LINKAGE GROUP—KARYOTYPE CORRELATION IN THE HOUSE FLY DETERMINED BY CYTOLOGICAL ANALYSIS OF X-RAY INDUCED TRANSLOCATIONS

DALE E. WAGONER

Radiation Biology and Insect Genetics Section, Metabolism and Radiation Research Laboratory, Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota 58102

Received July 28, 1967

THE house fly, *Musca domestica* L., has a diploid chromosome number of 12 (STEVENS 1908) that includes five pairs of autosomes and a pair of sex chromosomes. Normally, the female contains an XX and the male an XY chromosome complement (PERJE 1948); however, abnormal cases of sex chromosome constitution with spontaneous origin have been reported (HIROYOSHI 1964; RUBINI 1964; RUBINI and FRANCO 1966). The gene mutations studied have been assigned to five linkage groups (Table 1) that are believed to correspond to the five pairs of autosomes since no sex-linked inheritance has been demonstrated. These linkage groups were given different Roman numeral designations by three groups of workers (HIROYOSHI 1960; MILANI 1961; TSUKAMOTO, BABA, and HIRAGA 1961). TSUKAMOTO's group has since adopted the nomenclature of HIROYOSHI (TSUKAMOTO and HIROYOSHI 1964) (Table 2). Also, karyotype analyses were performed by two other independent research groups (PERJE 1948; BOYES and NALOR 1962). The two groups numbered the karyotype in different ways (Figure 1). Attempts are now underway by some of these authors to resolve the nomenclatural differences.

If the genetic work performed on *Musca domestica* is to be unified, the individual linkage groups must be assigned to particular members of the karyotype by cytological analysis. This correlation has not been attempted in work thus far reported. The experiments reported here were made (1) to draw together the work already performed on linkage group studies by HIROYOSHI, MILANI, and TSUKAMOTO *et al.*, and on karyotype analyses by PERJE and by BOYES and NAYLOR; (2) to assign the five linkage groups to the particular member of the chromosome complement to which each corresponds; and (3) to thereby unify the nomenclature and the genetic knowledge already obtained. Cytological analysis of induced translocations was used to determine which markers are located on particular chromosomes of the karyotype.

MATERIALS AND METHODS[1]

House flies were reared at 26.7°C ± 2.0° on standard CSMA (Chemical Specialties Manufacturing Association) larval medium mixed with water. Adult flies were held at the same tem-

[1] Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.

TABLE 1

Mutant markers and linkage groups of the house fly compiled from the work of MILANI *(1954, 1961, and other papers);* TSUKAMOTO, OGAKI, *and* KOBOYASHI *(1957, and other papers);* HIROYOSHI *1960;* SULLIVAN *and* HIROYOSHI *(1960);* FRANCO *and* OPPENOORTH *(1962);* TSUKAMOTO *and* SUZUKI *(1964, 1966);* OGITA *and* KASAI *(1965);* OGITA *and* HIROYOSHI *(1965);* HOYER *(1966);* WAGONER *(unpublished); and others*

1	2	3	4	5
**ac*—ali-curve (curled wings)	*a*—ali-esterase (activity)	*bw*—brown (eye color)	*Amy-A*—Amylase-A (group isozymes)	*cy*—curly (wings)
acv—anterior-crossveinless	**ar*—aristapedia (leg-like antennae)	**bwb*—brown-body	*Amy-B*—Amylase-B (group isozymes)	*Lp*—Loop (wing vein looped)
av—abnormal-veins	*Atp*—Antennapedia (leg-like antennae)	*Bx*—Beadex (wing margin snipped)	*Aw*—Airplane-wing (abnormal shape and position)	**ocra*—ocra (eye color)
bp—black-puparium	*bu*—brunette (eye color)	*cp*—clump (dots at wing tip)	**ct*—cut (cut wing tips)	
ctc—countercoiled (genitalia coiled in opposite direction)	**car*—carnation (eye color)	*Cw*—Cleft-wing (notched wing tips)	*cyw*—curly-wing (mimics Rl and ac)	
do—dark-orange (eye color)	*clw*—classic-wing (wings extended from body)	*dv*—divergent (wings)	*dld*—dieldrin-resistance	
it—interrupted (wing vein)	**cm*—carmine (eye color)	*ge*—green (eye color)	*ext*—extended (wings)	
itu—interruptus (wing vein interrupted)	D_2 (resistance to diazinon)	*Iv*—Irregular-veins	**rb*—ruby (eye color)	
**Rl*—Rolled (wings curled)	*Dch*—Dehydrochlorinase (DDT resistance)	*kdr*—knockdown-resistance (DDT resistance)	**ye*—yellow (eye color)	
sb—subcostal (vein broken)	*oc*—ocelliless	*kdr-o*—knockdown-resistance-Orlando (DDT resistance)		
sht—short (short wings and legs)	*Phos.*—Phosphatase (isozymes)	*lv*—linked-veins (mimic of av)		
	ps—pigmented-sternites	*N*—Notch (notched wing margins)		
	R-DDT (resistance to DDT)	*pcv*—posterior-crossveinless		
	stw—stubby-wings (semi-vestigal wings)	*ro*—rough (roughened eye)		
	tw—twisted (front legs twisted)	*Sc*—Scalloped (wing margin)		
		Sn—Singed (bristles shortened)		
		tin—organo-tin (resistance)		
		w—white (eye color)		
		w^{au}—white-auburn (eye color)		

* Used for markers in translocation induction tests.

