been completed.

The sex ratios from these TY-strains varied from generation to generation, and also from strain to strain, but, in general, the number of male progeny exceeded females. Extremely abnormal sex ratios were frequently found in the TY-Furen and TY-Akashi strains.

Analyses of abnormal sex ratios: The abnormal sex ratios found in the TY-strains were first thought simply to be due to viability differences, the viability of female progeny which carried *w-bwb* or *bwb-ge-ro-pcv*, possibly being lower than that of males, whose phenotype was always wild-type.

TABLE 3

Sex-ratios of F_1 offspring from crosses between normal-type females and single males from the TY-ND strain

Test number	Number of offspring Females	Males	Total	Sex ratio (♂/♀)	Chi-square (1:1)
1	166	135	301	0.81	3.2
2	151	128	279	0.85	1.9
3	208	179	387	0.86	2.2
4	148	136	284	0.92	0.5
5	572	532	1104	0.93	1.4
6	247	230	477	0.93	0.6
7	218	203	421	0.93	0.5
8	235	222	457	0.95	0.4
9	205	194	399	0.95	0.3
10	231	219	450	0.95	0.3
11	244	232	476	0.95	0.3
12	134	129	263	0.96	0.1
13	304	295	599	0.97	0.1
14	229	224	453	0.98	0.1
15	276	273	549	0.99	0.0
16	266	264	530	0.99	0.0
17	234	233	467	1.00	0.0
18	324	324	648	1.00	0.0
19	207	210	417	1.01	0.0
20	294	298	592	1.01	0.0
21	169	172	341	1.02	0.0
22	342	350	692	1.02	0.1
23	202	207	409	1.03	0.1
24	339	352	691	1.04	0.2
25	160	167	327	1.04	0.2
26	222	232	454	1.05	0.2
27	325	340	665	1.05	0.3
28	208	220	428	1.05	0.3
29	153	164	317	1.07	0.4
30	186	200	386	1.08	0.5
31	355	382	737	1.08	1.0
32	177	193	370	1.09	0.7
33	141	155	296	1.10	0.7
34	139	154	293	1.11	0.8
35	284	318	602	1.12	1.9
36	418	469	887	1.12	2.9
37	148	166	314	1.12	1.0
38	127	143	270	1.13	0.9
39	95	108	203	1.14	0.8
40	130	151	281	1.16	1.6
Pooled	9213	9303	18516	1.01	0.4

	Degrees of freedom	Chi-square	P	
Total	40	26.717	$0.95 > P > 0.90$	(nonsignificant)
Pooled	1	0.437	$0.70 > P > 0.50$	(nonsignificant)
Heterogeneity	39	26.280	$0.95 > P > 0.90$	(nonsignificant)

TABLE 4

Sex-ratios of F_1 offspring from crosses between normal-type females and single males from the TY-Furen strain

Test number	Number of offspring Females	Males	Total	Sex ratio (♂/♀)	Chi-square (1:1)	(2:1)
1	157	148	305	0.94	0.3	..
2	240	237	477	0.96	0.0	..
3	227	221	448	0.97	0.1	..
4	201	199	400	0.99	0.0	..
5	242	243	485	1.00	0.0	..
6	243	246	489	1.01	0.0	..
7	67	68	135	1.01	0.0	..
8	130	132	262	1.02	0.0	..
9	133	136	269	1.02	0.0	..
10	202	207	409	1.02	0.1	..
11	198	208	406	1.05	0.2	..
12	181	191	372	1.06	0.3	..
13	157	171	328	1.09	0.6	..
14	153	167	320	1.09	0.6	..
15	213	233	446	1.09	0.9	..
16	255	280	535	1.10	1.2	..
17	151	168	319	1.11	0.9	..
18	305	343	648	1.12	2.2	..
19	235	267	502	1.14	2.0	..
20	160	183	343	1.14	1.5	..
21	206	242	448	1.17	2.9	..
22	172	203	375	1.18	2.6	..
23	202	246	448	1.22	4.3*	..
24	82	101	183	1.23	2.0	..
25	170	213	383	1.25	4.8*	..
26	116	186	302	1.60	..	3.5
27	87	147	234	1.69	..	1.6
28	192	327	519	1.70	..	3.1
29	249	440	689	1.77	..	2.4
30	197	356	553	1.81	..	1.3
31	117	214	331	1.83	..	0.6
32	105	201	306	1.91	..	0.1
33	253	499	752	1.97	..	0.0
34	99	199	298	2.01	..	0.0
35	265	548	813	2.07	..	0.2
36	260	545	805	2.10	..	0.4
37	121	258	379	2.13	..	0.3
38	94	203	297	2.16	..	0.4
39	88	213	301	2.42	..	2.3
40	177	432	609	2.44	..	5.0*
Pooled						
(1–25)	4682	5053	9735	1.08	14.1	..
(25–40)	2420	4768	7188	1.97	..	0.4

