AN ANALYSIS OF TRANSLOCATIONS IN THE MOUSE

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INTRODUCTION

R ADIATION-INDUCED translocations in the mouse have been studied by SNELL, BODEMANN, and HOLLANDER (1934), SNELL (1935, 1939, 1941a, 1941b), SNELL and PICKEN (1935), SNELL and AMES (1939), HERTWIG (1938, 1940), KOLLER and AUERBACH (1941), and KOLLER (1944). The results of SNELL and co-workers have been summarized elsewhere (1941a).

HERTWIG (1940) has made a very thorough and detailed investigation of prenatal mortality, litter size, and inheritance of semi-sterility in 11 out of a total of 60 semi-sterile lines derived from irradiated males. The prenatal mortality was determined from sections of the uteri and ovaries of 300 normal females mated to semi-sterile males and killed ten days or less post-coitus. The sectioned uteri and ovaries of 211 normal females×normal males served as controls. Embryos were classified as normal, dead embryos, large implantations (that is, large uterine swellings containing only the remains of an embryo), and small implantations. A corpus luteum count indicated the number of ova fertilized, or at least available for fertilization. In the semi-sterile group, the embryos showed 36.10 ± 0.91 percent survival (number of normal embryos×100/number of corpora lutea); in the control group the figure was 75.37 ± 0.99 percent. Hence the fertility of the test animals was slightly less than 50 percent of the fertility of the controls.

The fertility of the test animals may be calculated also from the size of litters carried to term. The mating of semi-steriles, both males and females, from 11 translocation stocks,×normals gave 1462 litters with a mean litter size of 3.12 ± 0.002. Comparable normal matings gave 790 litters with a mean litter size of 6.86 ± 0.064. The fertility of the translocation heterozygotes is, therefore, 45.48 percent that of normal mice. Again the fertility is reduced by slightly more than one-half. This is the average figure for the 11 different stocks. Taking the stocks separately, the fertilities for the different translocations each in terms of its control are: 41.8 percent, 42.9 percent, 44.2 percent, 45.2 percent, 45.7 percent, 46.3 percent, 48.1 percent, 49.1 percent, 50.6 percent, 52.2 percent, 58.7 percent. If these figures are broken down still further according to which parent is the heterozygote and to whether, in the case of male heterozygotes, the female is a sister or unrelated, there is seen to be a considerable lack of consistency in the data. Hence the significance of the individual percentages is somewhat open to question. Nevertheless, a tendency for at least some translocations in the mouse to cause a reduction in fertility to something less than 50 percent of normal is very strongly indicated.

The litter size for the mating translocation heterozygote×translocation heterozygote was also investigated and found to be 37.17 percent of normal (average figure for 11 stocks).

From the mating heterozygote×normal, 1031 young were raised and tested for semi-sterility. The expected proportion of semi-sterile offspring is one-half. The observed figures were considerably less than this—namely, 31.6 percent to 50.6 percent for the different stocks, with a mean for all stocks of 43.9 percent. The number of litters raised in testing individual mice for semi-sterility or normal fertility is not indicated, nor is the mean litter size taken as the dividing line between the two groups. As an explanation for this deficiency of translocation heterozygotes, HERTWIG suggests that they are subject to a greater prenatal mortality than are homozygous normals. The mortality of this class of zygotes is in turn assumed to explain the reduction of litter size, where one parent is a translocation heterozygote, to less than one-half normal.

An attempt to obtain translocation homozygotes from six of the semi-sterile stocks was successful in two cases and probably successful in a third. Since the number of animals tested in each case was quite limited, it cannot be concluded that the other three stocks were incapable of yielding homozygotes.

The embryological material presented by HERTWIG will be summarized in a later paper.

KOLLER (1944) made a cytological and genetical analysis of three radiation induced semi-sterile lines in mice. On the basis of rather limited data, the fertilities of the three lines are found to be: line A, 29.9–39 percent of normal; line B, 45.7 percent; line T, 43.6–46.5 percent. An interesting correlation is found between the fertility percentage and the types of chromosome configurations observable at first meiotic metaphase. These configurations are classified as: disjunctional ring-of-four, non-disjunctional ring-of-four, disjunctional chain-of-four, non-disjunctional chain-of-four, normal figure-of-eight ("the result of five crossovers, one of which has taken place in an interstitial segment—that is, in the chromosome region between a centromere and the point of exchange,—while the four others are formed in the interchange segments and in the short arms"), abnormal figure-of-eight (similar to a normal figure-of-eight but showing non-disjunction of the centromeres), association-of-three, unequal bivalent, univalent, non-analyzed. Line A shows a particularly high proportion of non-disjunctional rings- and chains-of-four, of abnormal figures-of-eight, and of univalents, all of which produce all or predominantly unbalanced gametes. KOLLER concludes: "Chromosomes of the mouse are too small and numerous to enable very detailed and accurate observations to be made. The data which have been obtained are sufficient, however, to show that the differences in fertility between the lines are correlated with the frequency of disjunctional co-orientation of chromosomes within the translocation ring and may also be influenced by differences in the frequency of associations-of-three and of univalents."

WALETZKY and OWEN (1942) have described a partially sterile condition which appeared spontaneously in a single male rat and was transmitted to half his offspring. Normal females mated to partially sterile males and killed before term showed the same types of dead and abnormal embryos that are found in translocation stocks in mice.

STOCKS AND NOMENCLATURE

Of a number of translocations induced by the X-irradiation of males, the X-irradiation of females, and the neutron-irradiation of males, six were selected for detailed study. These are: $T(5;8)a$ (previously called $T\text{-}F_1146$) from an X-irradiated male, $T(1;?)c$ (previously called $T\text{-}R_112$) from a neutron irradiated male, $T(2;?)d$ (previously called $T\text{-}R_158$) from a neutron-irradiated male, $T\text{-}F_1111$ and $T\text{-}F_1194$ from X-irradiated females, and $T\text{-}R_18$ from a neutron-irradiated male. These translocations originally were designated by $T\text{-}$ followed by the number of the mouse in which they appeared. More recently, where one or both of the chromosomes involved in a translocation has been identified, the original symbol has been replaced by the type of designation used in Drosophila (BRIDGES and BREHME 1944). The numbers in parenthesis are the numbers of the chromosomes (as assigned by the Committee on Mouse Genetics Nomenclature, DUNN, GRUNEBERG, and SNELL 1940) involved in the translocation. A question mark in place of one of the numbers indicates that one of the chromosomes is unknown. The final letter is the known gene closest to the break.

A word is in order in regard to the chromosomes designated 8 and 9. The highest number assigned by the Committee on Mouse Genetics Nomenclature was 7. DUNN and CASPARI (1942) described a new linkage group comprising the genes T, Ki, and Fu. HERTWIG (1942) described a new linkage group comprising the genes an (Anamie) and b (brown) and assigned it the number 8. WOOLLEY (1943) reported a linkage between m (misty) and b, and, because HERTWIG's paper had not reached this country, assigned it, at the writer's suggestion, the number 9, treating the T, Ki, Fu group as 8. It would now appear that the best usage was to call the an, b, m group, chromosome 8, and this usage will be followed here.

There is also confusion in regard to the symbol m. WOOLLEY (1941) used this symbol for the new mutant "misty"; in 1942 HERTWIG assigned it to the mutation "mikrophthalmus." Its usage to denote misty has priority, and we shall employ it for this gene.

The following multiple factor stocks were used in linkage tests: CA stock: $CaCa\ Fufu\ Ww$; P stock: $aa\ bb\ dse/dse\ pp$; V stock: $aa\ lnln\ ss\ wa\text{-}1wa\text{-}1\ vv$; W stock: $ff\ sh\text{-}2\ wa\text{-}2/sh\text{-}2\ wa\text{-}2$.