TABLE 2

Arbitrary numbering of linkage groups of the house fly by two independent research groups

Author	Sex chromosomes	Mutant markers representing five linkage groups				
		Rl	*car*	*bwb*	*rb*	*ocra*
HIROYOSHI	I	VI	V	II	IV	III
MILANI	I	III	V	II	VI	IV

perature and at a relative humidity of 55% ± 5%, and were provided with water and a dry diet consisting of three parts powdered milk, three parts sugar, and one part dry egg yolk.

X-ray treatments for induction of translocations were administered to adult males with a General Electric Maxitron X-ray machine at 300 kv, 20 ma, at a distance of 35 cm with a filtration of 1 mm Al and 1 mm Cu. The flies were irradiated in gelatine capsules at an approximate dose rate of 280r/min.

Three of the five linkage groups were chosen for the first section of the experiment. A stock of house flies bearing two mutant markers, *bwb* (brown—body color) and *ocra* (ocra—eye color), both homozygous recessives representing different linkage groups, was mated reciprocally to a second stock carrying the dominant marker, *Rl* (Rolled—wings), in heterozygous form (*Rl*/*Rl*+) which represents a third linkage group. Controls and a series of treatments were set up for each reciprocal cross. Four- to six-day-old males were irradiated with 2000 to 2750r of X rays in the treated series.

P_1 matings were made in mass, and F_1 *Rl*/*Rl*+ males heterozygous for the recessives *bwb* and *ocra* were used to test for the presence of translocations. The F_1 *Rl*/*Rl*+ males were crossed individually to homozygous *bwb*; *ocra* virgin females (backcross), and the F_2 progeny were scored for the presence of translocations between the linkage groups containing the *bwb*+ and *Rl* markers, the *ocra*+ and *Rl* markers, the *bwb*+, *ocra*+, and *Rl* markers, and the Y chromosome and *Rl* marker (when *Rl*/*Rl*+ males were treated). Cultures containing translocations were multiplied by making appropriate crosses; they were then held in stock form in the heterozygous state until enough larvae and flies were available for cytological analyses.

The second section of the experiment was similar to the first, but a fourth linkage group containing *car* (carnation—eye color) was tested with the *Rl* marker. Translocations were recovered between the *car*+ and *Rl* markers, the Y chromosome and *Rl* marker, and the Y

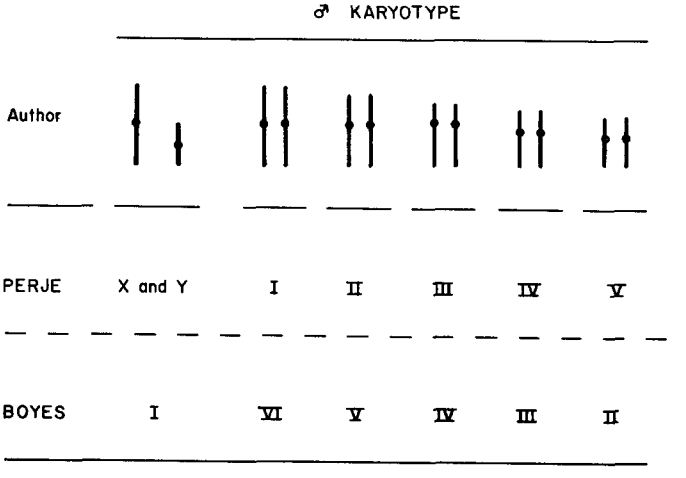


FIGURE 1.—Karyotype numbering of the house fly by two independent research groups.

chromosome and *car*+ marker; they were then multiplied for future cytological analyses.

In the third section of the experiment, the remaining linkage group containing *rb* (ruby—eye color) was tested with the linkage group containing the *Rl* marker. Translocations were recovered between the *rb*+ and *Rl* markers, the Y chromosome and *Rl* marker, and the Y chromosome and *rb*+ marker; these too were multiplied for future cytological analyses.

A representative diagram of the procedure used to recover translocations is given in Figure 2. The markers used in the diagram are taken from the second section of the experiment.

Theoretically, cytological analysis of the induced translocations from the three preceding sections of the work should provide the answer to the linkage group—karyotype relationship. However, to avoid any error in establishing this relationship because of the possibility of some chromosomal abnormality previously present in any of the stocks used in the foregoing work, we studied translocations induced in four additional crosses.