	Degrees of freedom	Chi-square	P	
Total (#1–#25)	25	27.579	$0.50 > P > 0.30$	n.s.
Pooled	1	14.138	$0.001 > P$	**
Heterogeniety	24	13.441	$0.98 > P > 0.95$	n.s.

	Degrees of freedom	Chi-square	P	
Total (#26–#40)	15	21.726	$0.20 > P > 0.10$	n.s.
Pooled	1	0.360	$0.70 > P > 0.50$	n.s.
Heterogeniety	14	21.366	$0.10 > P > 0.05$	n.s.

n.s. = not significant. * = significant at 5 percent level. ** = significant at 1 percent level.

In order to test the above explanation tests of single males were carried out. Among many tests, detailed data only from experiments with TY-Furen and TY-ND males are shown in Tables 3 and 4. The sex ratio of the original TY-Furen strain deviated extremely from the normal 1:1 ratio, and that of TY-ND strain was less extreme. Single males from these TY-strains were outcrossed to females from a normal-type $Lab_{em\text{-}7\text{-}em}$ strain (approximately 20 females each for a single male), and the sex ratios of the offspring were examined. By this method, the effect of supposed differences in viability between the sexes could be neglected, because both the male and female offspring were the same, wild-type. Therefore, if the sex ratio of offspring from these single-male tests turned out to be normal, the abnormal sex ratios originally found in a series of TY-strains could be attributed to mere differences in viabilities of the sexes. Contrary to this, if the sex ratio still remained abnormal after the single-male tests, the disturbing cause must be other than viability.

As shown in Table 3, all 40 single-male tests of the TY-ND strain gave the normal 1:1 sex-ratio among the offspring. Deviations are nonsignificant, and the average sex ratio was 1.01.

On the other hand, the TY-Furen males examined gave offspring having a variety of sex ratios (δ/φ) ranging from 0.94 to 2.44 (Table 4). Among these 40 tests, however, the lower 25 cases did not deviate significantly from the normal 1:1, but the other 15 cases were significantly abnormal. Homogeneity tests showed that the former 25 cases agreed with a 1:1 sex-ratio, and the latter 15 cases agreed with a 2:1 ratio. These results indicate that there existed two kinds of males in the TY-Furen strain, the one producing a 1:1 sex ratio, and the other a 2:1 ratio, for a reason other than mere differences in viability between the sexes.

Although detailed data are not shown, single male tests of two other TY-strains, TY-OL and TY-Akashi, were also carried out. The result of tests with TY-OL males was similar to that of TY-ND males, producing only 1:1 ratio offspring, but the result with TY-Akashi males resembles that of TY-Furen, producing either 1:1 or 2:1 sex ratios.

Cytological studies on TY-strains: In an attempt to prove the supposed translocation of the Y chromosome to the second chromosome in the abnormal males, cytological studies were carried out. Trials to observe giant chromosomes in the salivary glands, the intestines and the Malpighian tubes had been made with various techniques widely used for the study of Drosophila, but well spread and satisfying figures were difficult to obtain in the housefly. Hence, all the effort has been devoted to observing chromosomes of mitotic nuclei of the larval ganglia.

The diploid chromosome number in the normal housefly is 12, consisting of five pairs of autosomes and a pair of sex-chromosomes, heterozygous in the male; that is, XX-type for the female and XY-type for the male (PERJE 1948). The sex chromosomes show heteropicnosis, and can clearly be distinguished from autosomes when metaphase cells are stained with aceto-orcein. The Y chromosome is much shorter than the X chromosome, and the two are easily distinguished from each other. Therefore, if the Y chromosome was in fact translocated to an autosome, it should easily be detected microscopically.