The P stock is also rr, but this locus was not used in the tests.

To test males for semi-sterility, matings were usually made to females of the B alb C stock. This stock, originally developed by MACDOWELL, is particularly suited to this purpose because of the consistent and uniform fertility of the females.

METHODS

Pregnant females were isolated in separate pens and examined, with occasional exceptions, daily. Litters were thus usually recorded within 24 hours of birth.

The classification of mice as semi-sterile or normal on the basis of the mean size of litters produced is usually quite clear but occasionally involves a con-

TABLE 1

Linkage tests with three translocations; backcross mice tested for semi-sterility.

TRANSLO-CATION	MUTATION		$++$	$x+$	$+T$	xT	TOTAL	CROSSOVER PERCENTAGE
$T(5;8)a$	a	repulsion	8	0	0	20	28	0.0
	b	coupling	6	16	20	6	48	25.0
	Ca	repulsion	4	5	8	4	21	38.1
	Ca	coupling	2	9	10	11	32	59.4
	f	coupling	6	6	6	2	20	40.0
	Fu	repulsion	2	7	7	5	21	33.3
	ln	coupling	3	4	6	8	21	52.4
	p	repulsion	5	3	10	10	28	46.4
	s	coupling	3	4	6	8	21	52.4
	$sh\text{-}2$	coupling	6	6	6	2	20	40.0
	v	coupling	5	2	10	4	21	42.8
	W	repulsion	6	3	7	5	21	52.4
	$wa\text{-}1$	coupling	5	1	7	8	21	61.9
	$wa\text{-}2$	coupling	6	6	5	3	20	45.0
$T(1;?)c$	a	coupling	7	9	7	8	31	48.4
	b	repulsion	5	5	3	2	15	53.3
	Ca	repulsion	8	7	1	5	21	61.9
	d	coupling	3	3	2	5	13	61.5
	f	coupling	6	2	7	4	19	52.6
	Fu	repulsion	8	7	0	5	20	65.0
	je^*	coupling	4	2	1	2	9	66.7
	je^*	F_2 coupling	51	21	19	4	95	41.3
	ln	coupling	4	0	5	3	12	61.7
	p	coupling	2	4	6	1	13	23.1
	Re^*	coupling	24	15	25	25	89	44.9
	Re^*	repulsion	16	12	24	10	62	41.9
	s	coupling	3	1	6	2	12	41.7
	$sh\text{-}2$	coupling	5	3	5	6	19	57.9
	Sp^*	repulsion	18	10	25	9	62	43.6
	v	coupling	4	0	6	2	12	50.0
	W	repulsion	6	9	3	2	20	40.0
	$wa\text{-}1$	coupling	2	2	4	4	12	50.0
	$wa\text{-}2$	coupling	2	6	5	6	19	42.1
$T(2;?)d$	a	coupling	3	3	0	5	11	72.7
	b^{**}	repulsion	6	6	2	2	16	50.0
	Ca	repulsion	2	3	3	1	9	33.3
	Ca^{**}	repulsion	11	9	7	7	34	47.0
	d	coupling	0	12	9	2	23	8.7
	$d\dagger$	coupling	3	4	22	5	34	23.5
	f	coupling	6	6	6	3	21	42.8
	Fu	repulsion	3	2	2	2	9	55.6
	ln	coupling	3	8	4	6	21	42.8
	p	coupling	3	9	5	6	23	39.1
	s	coupling	5	6	4	6	21	52.4
	$sh\text{-}2$	coupling	6	6	7	2	21	38.1

TABLE 1—*Continued*

TRANSLO-CATION	MUTATION		$++$	$x+$	$+T$	xT	TOTAL	CROSSOVER PERCENTAGE
	v	coupling	8	3	7	3	21	52.4
	W	repulsion	2	2	2	1	7	42.8
	wa-1	coupling	5	6	5	5	21	47.6
	wa-2	coupling	3	9	6	3	21	28.6
	wa-2**	coupling	14	12	10	13	49	44.9

* Presence or absence of $T(1;?)c$ in backcross (or F_2) animals was determined by presence or absence of c, which was segregating in this cross and with which $T(1;?)c$ is very closely linked, and not by tests for semi-sterility. Coupling is used to refer to the relationship in which $(T1;?)c$ and the gene under test entered the cross. When this gene was recessive (that is, je), the relationship with c was actually one of repulsion.

** Presence or absence of $T(2;?)d$ in backcross animals was determined (with the probability of some error) by the presence or absence of d, which was segregating in this cross and with which $T(2;?)d$ is linked, and not by tests for semi-sterility.

† These animals are from litters from which most dilute mice were discarded without testing for semi-sterility. The animals were less thoroughly tested than in the cross listed above so that there is greater chance of error in the classification.

siderable element of judgment. Males are more easily classified than females; the latter, even when genetically normal, occasionally throw a succession of small litters due to unknown causes. Four or more litters were usually raised from each mouse. In the case of important and doubtful males, 12 or more litters were sometimes raised. In uncertain cases, the age, general condition, presence of detrimental recessives, and probable hybrid vigor of females were taken into consideration as well as the mean litter size. Mice throwing litters averaging less than 4.6 were always classed as semi-sterile; mice throwing litters of more than 6, as normal; within the range 4.6 to 6 the element of judgment entered (table 9).

LINKAGES

For initial linkage tests, semi-sterile mice of the six translocation stocks were crossed to mice of the CA, P, V and WA stocks, and a backcross raised and tested for semi-sterility. About 20 backcross animals were usually tested. This gave tests for a large number of combinations (six translocations tested against 15 genes on 12–13 chromosomes) but yielded insufficient data for the detection of anything except close linkages. This method was selected because loose linkages would not lend themselves to further tests, whereas close linkages would do so. In the case of $T(1;?)c$, tests were also made with je, Re and Sp (Sp=splotch, a new dominant spotting mutation, W. L. Russell, unpublished).

In the case of the translocations T-F_1111, T-F_1194, and T-R_18 no clear linkages were found. T-R_18 showed only 25 percent crossing over (24 animals) with W, suggesting a loose linkage, but because T-R_18 showed a higher mean litter size than any of the other translocations, making it difficult to work with, this cross was not pursued further. Other crossover percentages ranged

from 42.1 to 70.0 in the case of T-F_1111, from 38.9 to 61.2 in the case of T-F_1194, and from 27.8 to 68.4 in the case of T-R_18. Since clear linkages were not found, these translocation stocks were discarded. (A linkage between T-F_1111 and wa-2 reported in a preliminary communication (SNELL 1941b) as probable but requiring more data was not substantiated by further tests.)

The other three translocations all proved to involve tagged chromosomes (table 1). $T(5;8)a$ gave no crossovers with a, 25 percent crossovers with b. $T(1;?)c$ gave 23.1 percent crossovers with p. $T(2;?)d$ gave 8.7 percent crossing over with d in one test, 23.5 percent in a second test.

Tests were next set up with translocations $T(5;8)a$ and $T(1;?)c$ involving the other known genes on chromosomes 5, 8, and 1.

The mating Pa a $T(5;8)a$ B/pa $A+b \times pa$ $a+b/pa$ $a+b$ gave the results shown in table 2. The crossover value between pa and a was 19.8 ± 3.8 percent for the female and 15.8 ± 3.3 percent for the male (table 5). This compares with normal values of 21.2 ± 2.7 percent and 19.6 ± 2.6 percent, respectively (ROBERTS and QUISENBERRY 1935). The crossover value between a and b was 31.0 ± 4.3 percent in the female, 25.3 ± 3.6 percent in the male. These genes show no linkage in the normal mouse.