The following crosses were made in the same manner as the foregoing tests. (1) Fargo wild-type males were treated and crossed to a stock homozygous for the recessives, *car* and *ct* (cut—wing tips); translocations were recovered between the *car*+ and *ct*+ linkage groups, the Y chromosome and *car*+ marker, and the Y chromosome and *ct*+ marker. (2) Fargo wild-type males were treated and crossed to a stock homozygous for the recessives *bwb* and *ocra*; translocations were recovered between the *bwb*+ and *ocra*+ linkage groups, the Y chromosome and *bwb*+ marker, and the Y chromosome and *ocra*+ marker. (3) Fargo wild-type males were treated and crossed to a stock homozygous for the recessives, *cm* (carmine—eye color) and *bwb*; translocations were recovered between the *cm*+ and *bwb*+ linkage groups, the Y chromosome and *cm*+ marker, and the Y chromosome and *bwb*+ marker. (4) Fargo wild-type males were treated and crossed with a stock homozygous for the recessives, *ac* (ali-curve—curled wings), *ar* (aristapedia—leg-like antennae), and *ye* (yellow—eye color); translocations were recovered between the *ac*+ and *ar*+ linkage groups, the *ac*+ and *ye*+ linkage groups, the *ar*+ and *ye*+ linkage groups, the *ac*+, *ar*+, and *ye*+ linkage groups, the Y chromosome and *ac*+ marker, and the Y chromosome and *ar*+ marker.

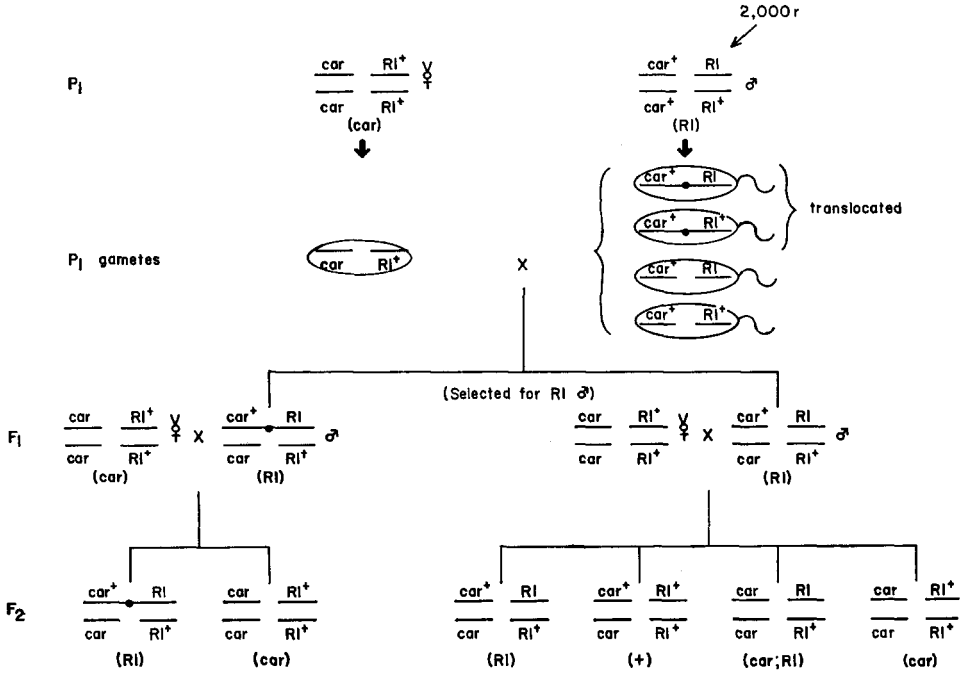


FIGURE 2.—Diagram of translocation recovery between two linkage groups in the house fly.

TABLE 3

Relative lengths and arm ratios of house fly autosomes using PERJE's *numbering system*

	PERJE's autosome numbering system				
	I	II	III	IV	V
Relative autosome lengths	1.5	1.4	1.25	1.1	1.0
Relative arm ratios	1 : 1.1	1 : 1.5	1 : 2.4	1 : 1.7	1 : 1.4

Y chromosome-autosome translocations were held in stock form by making brother-sister matings each generation. The Y chromosome-autosome translocations described by SULLIVAN (1958, 1961), MILANI and FRANCO (1959), TSUKAMOTO *et al.* (1961), KERR (1960, 1961), and HIROYOSHI (1964) have been hypothesized to involve a male-determining mechanism that spontaneously links to an autosome. Progeny receiving the translocated autosome are male; progeny receiving the nontranslocated autosome in Y-autosomal translocation stocks are female. This phenomenon has not yet been demonstrated cytologically and is sometimes accompanied by entire loss of the Y chromosome, at least in respect to cytological visibility (Orlando regular wild-type stock, personal observation), and by the presence of extra heterochromosomes (HIROYOSHI 1964; RUBINI 1964; RUBINI and FRANCO 1965).