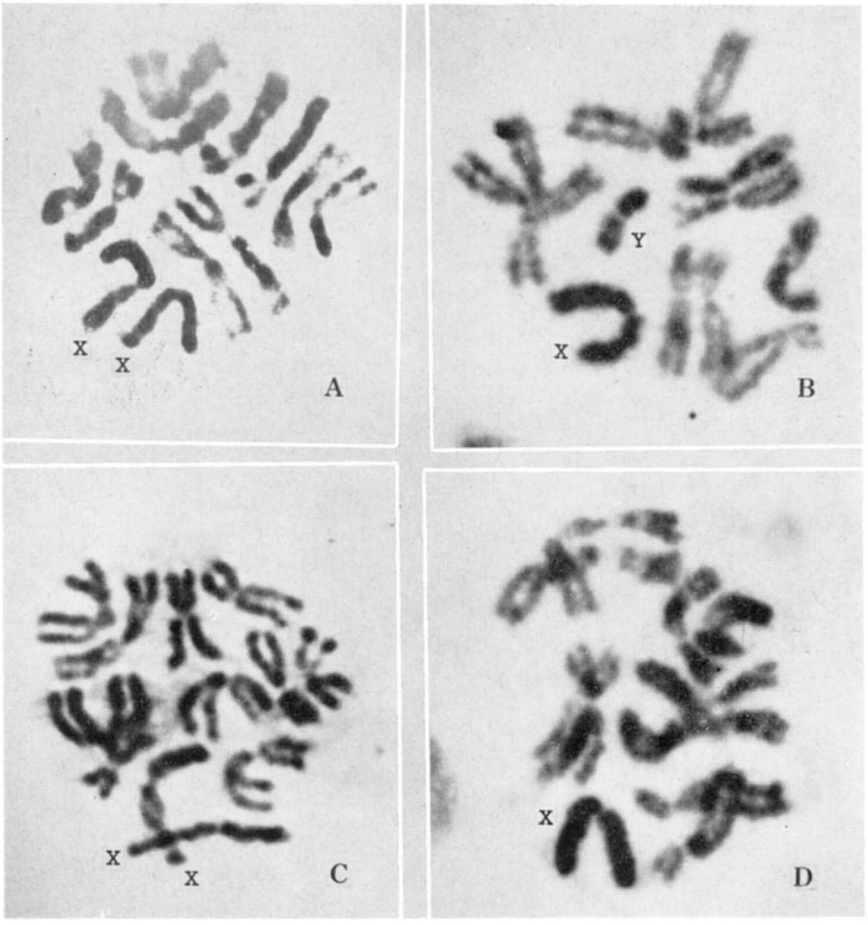

FIGURE 1.—Photographs of mitotic chromosomes in the larval ganglion of the housefly. A and B: the female and the male of a normal strain, C and D: males from TY-strains.

The female and male chromosomes of a normal strain $Lab_{em\text{-}7\text{-}em}$ are shown in Figures 1A and B. Although larvae of this strain could not be sexed, the one that possesses two X chromosomes of equal length is no doubt female, and the other, with one X and a short Y chromosome, is male.

Larvae of the TY-strains were sexed by means of the pigmentation of chitinous ring of the spiracle. The chitinous ring of male larvae of these strains was colored dark black, characteristic of wild-type larvae, while that of females was pale yellowish-brown, owing to a pleiotropic expression of *bwb* genes which were carried in homozygous condition by the female but not by the male.

In all female larvae of the TY-strains, two X chromosomes and five pairs of autosomes were detected, just as in the normal female.

On the other hand, the observation of male larvae of the TY-strains was somewhat fascinating. In TY-OL and TY-ND males, the free Y chromosome was never detected, as was expected, but neither was it evident that the Y was trans-

located to an autosome. Instead, two X chromosomes were observed in these male materials (Figure 1C). No Y chromosome, nor any indication of its translocation to another chromosome, was detected in males of the TY-Akashi and TY-Furen strains, either. But two kinds of males were found in these strains, one possessing two X-chromosomes and the other, only one X-chromosome (Figure 1D).