TABLE 2

Segregants from the mating $+a$ $T(5;8)a+/pa++b \times pa$ a b/pa a b.

HETERO-ZYGOUS SEX	$+a+$	$pa+b$	$pa\ a+$	$++b$	$+a\ b$	$pa++$	$pa\ a\ b$	$+++$	TOTAL
Female	39	27	3	11	8	19	4	5	116
Male	48	42	8	11	16	17	2	2	146

The crosses listed in table 1 showed zero crossovers between a and semi-sterility in a total of 28 mice, establishing a as close to the break in chromosome 5. The female was the heterozygous parent in this case. Further data were obtained by testing for semi-sterility mice of the phenotypes $+a\ b$, $pa\ ++$, $pa\ a\ b$, and $+++$ from the cross discussed in the previous paragraph. Table 2 shows 73 mice in these four classes. Tests were completed on 53 of these, showing one male, No. T01255, to be a crossover of the genotype $pa\ A$ $T(5;8)a$ B/pa $a+b$. This male was from a mating in which the male was the heterozygous parent. He gave 12 litters averaging 4.3 young and transmitted semi-sterility to his descendants, the semi-sterility remaining associated with A except in the case of one daughter which was apparently a second crossover (one out of 11 possible cases). Allowing for the untested mice of the four classes named above, we may estimate a frequency of zero crossovers in 77 mice where the female was the heterozygous sex, one crossover in 114 mice where the male was the heterozygous sex. It should be noted that the above method of testing four out of the eight classes will detect all crossovers between a and $T(5;8)a$ only if a and pa lie on the same side of the break. If they lie on

opposite sides of the break, and if crossovers can occur simultaneously between both loci and the break, some, although presumably a minority, of the crossovers may have the phenotypes $pa\ a+$ and $++b$ and hence go undetected.

Additional data on the linkage of a and the break came from two matings, $pa\ a\ T(5;8)a\ B/pa\ A+B \times pa\ A+B/pa\ A+B$ and $Pa\ a\ T(5;8)a\ b/Pa\ A+b \times Pa\ A+b/Pa\ A+b$, designed primarily not to provide linkage data, but to provide mice for a cross to be described in a later section. The offspring were tested for the presence of a and, as a by-product of this and of other matings in which some of them were used, for semi-sterility. Thirteen tested offspring where the female was the heterozygous sex yielded one probable crossover; 139 tested offspring where the male was the heterozygous sex yielded two

TABLE 3

*Segregants from the mating ♀ $T(5;8)a++/+m\ b \times ♂ +m\ b/+m\ b$.**

$++$	$m\ b$	$T+b$	$+m+$	$T\ m+$	$++b$	$?+b$	$?\ m+$	TOTAL
85	87	4	6	o	1	1	1	185

* Only crossovers between m and b were tested for semi-sterility. The four tested mice classed as semi-sterile gave litters as follows: 3.5 (four litters), 4.5 (three), 4.8 (five), 5.3 (three). The corresponding figures for the seven normals: 5.7 (six), 6.5 (two), 6.6 (five), 7.5 (four), 8.0 (four), 8.3 (seven), 9.0 (three). One crossover was killed untested, one was sterile.

probable crossovers. Two of the three probable crossovers were further checked (and confirmed) by a test of their progeny. As previously noted, there were no crossovers in 28 mice in the original linkage test.

The totals for crossovers between a and the break are as follows: one in 124, or 0.8 percent, where the female was the heterozygous parent; four in 258, or 1.5 percent, where the male was the heterozygous parent. Four of these crossovers were checked by testing of progeny; one being unchecked is slightly open to question.

One more probable crossover, which may have occurred in either sex, was found among five tested aa mice from the cross of semi-sterile×semi-sterile discussed in a later section.

None of the crossovers serve to prove whether the break is between pa and a, or on the opposite side of a from pa. However, since on the latter assumption ♂ No. To1255 (see above) would be produced by a single crossover, whereas on the former two crossovers on adjacent arms would be required, there is perhaps a slight presumption in favor of the latter alternative.

The relation of misty (m), an eighth chromosome gene, to b and the break was determined by the two crosses listed in tables 3 and 4. These tables are self-explanatory. They show m to lie between b and the break, with 8.0 ± 1.9 percent crossing over between m and b in the female (normal value 7.1 percent, WOOLLEY 1945), and 21.4 ± 7.7 percent crossing over between a and m. The figures for the male are 6.7 ± 6.3 percent and 20.3 ± 10.4 percent. One double crossover was found.

TABLE 4

Segregants from the mating a $T(5;8)a++/++m\ b \times a+m\ b/a+m\ b$.

HETERO-ZYGOUS SEX	$a++$	$+m\ b$	$+++$	$a\ m\ b$	$+m+$	$a+b$	$a\ m+$	$++b$	TOTAL
Female	10	8	5	1	1	3	0	0	28
Male	7	4	2	1	0	1	0	0	15

The results of linkage tests with $T(5;8)a$ are summarized in table 5.

TABLE 5

Crossing over in chromosomes 5 and 8 in mice heterozygous for $T(5;8)a$.

HETEROZYGOUS SEX	CROSSOVER PERCENTAGE BETWEEN				
	pa AND a	a AND BREAK	a AND m	m AND b	a AND b*
Female	$19.8 \pm 3.7\%$	$0.8 \pm 0.8\%$	$21.4 \pm 7.7\%$	$8.0 \pm 1.9\%$	$31.0 \pm 4.3\%$
Female (normal)	$21.2 \pm 2.7\%$			$7.1 \pm 1.1\%$	
Male	$15.8 \pm 3.3\%$	$1.5 \pm 0.8\%$	$20.3 \pm 10.4\%$	$6.7 \pm 6.3\%$	$25.3 \pm 3.6\%$
Male (normal)	$19.6 \pm 2.6\%$			$8.2 \pm 3.5\%$	

* This column is calculated from data from crosses in which m was not included. In the crosses in which $T(5;8)a$, a, m and b, or $T(5;8)a$, m and b, were all included, there was one double crossover in 226 mice.

The backcrosses made with $T(1;?)c$ showed 23.1 percent crossing over between p and semi-sterility in a total of 13 mice (table 1). Additional data not summarized showed a very close linkage of c and semi-sterility. To analyze these linkage relations in detail, the following cross was made:

$$Sh\text{-}1\ c\ T(1;?)c\ P/sh\text{-}1\ c^{ch}+p \times sh\text{-}1\ c^{ch}+p/sh\text{-}1\ c^{ch}+p$$

Animals from this mating showing a crossover between $Sh\text{-}1$ and c or between c and P were saved for a test as to semi-sterility. Of six animals involving a crossover in the first named segment, tests were completed on all six. Of 56 animals involving a crossover in the second named segment, tests were completed on 45. In no case did semi-sterility separate from c. It may be added that in several other fairly extensive crosses, not designed primarily for

TABLE 6

Segregants from the mating $+c\ T(1;?)c+/sh\text{-}1\ c^{ch}+p \times sh\text{-}1\ c^{ch}\ p/sh\text{-}1\ c^{ch}p$.

HETEROZYGOUS SEX	$+c+$	$sh\text{-}1\ c^{ch}\ p$	$+c\ p$	$sh\text{-}1\ c^{ch}+$	$sh\text{-}1\ c+$	$+c^{ch}\ p$	TOTAL
Female	151	150	16	25	3	2	347
Male	46	31	8	7	1	0	93

linkage purposes, c has always remained associated with semi-sterility. The results of the test cross described above are summarized in tables 6 and 7, which are self-explanatory.

TABLE 7

Crossing over in chromosome 1 in mice heterozygous for $T(1;?)c$.