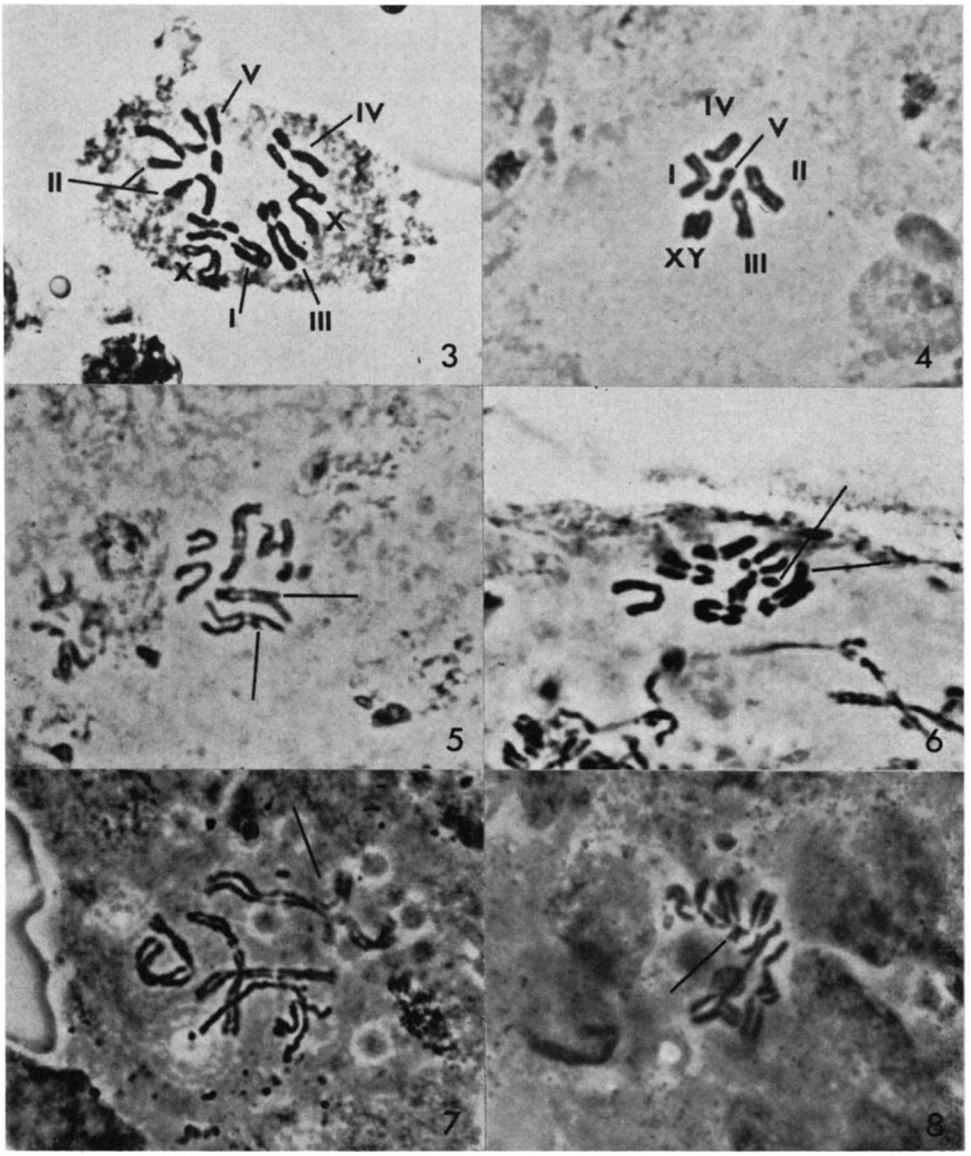
Translocations between autosomes (either marked with *Rl* and one or more recessive markers or with two or more recessive markers) had to be selected each generation to perpetuate them in heterozygous stock form by mating *Rl* or + males from translocation-containing stocks to virgin females homozygous for the recessive marker or markers used in the original P_1 cross. Males were used to avoid the loss or complication of the translocation by crossing over, shown to take place only in female house flies (HIROYOSHI 1960; WAGONER unpublished).

Cytological analysis consisted of microscopic examination of larval ganglia and spermatogonial cells in mitotic divisions and of first meiotic divisions in testes. Because of somatic pairing in Diptera, mitotic rather then meiotic cells were used for most of the analyses; meiotic chromosomes are much smaller and more difficult to interpret, especially in the house fly, because the morphological differences of some of the chromosomes are quite small (Table 3). Squashes of the tissues stained with lacto-aceto-orcein were sufficient for us to determine which chromosomal pairs were involved in mitotic and meiotic interpairing in induced translocations. The testes or ganglia were dissected in Belar's saline solution, cleaned, put into a drop of stain for 25 seconds, squashed under a coverslip, and sealed with paraffin. Only autosome-autosome translocations were used for final cytological analyses.