DISCUSSION

In order to explain both genetical and cytological findings on the sex-limited inheritance and the abnormal sex ratio of the housefly strains described above, the following hypotheses have been made: (1) In the housefly, the X chromosome plays no major part in sex-determination, and the Y chromosome carries the male determining factor(s). The male determinant(s) is located in a limited region of the Y chromosome, and the remainder of the Y chromosome is not concerned with sex-determination and is not essential for fertility and viability. (2) The Y chromosome, or a part of the Y chromosome on which the male determinant is supposed to be located, has some affinity for the second chromosome, and is occasionally translocated to it. The male determinant once translocated to the second chromosome is rarely separated from the chromosome to which it is translocated. The Y chromosome (or the male determinant) in such state is symbolized as Y′ in this paper. (3) At the nuclear division, the movement of Y′ (combined with the second chromosome) is independent from the X chromosome; that is, the Y′ does not necessarily segregate in repulsion with respect to the X. (4) The X and Y chromosomes each carry one viability factor (*V*), and at least two *V* factors are necessary for the fly to be viable. Consequently, no X0-type female exists. The *V* factor on the Y chromosome is tightly linked with the male determinant, or *V* may be the male determinant itself.

These hypotheses would provide the mechanisms whereby TY-strains produce only mutant-type females and wild-type males in either 2:1 or 1:1 ratios as shown schematically in Figures 2 and 3.

In male ancestors of the TY-strains, the elimination of a large part of the Y chromosome from the cell might have occurred, leaving a small fragment of the Y, in which the male determinant and the viability factor are included. The

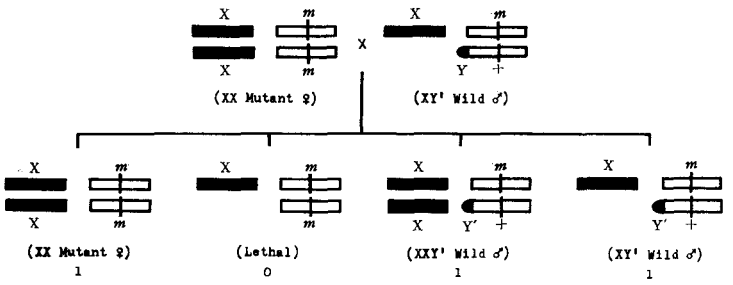

FIGURE 2.—Schematic explanation that the supposed XY′-type male in TY-strains of the housefly produces only mutant female and wild-type male offspring with a 1:2 sex ratio.

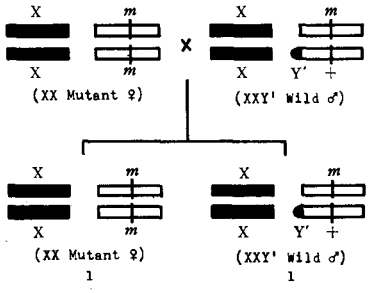

FIGURE 3.—Schematic explanation that the supposed XXY'-type male in TY-strains of the housefly produces only mutant-type female and wild-type male offspring with the normal 1:1 sex ratio.

small fragment of the Y chromosome might have been translocated to the second chromosome to form Y'. The Y' must be too small to be detected under a microscope with the technique used for our cytological studies of the TY-strain.

As shown in the Schemata, the XY'-type male in TY-strains, where only one X chromosome was observed but no Y chromosome, might produce offspring with a 2:1 sex ratio (1 XY'- and 1 XXY'-type wild males : 1 XX-type mutant female), and the XXY'-type male, where two X chromosomes were observed, might produce offspring with a 1:1 sex ratio (1 XXY' male : 1 XX female).

If the viability and the fertility of the XY'- and XXY'-type males are the same, XXY' males may increase very rapidly and soon outnumber the XY' types even in a population started with only XY' males, and the sex ratio of the population may change and approach the normal 1:1 generation by generation, as shown in Figure 4.

Experiments to confirm these theoretical changes in sex ratios and in frequencies of the XXY' males in a population started with XY' males are being carried on from the viewpoint of population genetics. If the theoretically predicted changes are proved to be correct, the presence of the XY'-type male in a population may mean that the translocation of the male determinant to an autosome (Y') has occurred relatively recently in that population. Thus, in Akashi and