HETER-OZYGOUS SEX	p AND c	DIF.	P	c AND BREAK	c AND sh-1	DIF.	P	TOTAL NUMBER OF MICE
Female	$11.8 \pm 2.0\%$			0%	$1.4 \pm 1.1\%$			347
		-4.3	.033			-2.71	.017	
Female (normal)*	$16.10 \pm 0.503\%$				$4.11 \pm 0.291\%$			
Male	$16.1 \pm 3.4\%$			0%	$1.1 \pm 1.8\%$			93
		$+4.2$	.22			-1.94	.30	
Male (normal)*	$11.90 \pm 0.608\%$				$3.04 \pm 0.499\%$			

* Combined totals from GRÜNEBERG (1943).

No tests in addition to the backcross have been made with $T(2;?)d$.

The results of the linkage studies may be summarized as follows:

The crossing over in marked chromosome segments is reduced as compared with the normal value in all cases except two. One exception is the $p-c$ segment in the male of $T(1;?)c$, where the observed value is 16.1 ± 3.4 percent as compared with the normal value of 11.90 ± 0.608 percent (GRÜNEBERG 1943). The increase is not significant (P = .22). The decrease for the $c-sh$-1 segment in the female is significant (P = .017), and probably also that for the $p-c$ segment in the female (P = .033). The second segment showing an increased crossing over is the $m-b$ segment in the female of $T(5;8)a$. Again the increase is not significant. It is noteworthy that this is the only segment some distance removed from the break. This suggests that the translocation does not interfere with normal pairing in this arm, but the data are insufficient to establish this point beyond question. The reduced crossing over in other segments may be due to the mechanical action of the break rather than to reduced pairing.

The existence in $T(5;8)a$ of a linkage between the normally unlinked genes a and b is proof that a translocation has occurred.

SEMI-STERILE: NORMAL RATIO

The mating semi-sterile×normal, according to theory, should give a ratio of one semi-sterile to one normal (in addition to non-viable embryos). HERTWIG (1940), as stated in the introduction, found evidence for a deficiency of semi-sterile mice. Her evidence is derived from the mean-litter-size test for semi-sterility. Evidence from this source, no matter how much care is used in gathering it, is open to the objection that there is a region of overlap in the mean size of litters produced by semi-sterile and normal mice (see, for example, table 9).

TABLE 8

Ratio of semi-steriles to normals from mating $T/+ \times +/+$ as determined by linked genes.

MATING	MARKER GENES	OBSERVED RATIO	UNCLASSIFIED	PERCENTAGE SEMI-STERILE (OR CROSSOVERS)
$+a\ T(5;8)a+/pa++b \times pa\ a+b/pa\ a+b$	$pa:Pa$†	140:145	3	50.9±3.0
Same	$A:a$	134:128	26	
$a\ T(5;8)a/++ \times a+/a+$	$A:a$	28:43	5	50.7±2.3
$a\ T(5;8)a/++ \times ++/++$ fully tested*	$A:a$	74:68	159	
Same test incomplete*	$A:a$	4:7		
$T(5;8)a+/+m \times +m/+m$	$m:M$	109:119	5	52.2±3.3
$+c\ T(1;?)c+/sh\text{-}1\ c^{ch}+p \times sh\text{-}1\ c^{ch}\ p/sh\text{-}1\ c^{ch}\ p$	$p:P$†	214:241	10	53.0±2.3
Same	$c^{ch}:c$	215:225	25	52.3±2.1
$c\ T(1;?)c/c^{ch}+ \times c^{ch}+/c^{ch}+$	$c^{ch}:c$	67:84	4	

† With a few exceptions, pregnant females were checked daily and newborn litters classified as to eye color.

* Offspring from this cross were phenotypically agouti and had to be classified as AA or Aa by a genetic test. The test consisted of mating to aa mice; five A young were regarded as proof that the parent was AA. A few mice giving four A young, or proven semi-sterile or normal by litter size test only, are classified as "test incomplete."

The establishment of genes linked with $T(5;8)a$ and $T(1;?)c$ made it possible to use genetic markers to establish the frequency of the two classes. The genes a and c, because of their close linkage with the breaks in the two translocations, are particularly suitable for this purpose. The genes pa, m, and p can also be used. These markers have the disadvantage, as compared with a and c, that they are farther from the breaks; the first and third have the advantage that, in the crosses used, they could be identified in new-born animals. It will be seen from table 8 that in every case there was a slight but not significant excess of the gene that entered the cross with semi-sterility. There is no evidence for a departure from the expected 1:1 ratio.

PERCENTAGE FERTILITY OF SEMI-STERILES

KOLLER (1944) has summarized much of the literature dealing with the fertility of translocation heterozygotes in different species. No further summary will be given here. Suffice it to say that in many cases, within the limits of statistical error, there is just 50 percent reduction in fertility. In some species there is much less than 50 percent reduction in fertility; in certain cases there is more than 50 percent reduction.

As the measure of fertility of translocation heterozygotes in mice we have used the "percentage fertility," defined as the mean litter size of semi-sterile mice, divided by the mean litter size of a comparable group of normal mice, times 100. The data in regard to the percentage fertility of the six translocation stocks are summarized in tables 10 and 11. The values range, for semi-

TABLE 9

Frequency distribution of backcross animals with respect to the mean sizes of their litters.

	TOTAL	1.1–1.5	1.6–2	2.1–2.5	2.6–3	3.1–3.5	3.6–4	4.1–4.5	4.6–5	5.1–5.5	5.6–6	6.1–6.5	6.6–7	7.1–7.5	7.6–8	8.1–8.5	8.6–9	9.1–9.5	9.6–10	10.1–10.5	10.6–11	11.1–11.5	11.6–12	12.1–12.5	12.6–13	13.1–13.5
T(5;8)a normal	53									1	3	3	10	2	7	7	7	6	2	1	4					
semi-sterile	60		1	0	7	5	12	13	12	8	2															
T-F₁III normal	43										1	3	3	6	7	6	9	3	1	2		1		1		
semi-sterile	54		2	7	11	16	9	3	4	0																
T-F₁194 normal	35								2	4	0	5	5	5	6	2	1	2					1		1	1
semi-sterile	40			5	11	10	10	2	1	1																
T-R₁8 normal	37										2	5	6	4	5	5	2	5	3							
semi-sterile	43			1	3	2	6	4	8	11	8															
T(1;?)c normal	43										2	3	4	8	9	5	2	5	3	2						
semi-sterile	40	1	2	4	11	4	6	7	2	3																
T(2;?)d normal	40										1	2	5	9	6	7	4	1	2	2			1			
semi-sterile	34		1	1	6	7	12	2	2	3																

TABLE 10

Percentage fertility of semi-sterile mice from six translocation stocks.