RESULTS AND DISCUSSION

In 3,865 tests, 177 translocations were recovered in the different irradiated series of the following types:

Linkage groups	No. of translocations recovered	Linkage groups	No. of translocations recovered	Linkage groups	No. of translocations recovered
bwb+-*Rl*	12	*car*+-*ct*+	12	Y-*bwb*+	2
ocra+-*Rl*	18	Y-*car*+	8	*ac*+-*ar*+	23
bwb+-*ocra*+-*Rl*	4	Y-*ct*+	3	*ac*+-*ye*+	12
car+-*Rl*	5	*bwb*+-*ocra*+	15	*ar*+-*ye*+	8
rb+-*Rl*	5	Y-*bwb*+	5	*ac*+-*ar*+-*ye*+	1
Y-*Rl*	21	Y-*ocra*+	3	Y-*ac*+	7
Y-*car*+	3	*cm*+-*bwb*+	6	Y-*ar*+	2
Y-*rb*+	1	Y-*cm*+	1	Y-*ye*+	0
				TOTAL	177



FIGURES 3–8. FIGURE 3.—Normal female house fly chromosomes seen in a larval ganglion cell taken from the Orlando regular wild-type stock, mitotic metaphase, ×1000. FIGURE 4.—Normal house fly chromosomes seen in metaphase of meiosis I in a testis cell, ×1200. FIGURE 5.—I–III translocation, arms of unequal length, spermatogonial metaphase, ×1300. FIGURE 6.—I–III translocation, arms of unequal length, spermatogonial metaphase, ×1500. FIGURE 7.—I–III translocation, spermatogonial prophase, ×1350. FIGURE 8.—I–III translocation, spermatogonial metaphase, ×1200.

The majority of translocations were sufficiently fertile to be multiplied and kept as stocks in heterozygous form. No translocations were recovered in any control series (684 tests). Reciprocal crosses showed no differences.

For our discussion, we will use PERJE's (1948) method of karyotype numbering (Figure 1) using X and Y to represent the sex chromosomes and Roman numerals I to V to represent the five pairs of autosomes, starting with the longest pair and ending with the shortest pair.

The normal chromosomes of a wild-type female house fly are shown in Figure 3. The normal karyotype which we observed agreed with the description of BOYES and NAYLOR (1962) concerning relative chromosome lengths and centromere positions (Table 3). Normal meiotic metaphase I is shown in Figure 4.

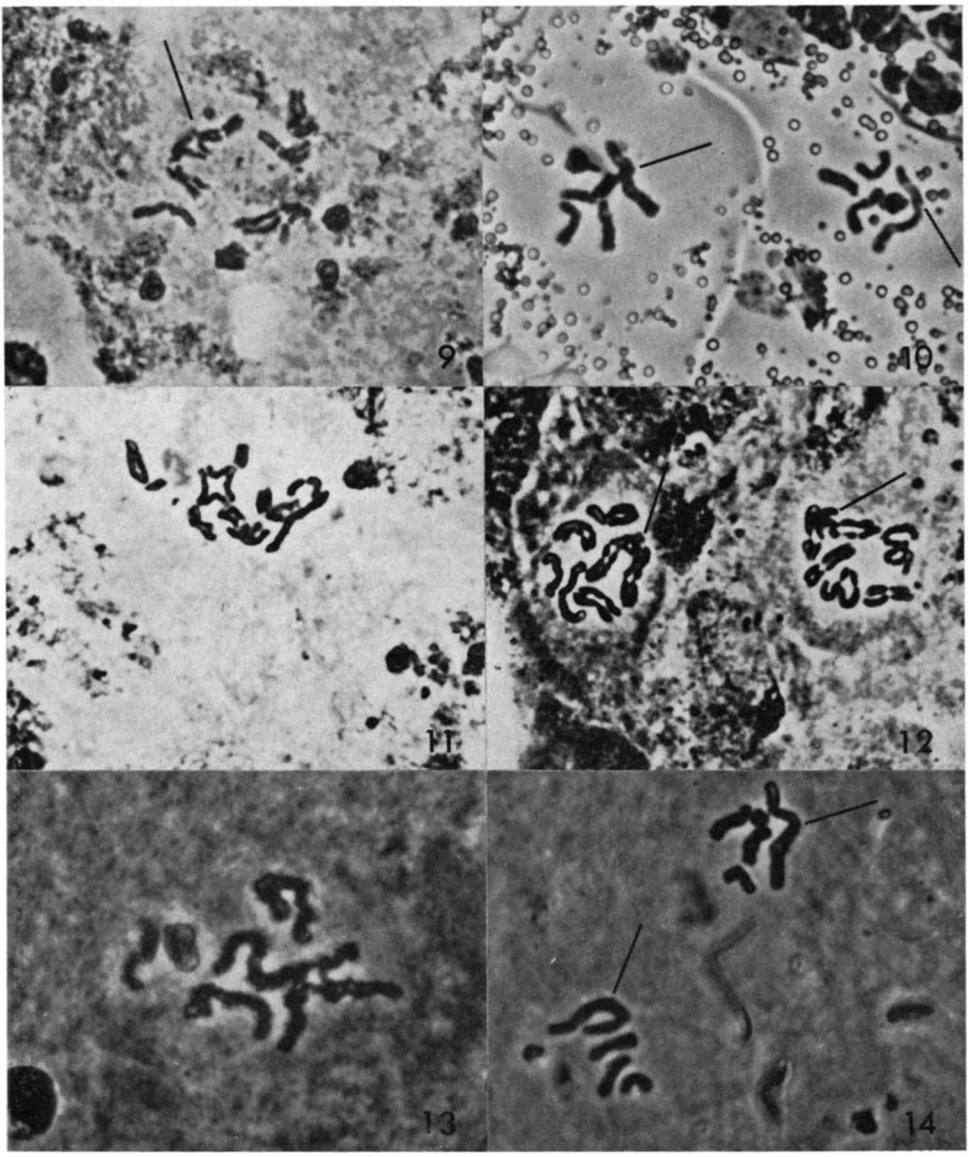
Cytological analyses of translocations recovered in the first section of the experiment showed that the *Rl* marker and the *bwb* marker were located on chromosomes I and III. The pairs were either joined in gonial metaphase or, if separate, had chromosome arms of unequal length in *Rl-bwb*$^+$ translocations (Figgures 5, 6, 7, 8). Analysis of translocations between the *Rl* and *ocra*$^+$ markers showed chromosome I and V joined in gonial metaphase and late prophase (Figures 9, 10, 11). Thus the *Rl* linkage group is located on chromosome I since this chromosome was involved in both *Rl-bwb*$^+$ and *Rl-ocra*$^+$ translocations. The *bwb*$^+$ marker must then have a position on chromosome III, and *ocra*$^+$ maintains a position on chromosome V.