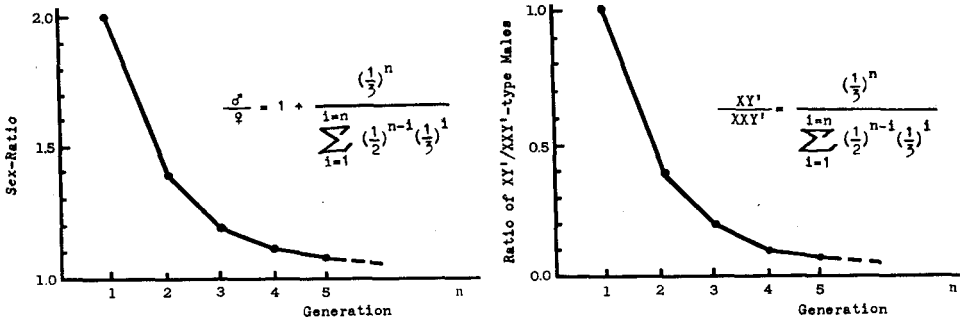

FIGURE 4.—Expected changes in the sex ratio and the frequency of XY'- and XXY'-type males in a housefly population started with only XY'-type males and the normal XX-type females.

Furen strains from which the TY-Akashi and TY-Furen strains were derived, the presence of the supposed XY′-type males was detected. Therefore, the translocation of the male determinant and the viability factor to the second chromosome might have occurred not so long before these strains were first examined. On the other hand, in the OL and ND strains from which the TY-OL and TY-ND strains were derived, only the supposed XXY′ males were found. Therefore, the translocation might have occurred many generations before they were first tested.

At any rate, the Y chromosome of the housefly seems to have an affinity for the second chromosome and to frequently be translocated to it, and there is a strong suspicion that, besides the normal XX-type females and XY-type males, a considerable number of XY′- and XXY′-type males are newly occurring and are mixed even in natural populations of the housefly.

As for the mechanism of sex-determination in organisms with the XY-type sex-chromosome constitution, several instances in which the Y-chromosome is supposed to be bearer of male determining factor (male-differentiator, or male-realiser) have recently been known, such as in the mouse, human being etc. More recently, ULLERICH (1963) demonstrated that unusual XXY-type individuals of *Phormia regina* (*Calliphoridae*, *Diptera*: more closely related to the housefly than is Drosophila) were male-like and X0-individuals were morphologically normal and fertile females, and concluded that the Y is carrier of male-differentiator. These facts may strongly support one of our major hypotheses, that the Y-chromosome of the housefly is bearer of the male determinant(s). No X0-type female, however, has yet been found in the housefly, but the indication was obtained that this type of female is lethal. Thus, the housefly seems interesting material for the study of sex-determining mechanisms of organisms.

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SUMMARY

Unusual males of the housefly, *Musca domestica* L., were found in five strains of North American and Japanese origin. These males cause sex-limited inheritance, and when they were crossed to females carrying recessive markers on the second chromosome, the mutant characters failed to be recovered in their male progeny. Male offspring from these abnormal males were frequently twice as numerous as female offspring.

Genetical and cytological analyses indicated that the sex-limited inheritance and the abnormal sex-ratio associated with it were due to translocation of a part of the Y chromosome, on which the male determinant(s) and the viability factor were supposedly located, to the second chromosome.

LITERATURE CITED

HIROYOSHI, T., 1960 Some new mutants and linkage groups of the housefly. J. Econ. Entomol. **53**: 985–990. —— 1961 The linkage map of the house fly, *Musca domestica* L. Genetics **46**: 1373–1380.

KERR, R. W., 1960 Sex-limited DDT-resistance in house flies. Nature **185:** 868. —— 1961 Inheritance of DDT-resistance involving the Y-chromosome in the housefly (*Musca domestica* L). Austral. J. Biol. Sci. **14:** 605–619.

MILANI, R., and M. G. FRANCO, 1959 Fertilita, rapporto-sessi e segregazione del mutante *bwb* in incroci tra ceppi geograficamente separati di *Musca domestica* L. Symposia Genet. **6:** 249–268.

PERJE, A. M., 1948 Studies on the spermatogenesis in *Musca domestica*. Hereditas **34:** 209–232.

SULLIVAN, R. L., 1958 Sex limitation of several loci in the housefly. (Abstr.) Proc. 10th Intern. Congr. Genet. **2:** 282. —— 1961 Linkage and sex limitation of several loci in the housefly. J. Heredity **52:** 282–286.

TSUKAMOTO, M., Y. BABA, and S. HIRAGA, 1961 Mutations and linkage groups in Japanese strains of the house fly. Japan. J. Genet. **36:** 168–174.

ULLERICH, F.-H., 1963 Geschlechtschromosomen und Geschlechtsbestimmung bei einigen Calliphorinen (Calliphoridae, Diptera). Chromosoma **14:** 45–110.