CROSS NUMBER*	TRANSLOCATION	FEMALES					MALES				
		NORMAL		SEMI-STERILE		PERCENTAGE FERTILITY**	NORMAL		SEMI-STERILE		PERCENTAGE FERTILITY**
		NO. OF LITTERS	MEAN LITTER SIZE	NO. OF LITTERS	MEAN LITTER SIZE		NO. OF LITTERS	MEAN LITTER SIZE	NO. OF LITTERS	MEAN LITTER SIZE	
I	$T\text{-}F_1194$	30	9.37±.53	79	3.61±.19	38.4±2.9	125	7.22±.20	126	3.04±.13	41.3±1.8
I	$T\text{-}F_1111$	26	10.11±.30	42	4.33±.31	42.9±3.3	77	7.51±.25	108	3.31±.14	45.0±1.9
I	$T(2;?)d$	131	8.66±.19	100	4.25±.16	49.1±2.1	176	7.48±.17	93	3.48±.18	47.3±2.5
I	$T(1;?)c$	102	7.84±.21	82	3.51±.17	44.7±2.6	220	7.29±.15	117	3.88±.17	52.7±2.4
2		65	7.74±.28	187	3.52±.11	45.4±2.2	108	7.24±.22	127	3.84±.15	53.0±2.6
I	$T(5;8)a$	174	8.34±.15	168	4.20±.14	50.4±1.9	190	7.39±.17	253	4.55±.12	61.8±1.7
3		50	7.20±.29	46	3.33±.23	46.2±3.7	82	7.83±.43	56	4.68±.44	59.8±6.5
4		89	6.69±.19	85	3.38±.15	50.5±2.7	32	6.56±.35	104	3.90±.14	59.5±3.8
I	$T\text{-}R_18$	59	8.44±.25	61	5.25±.21	62.2±3.1	185	7.25±.17	230	5.07±.13	68.9±1.9
total (cross I)							973	7.36±.07			

* Cross I is the group of linkage crosses listed in table I and other similar crosses not listed, with the exception that females from the backcrosses to the WA-stock and the V-stock are not included because many of them were homozygous for detrimental recessives tending to cut down litter size. Under cross 2 are listed litters by tested offspring from the mating $+c\ T(1;?)a+/sh\text{-}1\ c^{ch}+p\times sh\text{-}1\ c^{ch}+p/sh\text{-}1\ c^{ch}+p$. Under cross 3 are listed litters by tested offspring from the mating $+a\ T(5;8)a+/pa\ a+b/pa\ a+b$. For these three crosses, all males were tested by matings to B alb C females. Note the resulting uniform mean litter size for the normal males. Cross 4 was the test for the presence of A or a in young from the matings $+a\ T(5;8)a\ b/+++b\times+++b/+++b$ and $pa\ a\ T(5;8)a+/pa+++\times pa+++/pa+++$. Lack of hybrid vigor in the females and the large proportion of first litters accounts for the small litter size for females. Males were tested by mating to stocks other than the B alb C, mostly of lower fertility. In cross I, the identification of mice as semi-sterile or normal was based solely on the mean size of their litters; in crosses 2, 3, and 4 it was possible to check the identification on the basis of litter size by means of a very closely linked gene.

** The standard error of the percentage fertility is calculated from the formula $E_{x/y}=100\frac{\bar{X}}{\bar{Y}}\sqrt{\left(\frac{E_x}{\bar{X}}\right)^2+\left(\frac{E_y}{\bar{Y}}\right)^2}$ where $\bar{X}$ is the semi-sterile mean litter size and $\bar{Y}$ the normal mean litter size. For cross I males, the value used for $\bar{Y}$ is 7.36±.07, the normal mean litter size for all cross I males combined.

sterile females, from 38.4±2.9 percent to 62.2±3.1 percent; for males, from 41.3±1.8 percent to 68.9±1.9 percent. There is no question that these extreme values differ from 50 percent. From table 11 it will be seen that P, the probability of a deviation from 50 percent as great as or greater than that observed, occurring due to chance alone, is, for semi-sterile males, <.0001 for T-F_1194, .0085 for T-F_1111, <.0001 for $T(5;8)a$ (cross 1), and <.0001 for T-R_18. It can safely be concluded that for the first two of these translocations the mean size of litters produced by heterozygous males is less than 50 percent of normal, and for the last two more than 50 percent of normal.

Tables 10 and 11 reveal another interesting fact—namely, a difference in percentage fertility between sexes. In every case except one, $T(2;?)d$, the percentage is higher in the male than in the female. The difference is greatest in the case of $T(5;8)a$, being 11.4, 13.6, and 9.0 for the three crosses in which this translocation was used. The value of P for the first of the crosses is <.0001, so that in this case the difference is unquestionably significant.

That these differences in fertility between translocations and between sexes have real biological, as well as statistical, significance, is indicated by the

TABLE 11

Significance of differences in fertility between translocations and between sexes.

CROSS NUMBER*	TRANSLOCATION	PERCENTAGE FERTILITY SEMI-STERILE FEMALES	DIFFERENCE FROM 50%	P**	PERCENTAGE FERTILITY SEMI-STERILE MALES	DIFFERENCE FROM 50%	P**	DIFFERENCE BETWEEN MALES AND FEMALES	P†
1	T-F_1194	38.4±2.9	−11.6	.0001	41.3±1.8	−8.7	<.0001	2.9	.39
1	T-F_1111	42.9±3.3	−7.1	.032	45.0±1.9	−5.0	.0085	2.1	>.5
1	$T(2;?)d$	49.1±2.1	−0.9	>.5	47.3±2.5	−2.7	.31	−1.8	>.5
1	$T(1;?)c$	44.7±2.6	−5.3	.042	52.7±2.4	+2.7	.26	8.0	.023
2		45.4±2.2	−4.6	.037	53.0±2.6	+3.0	.25	7.6	.025
1	$T(5;8)a$	50.4±1.9	+0.4	>.5	61.8±1.7	+11.8	<.0001	11.4	<.0001
3		46.2±3.7	−3.8	.31	59.8±6.5	+9.8	.13	13.6	.07
4		50.5±2.7	+0.5	>.5	59.5±3.8	+9.5	.013	9.0	.06
1	T-R_18	62.2±3.1	+12.2	.0002	68.9±1.9	+18.9	<.0001	6.7	.07

* See footnote table 10.

** P is derived from the Dif./SE, the Dif. being the difference from 50 per cent fertility and the S.E. the standard error of the observed percentage fertility.

† P is derived from the Dif./S.E., the Dif. being the difference in percentage fertility between males and females, and the S.E. the standard error of the difference calculated from the formula $S.E._{x-y} = \sqrt{(S.E._x)^2 + (S.E._y)^2}$.

excellent agreement between fertility percentages observed with the same translocation in different crosses. Thus in three entirely different crosses, males heterozygous for $T(5;8)a$ showed percentage fertilities of 61.8±1.7, 59.8±6.5, and 59.5±3.8 (table 10).

The question next arises whether the percentage fertility coincides with the percentage of orthoploid gametes. (Orthoploid gametes are those carrying the normal haploid complement of genes, whether or not rearranged. Aneuploid gametes are those which do not contain a normal haploid complement of genes, but carry duplications and/or deficiencies of some. The first two types of sperm in figure 1 are orthoploid, the last four aneuploid.) Direct evidence on this point cannot be obtained. Some indirect evidence is furnished by an