Cytological results from the second section of the experiment showed chromosome II involved with chromosome I in *car*$^+$*-Rl* translocations (Figures 12, 13, and 14), so *car*$^+$ must maintain a position on chromosome II. When *rb*$^+$*-Rl* translocations were induced in the third section of the experiment, cytological analysis showed chromosome IV joined with chromosome I. Therefore, *rb*$^+$ is located on the remaining pair of autosomes, IV. Additional studies have shown that the *rb* phenotype is actually determined by recessive loci on two chromosomes. These data will be presented elsewhere.

Cytological analyses of the translocations induced in the four crosses where the Fargo wild-type strain was treated agreed with the linkage group——karyotype relationship established from analyses in the first three sections of the experiment.

Translocations between *car*$^+$ and *ct*$^+$ involved chromosomes II and IV (check Table 1 for linkage relationships); those between the *bwb*$^+$ and *ocra*$^+$ markers involved chromosomes III and V (Figures 15, 16); those between the *cm*$^+$ and *bwb*$^+$ markers involved chromosomes II and III (Figure 17); those between the *ac*$^+$ and *ar*$^+$ markers involved chromosomes I and II; those between *ac*$^+$ and *ye*$^+$ markers involved chromosomes I and IV; and those between the *ar*$^+$ and *ye*$^+$ markers involved chromosomes II and IV. Analysis of the translocation between the *ac*$^+$, *ar*$^+$, and *ye*$^+$ markers has not yet been completed.

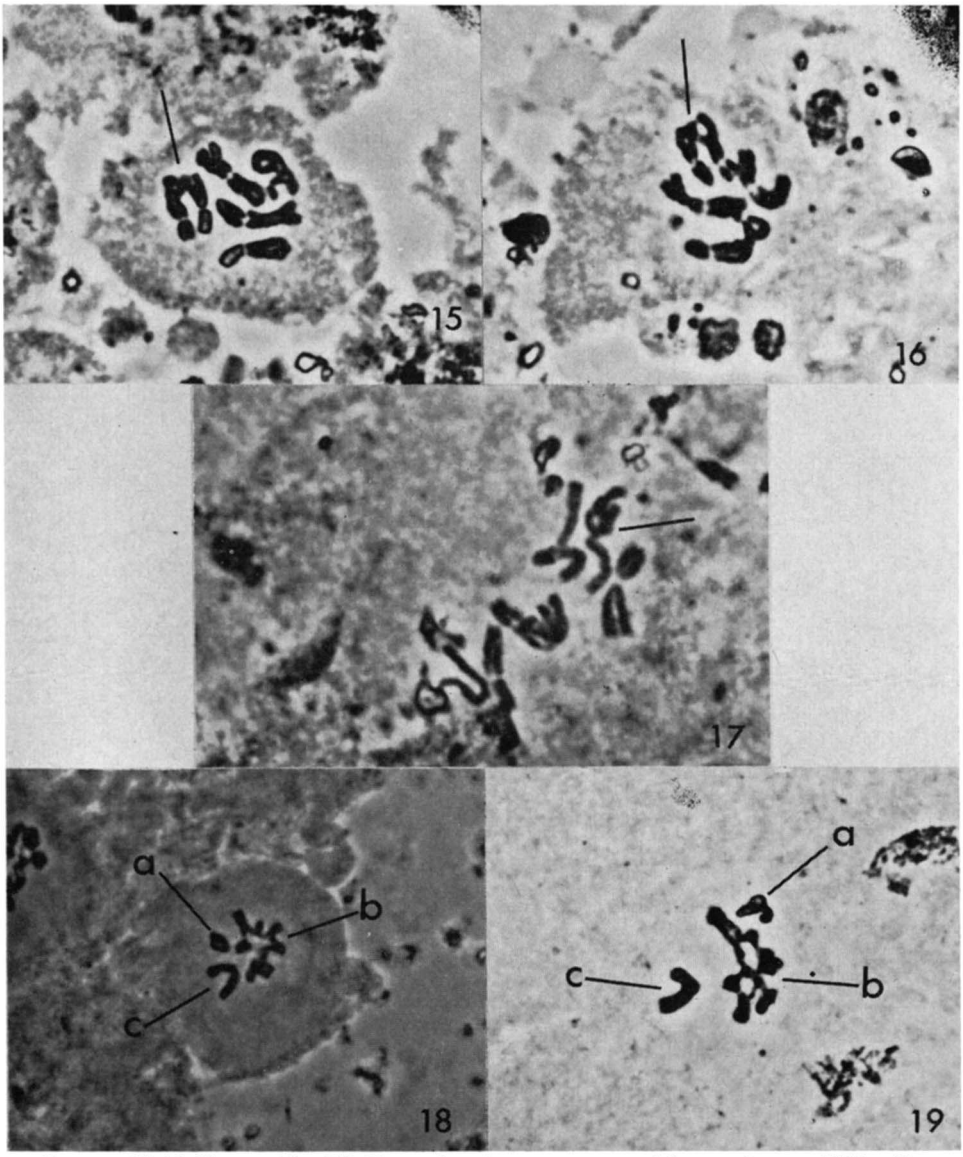
Five triple translocations and two quadruple translocations (Figures 18, 19) were recovered by cytological analysis from stocks in which two chromosome pairs were marked. In all cases, the additional chromosome pairs involved were