earlier embryological study with $T(5;8)a$ (SNELL, BODEMANN, and HOL-LANDER 1934). Seventeen females mated to a semi-sterile male and killed at ten to 13 days after mating yielded an average of 5.22 normal embryos per female. Eleven control females yielded 8.82 normal embryos per female. The percentage fertility calculated from these data is 59.2 percent, an excellent check with the values of 61.8, 59.8, and 59.5 shown in table 10. Further embryological studies are projected, but these earlier results are sufficient to indicate that differential mortality does not materially alter the percentage fertility between 10–13 days embryo age and term.
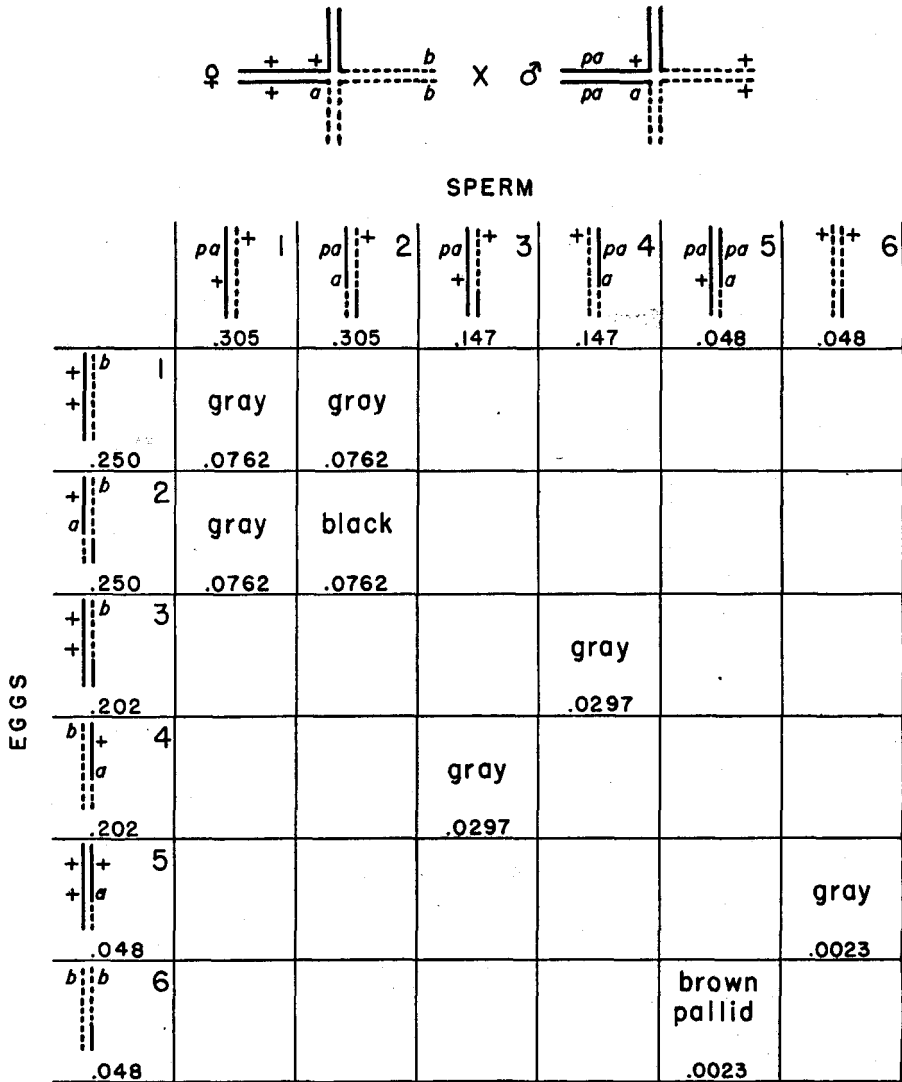
$$\varphi \quad \frac{+ \quad +}{+ \quad a} \bigg|\bigg| \begin{array}{c} b \\ \cdots\cdots \\ b \end{array} \quad X \quad \sigma \quad \frac{pa \quad +}{pa \quad a} \bigg|\bigg| \begin{array}{c} + \\ \cdots\cdots \\ + \end{array}$$

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EGGS	$pa \mid + / + $ 1 .305	$pa \mid + / a$ 2 .305	$pa \mid + / +$ 3 .147	$+ \mid pa / a$ 4 .147	$pa \| pa / + a$ 5 .048	$+ \| +$ 6 .048
$+\mid b / +\mid$ 1 .250	gray .0762	gray .0762				
$+\mid b / a$ 2 .250	gray .0762	black .0762				
$+\mid b / +$ 3 .202				gray .0297		
$b\mid + / a$ 4 .202			gray .0297			
$+\|+ / +\|a$ 5 .048						gray .0023
$b\mid b$ 6 .048					brown pallid .0023	

FIGURE 1.—Checkerboard showing expected results from cross of $T(5;8)a$ heterozygote×heterozygote. See text for explanation.

There are probably proportionately more still births, and hence more young eaten by the mother, in very small litters than in normal sized ones, but since litters were recorded soon after birth, and pains taken to locate the remains of any partly eaten young, this is probably a very minor disturbing factor.

In the next section it will be shown that the assumption that percentage fertility accurately measures the percentage of orthoploid gametes checks very well with evidence from other sources.

THE MATING SEMI-STERILE × SEMI-STERILE

Two final matings were made with $T(5;8)a$ and $T(1;?)c$. These were $Pa\ a$ $T(5;8)a\ b/Pa\ A+b\times pa\ a\ T(5;8)a\ B/pa\ A+B$ and Sh-$1\ c\ T(1;?)c\ p/Sh$-1 $c^{ch}+p\times sh$-$1\ c\ T(1;?)c\ P/sh$-$1\ c^{ch}+P$. The crosses were made reciprocally. The expected results are indicated in figures 1 and 2 and tables 12 and 14, and the observed results in tables 12 and 13.

The expected results for $T(5;8)a$ are calculated on the basis of two hypotheses. Since the appearance of two pallid brown mice proves that the pallid and brown loci are on opposite rather than adjacent arms, this is assumed for both hypotheses.

TABLE 12

Segregants from the mating $+a\ T(5;8)a\ b/+++b\times pa\ a\ T(5;8)a+/pa+++$.

FEMALE PARENT	+++	+a+	pa+b	+??	???
$+a\ T(5;8)a\ b/+++b$	228	62	2	174	8
$pa\ a\ T(5;8)a+/pa+++$	28	4	0	6	1
Totals	256	66	2	180	9
Expected, hypothesis 1	255.1	66.9	2.0		
hypothesis 2	264.3	57.7	2.0		

TABLE 13

Segregants from the mating $+\ c\ T(1;?)a\ p/+c^{ch}+p\times sh$-$1\ c\ T(1;?)c+/sh$-$1\ c^{ch}\ ++$.

FEMALE PARENT	$+c^{ch}c^{ch}+$	$+c^{ch}c+$	$+cc?$	$?c^{ch}+$	$?cc?$ (OR ??p)	? ? ?
$+c\ T(1;?)a\ p/+c^{ch}+p$ •	20	49	11	26	8	8
sh-$1\ c\ T(1;?)a+/sh$-$1\ c^{ch}++$	7	19	5	1	4	3

Hypothesis 1

The proportion of orthoploid sperm (first two types in figure 1) is taken as 0.61, the proportion of orthoploid eggs as 0.50. These figures are based on the fertility percentages shown in table 10. The proportions of the different types of aneuploid sperm assumed for hypothesis 1 are: type 3—.147, 4—.147, 5—.048, 6—.048. The corresponding figures for eggs are: 3—.202, 4—.202, 5—.048, 6—.048. These figures are selected so as to make the expected proportion of brown pallid mice agree with the observed (table 12). The agreement of these figures is thus, of course, without theoretical significance. Hy-

TABLE 14

Comparison of observed results from the mating +c T(1;?)c p/ +c^ch+p×sh-1 c T(1;?)c +/sh-1 c^ch++ with results expected on various hypotheses.

HYPOTHESIS	ASSUMPTIONS (SEE ALSO TEXT)								RESULTS (OBSERVED AND EXPECTED)						MEAN LITTER SIZE AT BIRTH*
	FREQUENCY OF GAMETES						% SURVIVAL cc COMPARED TO c^ch		Sh-1 c^hc^ch P	Sh-1 c^ch c P	Sh-1 cc P	P	sh-1sh-1 AND pp	P	
	1 & 2		3 & 4 (OR 5 & 6)		5 & 6 (OR 3 & 4)		AT BIRTH	AT WEANING							
	♂	♀	♂	♀	♂	♀									
Observed at weaning									27	68	16		0		1.55±.13 or 2.15±.13
1	.265	.225	.235	.275	0	0	100	100	18.0	55.5	18.0		19.5	<.0001	2.84
2	.265	.225	.117	.137	.117	.137	100	100	21.8	55.5	21.8		11.8	<.0001	2.35
3	.265	.225	.117	.137	.117	.137	100	100	21.8	67.3	21.8		0		2.35
4	.265	.225	.117	.137	.117	.137	100	100	27.7	55.5	27.7	.02	0		1.84
5	.265	.225	.117	.137	.117	.137	74.0	59.0	26.9	68.4†	15.8		0		1.97
6	.265	.225	.117	.137	.117	.137	68.5	50.5	31.7	63.3	16.0		0		1.70
7	.265	.225	.235	0	0	.275	68.5	50.5	31.7	63.3	16.0		0		1.70
8	.265	.225	.235	.275	0	0	68.5	50.5	31.7	63.3	16.0		0		1.70

* The first observed mean litter size, 1.55, is that obtained when "litters of o" (see text) are included, the second, 2.15, when "litters of o" are excluded. The normal litter size used in calculating the expected litter size is 7.74 (table 10, cross 2).

† Of these c^ch c mice, 14.6 percent will be exceptional in that they will be Sh-1Sh-1 or PP instead of Sh-1Sh-1 or Pp.

pothesis 1 is the one on which figure 1 is based. The blank spaces in the checkerboard in this figure represent unbalanced and hence presumably inviable zygotes. The black (non-agouti or *aa*) mice, unless derived from gametes one of which was a crossover between *a* and the break, are homozygous for the translocation.

Hypothesis 2

The proportion of orthoploid eggs and of orthoploid sperm are both taken as 0.5, corresponding to a fertility percentage of 50 for both sexes. Otherwise the expected results are derived as in hypothesis 1.

Hypothesis 1 gives a very close fit, hypothesis 2 a somewhat poorer fit. This tends to confirm the conclusion drawn from litter size data that the higher percentage fertility of males as compared with females is a real phenomenon, and further tends to show that it is due to the production of a higher proportion of orthoploid gametes.

The proportion of gametes of types 5 and 6 assumed for hypothesis 1 is perhaps too large for the following reason. There was a high early mortality in the cross semi-sterile $\times$ semi-sterile apparently in some way correlated with difficulties in parturition due to the small litter size. The young that died during the first few days could always, and the still-born young usually, be classified by the presence of eye pigment as *Pa*. There were 174 animals so classified, and no pallid animals, among the mice that failed to reach the age (about six to ten days) when coat colors were classified. If these animals are added to the gray coated and black mice in figuring the gamete frequencies, these become for the sperm: 1—.305, 2—.305, 3—.156, 4—.156, 5—.039, 6—.039; for the eggs: 1—.25, 2—.25, 3—.211, 4—.211, 5—.039, 6—.039. This is perhaps a better estimate than that used in figure 1, but the standard error, except for gametes of types 1 and 2, would be rather high in either case.

The problem of the possible types of segregation that can be shown by the four chromosomes, or eight chromatids, which are involved in a reciprocal translocation heterozygote at first meiotic metaphase is a complex one. KOLLER (1944) reported the rather common occurrence of "numerical non-disjunction, by which three chromosomes went to one nucleus and one to the other." This would give rise to some gametes with three of the translocation chromosomes, others with only one. The higher fertility of $T(5;8)a$ as compared with the interchanges studied by KOLLER, and the nearly normal crossing over, suggest that such numerical non-disjunction may be infrequent in this translocation. In any case, the union of the appropriate complementary three and one gametes to produce a viable zygote, although it can give rise theoretically, in the case of the above cross, to brown, pallid, non-agouti, non-agouti brown and non-agouti pallid mice, cannot give rise to the actually observed class, brown pallid.

KOLLER also noted that "configurations, very similar to a figure-of-eight with non-disjunctionally arranged chromosomes were often encountered." (See KOLLER's figure 4C.) If these are interpreted as involving one chiasma, or two disparate chiasmata, between a pair of centromeres and the break, and

if the non-disjunction means non-disjunction of the centromeres at the first meiotic division, then, besides gametes of classes 5 and 6 (or 3 and 4), one must infer the possibility of four new classes of gametes each carrying one of the four translocation chromosome *represented twice*, but none of the other three translocation chromosomes. The union of the appropriate complimentary gametes can then give rise to viable zygotes of the phenotypes non-agouti, non-agouti brown pallid, type, and brown pallid. The last class, though phenotypically the same as one of the classes in figure 1, is genetically different, being normally fertile and homozygous agouti instead of semi-sterile and heterozygous for non-agouti. Of the two mice in this phenotypic class, one died shortly after weaning, and the other was tested and proved to be semi-sterile and heterozygous for non-agouti.

It is worthy of note that, because of the absence of crossing over in the male Drosophila, KOLLER's "abnormal figure-of-eight" cannot occur in the male of this species, and hence the four unusual aneuploid gametes produced by this figure are not to be expected. They are a possibility in the case of the female Drosophila, but since the complementary classes of sperm could not be formed, they would necessarily go undetected. Unfortunately for genetic demonstration of their occurrence in mice, their frequency is presumably low and the chance of finding a zygote of a genotype derivable from them alone very slight indeed.

If KOLLER's abnormal figure-of-eight produces gametes 3 and 4 (fig. 1), the normal figure-of-eight produces 5 and 6, and *vice versa*. In two of his three translocations he observed both figures. The ring-of-four and chain-of-four might also produce both groups of gametes, but it would appear from KOLLER's description that they cannot actually be identified as so doing. The two types of figure-of-eight, however, are evidence that KOLLER's interchange lines B and T, like our $T(5;8)a$, produce the four classes of aneuploid gametes shown in figure 1.

In conclusion, although it would appear to be theoretically possible for as many as 26 types of gametes to be formed by a translocation heterozygote instead of merely the six represented in figure 1, and although some of these may be formed occasionally in the case of $T(5;8)a$, it seems probable that the frequency of the different types of gametes indicated for this translocation in figure 1 is a good approximation to the actual situation. In the case of less fertile translocations, such as those studied by KOLLER or as $T-F_1194$, there is some reason to suppose that some of the possible gametes other than those shown in figure 1 are fairly common.

If it could be shown which of the aneuploid classes of figure 1—namely, 3 and 4 or 5 and 6—resulted from centromere non-disjunction, it would become possible to infer at which ends of chromosomes 5 and 8 the centromeres are located. Thus, if classes 5 and 6 are the centromere non-disjunction classes, the centromere is located at the pallid end of chromosome 5 and the brown end of chromosome 8. There is a possibility that a cytological study can elucidate this point.

The occurrence of black (*aa*) mice in the cross discussed above proves that the translocation homozygote is viable in the case of $T(5;8)a$. This was further checked by testing five black mice from this cross for semi-sterility. Four were normally fertile as expected, one probably semi-sterile (mean size of four litters, 3.5) and hence produced by a crossover between *a* and the break. Fourteen mice from the mating translocation homozygote times normal were all semi-sterile as expected.

The mean litter size for the mating semi-sterile × semi-sterile can be calculated in two ways. The first is simply to average the observed litters. The second takes into consideration the fact that, in this cross, pregnancies in which all embryos were inviable would occur with appreciable frequency, and includes as litters of zero cases in which females were set out as pregnant but failed to give birth to a litter. There were 15 such cases as compared with 197 cases of litters of one or more. The two averages for $T(5;8)a$ are 2.60±.08 and 2.41±.09. The mean litter size for normal females of cross 4, table 10 (6.69±.19), may be used as a control, but it is probably a little low because of the large proportion of first litters. Using this figure as it stands, the expected mean litter size for the mating $T(5;8)a/+ \times T(5;8)a/+$, calculated on the basis of the gamete frequencies shown in figure 1, is 2.47. The agreement with the observed litter size, either with or without litters of zero, is satisfactory.
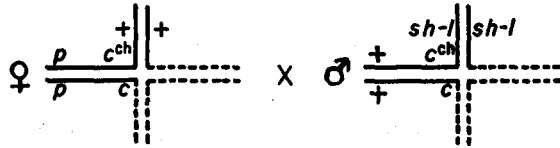
To test the segregation of $T(1;?)c$, the mating shown in figure 2 was made. The results are summarized in table 13. It will be seen that, as in the case of $T(5;8)a$, the translocation homozygote, recognizable by its albinism, was obtained, although the fact that there were only 16 of these at weaning as compared with 27 $c^{ch}c^{ch}$ mice suggests a reduced viability. The *cc* mice bred, as expected, like normals.