FIGURES 9–14. FIGURE 9.—I–V translocation, spermatogonial metaphase, ×1200. FIGURE 10.—I–V translocations, meiosis I metaphase, testis, ×1200. FIGURE 11.—I–V translocation, spermatogonial metaphase, ×1300. FIGURE 12.—I–II translocations, spermatogonial metaphase, ×1200. FIGURE 13.—I–II translocation, meiosis I prophase, testis, ×1550. FIGURE 14.—I–II translocations, meiosis I metaphase, testis, ×1200.

related to predicted linkage groups from cytological analysis after appropriate genetic crosses were made to test the predictions, further evidence supporting the correct assignment of linkage groups to particular members of the karyotype.

The nomenclatural differences can now be resolved. This is shown by combin-



FIGURES 15–19. FIGURE 15.—III–V translocation, spermatogonial metaphase, ×1550. FIGURE 16.—III–V translocation, spermatogonial metaphase, ×1550. FIGURE 17.—II–III translocation, spermatogonial metaphase, ×1550. FIGURE 18.—II–III–IV–V translocation, meiosis I metaphase, testis (a indicates the sex chromosomes; b indicates ring of 8; and c indicates chromosome I), ×1000. FIGURE 19.—I–III–IV–V translocation, meiosis I metaphase, testis (a indicates the sex chromosomes; b indicates ring of 8; and c indicates chromosome II), ×1200.

ing Table 2 and Figure 1 (Figure 20) with the new assignment of linkage groups added using PERJE's method of numbering the karyotype. Chromosome I contains *Rl* and *ac*; chromosome II, *car*, *cm*, and *ar*; chromosome III, *bwb*; chromosome IV, *rb*, *ct*, and *ye*; and chromosome V, *ocra*.

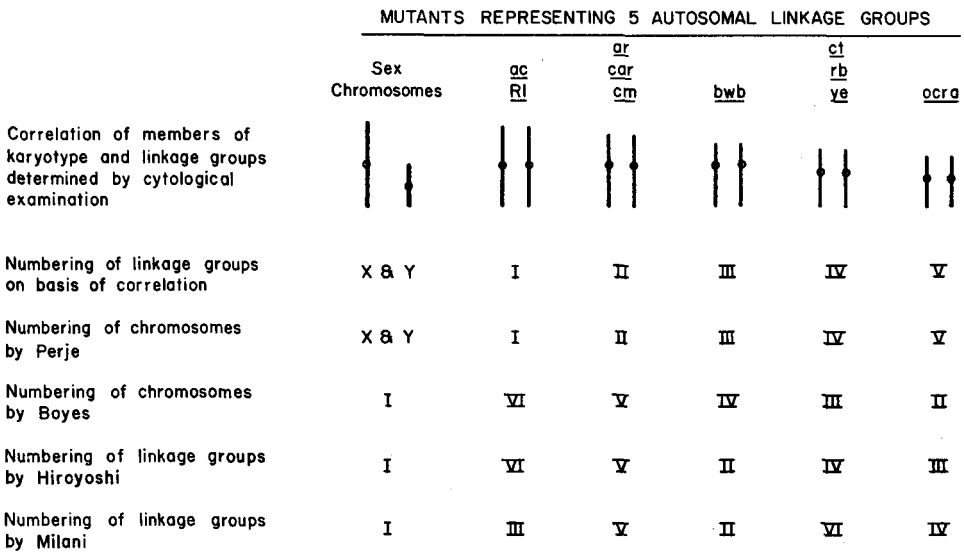


FIGURE 20.—Linkage group and karyotype numbering by four independent investigators and the linkage group—karyotype correlation determined by the cytological analysis of induced translocations.

I would like to acknowledge the excellent technical assistance of MR. CURTIS NICKEL and MR. ODELL JOHNSON of this Division during the progress of this research. I am indebted to DR. R. MILANI of the Istituto di Zoologie, Università di Pavia, Pavia, Italy, and DR. T. HIROYOSHI of the Department of Genetics, Osaka University Medical School, Osaka, Japan, for originally supplying some of the mutant stocks used in this research and for critically reviewing the manuscript. I would also like to thank DR. L. E. LACHANCE, DR. A. C. BARTLETT, and MR. R. HOYER, all of this Division, for their critical review and helpful comments in the preparation of this manuscript, MISS ANN LEVERICH and MRS. VIVIAN CHASKA of this Division for preparation of tables and figures, MISS ARLENE CONKLIN for cytological preparations, MR. GARY HILBER and MR. GENE SKAARE for stock maintenance, and MISS SANDY CVANCARA for photographic assistance.

SUMMARY

Cytological studies of induced translocations in the house fly, *Musca domestica* L., showed that the five linkage groups previously reported by other workers belong to the five pairs of autosomes also previously reported. No sex-linked mutants have been found thus far. The correlation of the linkage groups to particular chromosomes as seen cytologically had not been previously attempted and was the objective in this work. The new assignment of linkage groups to particular chromosomes (made by using PERJE's numbering of the house fly karyotype with X and Y representing the sex chromosomes and Roman numerals I to V representing the autosomal pairs starting with I as the longest pair and ending with V as the shortest pair) shows the *Rl* marker and its associated linkage group located on chromosome I, the *car* marker and its linkage group on chromosome II, the *bwb* marker and its linkage group on chromosome III, the *rb* marker and its linkage group on chromosome IV, and the *ocra* marker and its linkage group on chromosome V.

LITERATURE CITED

BOYES, J. W., and A. F. NALOR, 1962 Somatic chromosomes of higher diptera. VI. Allosome-autosome length relationships in *Musca domestica* L. Can. J. Zool. **40**: 777–784.

FRANCO, M. G., and F. J. OPPENOORTH, 1962 Genetical experiments on the gene for low aliesterase activity and organophosphate resistance in *Musca domestica* L. Entomol. Exptl. Appl. **5**: 119–123.

HIROYOSHI, T., 1960 Some new mutants and linkage groups of the house fly. J. Econ. Entomol. **53**: 985–990. —— 1964 Sex-limited inheritance and abnormal sex ratio in strains of the house fly. Genetics **50**: 373–385.

HOYER, R. F., 1966 Some new mutants of the housefly, *Musca domestica*, with notations of related phenomena. J. Econ. Entomol. **59**: 133–137.

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.). Australian J. Biol. Sci. **14**: 605–619.

MILANI, R., 1954 The genetics of the house fly. Preliminary note. Proc. 9th Intern. Congr. Genet. (Caryologia **6** Suppl. Vol): 791–796. —— 1961 Results of genetic research on *Musca domestica* L. Atti Assoc. Genet. Ital. **6**: 427–438.

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

OGITA, Z., and T. HIROYOSHI, 1965 Further genetical-biochemical study on amylase-isozymes in the house fly. Japan. J. Genet. **40**: 411–412.

OGITA, Z., and T. KASAI, 1965 Genetic control of multiple molecular forms of the acid phosphomonoesterases in the house fly, *Musca domestica*. Japan. J. Genet. **40**: 185–197.

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

RUBINI, P. G., 1964 Polimorfismo cromosomico in *Musca domestica* L. Bull. Zool. **31**: 679–693.

RUBINI, P. G., and M. G. FRANCO, 1966 Polymorphism and aneuploidy of the heterochromosomes in *Musca domestica* L. pp. 89–92. *Mutation in Population*. Edited by R. HONČARIV. Symp. Mutation Process, Prague, 1965. Academia, Praha.

STEVENS, N. M., 1908 A study of the germ cells of certain Diptera with reference to the heterochromosomes and the phenomenon of synapsis. J. Exptl. Zool. **5**: 359–374.

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

SULLIVAN, R. L., and T. HIROYOSHI, 1960 Preliminary report on mutations in the housefly. J. Econ. Entomol. **53**: 213–215.

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.

TSUKAMOTO, M., and T. HIROYOSHI, 1964 Re-examination on linkage groups of the housefly. (Abstr. in Japanese). Japan. J. Genet. **39**: 374.

TSUKAMOTO, M., M. OGAKI, and H. KOBAYASHI, 1957 Malaria control and the development of DDT resistant insects in Hikone City, Japan. Japan. Sanit. Zool. **8**: 118–122.

TSUKAMOTO, M., and R. SUZUKI, 1964 Genetic analyses of DDT-resistance in two strains of the housefly, *Musca domestica* L. Botyu-Kagaku **29**: 76–89. —— 1966 Genetic analyses of Diazinon-resistance in the house fly. Botyu-Kagaku **31**: 1–14.