While the three classes produced by the union of balanced gametes were all obtained, the two classes, *pp* and *sh-1sh-1*, recognizable as the product of the union of complementary classes of unbalanced gametes, were both lacking. This result was totally unexpected. As may be seen from figure 2, the production of gametes 3 and 4, or 5 and 6, or 3, 4, 5, and 6 by both males and females should lead to the appearance of one or both of these particular types. Possible explanations will be considered latter.

As in the case of $T(5;8)a$, mean litter size can be calculated either with or without the inclusion of "litters of zero." The frequency distribution of litters of different sizes was: 0, 29; 1, 24; 2, 29; 3, 13; 4, 6; 5, 2; 6, 1. The mean litter size including litters of 0 is 1.55±.13; excluding them, it is 2.15±.13. Some embryological data, for which the writer is indebted to MISS GLORIA BRADY, show that in this cross, litters in which all embryos degenerate are not infrequent; hence the inclusion of some litters of zero is certainly justified. Perhaps as good a guess as any of the true mean litter size would be a value midway between the two figures given above, or 1.85. It seems reasonable to suppose that this unusually small litter size is due to the same cause, whatever it may be, that leads to the absence of the *pp* and *sh-1sh-1* classes.

DISCUSSION OF RESULTS WITH $T(1;?)c$

Below, and in table 14, are presented and discussed various possible hypotheses to explain the absence of both pp and sh-$1sh$-1 mice in the progeny

SPERM

	$+$ / c^{ch} / sh-1 1 .265	$+$ / c / sh-1 2 .265	$+$ / c^{ch} / sh-1 / sh-1 3 .235 or .117	$+$ / c 4 .235 or .117	$+$ / c^{ch} / c / sh-1 5 0 or .117	/ sh-1 6 0 or .117
p / c^{ch} / $+$ 1 .225	Pp $c^{ch}c^{ch}$ Sh-1 sh-1 .0596	Pp $c^{ch}c$ Sh-1 sh-1 .0596				
p / c / $+$ 2 .225	Pp $c^{ch}c$ Sh-$1sh$-1 .0596	Pp cc Sh-$1sh$-1 .0596				
p / c^{ch} / $+$ / $+$ 3 .275 or .137				Pp $c^{ch}c$ Sh-$1Sh$-1 .0647 or .0161		
p / c 4 .275 or .137			Pp $c^{ch}c$ sh-$1sh$-1 .0647 or .0161			
p / p / c^{ch} / c / $+$ 5 0 or .137						pp $c^{ch}c$ Sh-1 sh-1 0 or .0161
/ $+$ 6 0 or .137					PP $c^{ch}c$ Sh-$1sh$-1 0 or .0161	

EGGS

FIGURE 2.—Checkerboard showing expected results from cross of $T(1;?)c$ heterozygote×heterozygote, based on two possible frequencies of gametes 3, 4, 5, and 6. See text for explanation.

of the cross shown in figure 2. In all cases it is assumed that gametes 1 and 2 are produced with a frequency of .265 in the male and .225 in the female (table 14 and fig. 2), these figures being derived from the fertility percentage (table 10).

Hypotheses 1 and 2

Assume that homozygous *pp* and *sh-1sh-1* zygotes in fact are produced and are viable but failed to appear as a matter of chance. Assume the frequency of gametes 3, 4, 5, and 6 shown in table 14. These hypotheses are untenable, the probability of the expected classes failing to appear due to chance alone being $<.0001$.

Hypothesis 3

Assume that gametes 3, 4, 5, and 6 are produced with equal frequency (.1175 in the male and .1375 in the female) and that all zygotes shown as balanced in figure 2 survive normally but for some unexplained reason produce no *pp* or *sh-1sh-1* young. This hypothesis leads to an expected litter size of 2.35, as compared with an observed litter size of $1.55\pm.13$ or $2.15\pm.13$. Any frequency of gametes 3, 4, 5, and 6 other than that assumed will lead to a still larger litter size. The discrepancy in litter size renders the hypothesis somewhat unlikely if not untenable. Moreover, the difficulty of imagining a mechanism which would cause the failure of *pp* and *sh-1sh-1* young to appear is against it.

Hypothesis 4

Assume that gametes 3, 4, 5, and 6 occur with equal frequency but that the balanced as well as the unbalanced zygotes produced by them are inviable. Assume normal viability for all other balanced zygotes. This hypothesis gives an expected frequency, $c^{ch}c^{ch}:c^{ch}c:cc::27.7:55.5:27.7$, whereas the observed frequency is $27:68:16$. There is a significant deficit of the *cc* (translocation homozygote) class ($P=.02$). This deficit is somewhat less at birth, the *cc* class showing a higher mortality between birth and weaning than the other classes. The translocation homozygote is not infrequently non-viable in Drosophila. It seems probable, although it cannot be regarded as proved, that the *cc* type is less viable than the $c^{ch}c^{ch}$ or $c^{ch}c$ types, and in hypotheses 5 to 8 a correction is made for this as indicated in the column headed "percent survival *cc* compared to $c^{ch}c$." The remaining hypotheses, but especially hypothesis 5, will still give a fairly satisfactory agreement with observation if this correction is not made.

Hypothesis 5

Assume that gametes 3, 4, 5, and 6 are produced with equal frequency, that *cc* mice show a 74 percent survival at birth (that is, that for every 100 $c^{ch}c$ zygotes that survive to birth, 74 *cc* zygotes survive to birth) and a 59 percent survival to weaning. Assume further that in the development of one of the two parent stocks (fig. 2)—that is, while shaker-1 or pink-eye was being introduced by crossing over, a further translocation or transposition occurred such that one of the two translocation chromosomes is hyperploid and the other hypoploid as compared with the original condition of these chromosomes, and as compared with their condition in the other parent stock. The transposition must be of such a nature as not to be lost by crossing over. Then while

gametes 1 and 2 will give rise to the usual balanced classes of zygotes (fig. 2), gametes 3, 4, 5, and 6 will give rise to unbalanced zygotes only. However, the four zygotes from gametes 3, 4, 5, and 6 shown as viable in figure 2 will in two cases be hyperploid for the new transposition only, in the other two cases hypoploid for this only. Assume, finally, that the ·new hyperploid class is viable, the hypoploid class inviable. The expected results under this hypothesis, both as to mean litter size and frequency of the viable classes, are in satisfactory accord with the observed results.

Hypothesis 6

Same as 5 except that both the new hyperploid and new hypoploid classes are assumed inviable, and a slightly different percentage survival of the *cc* class is postulated. This also fits the facts, the agreement as to litter size being perhaps slightly better than in the case of hypothesis 5. If hypothesis 5 or 6 is correct, the repetition of the cross of figure 2 with newly derived stocks should result in the appearance of pink-eye or shaker-1 mice.

Hypothesis 7

The special feature of this hypothesis is the assumption that the male produces gametes 3 and 4 (or 5 and 6) only, whereas the female produces 5 and 6 (or 3 and 4) only. It fits the observed data, but is rendered somewhat improbable by the fact that there is, so far as the writer is aware, no precedent for the basic assumption. A possible test for it could come from a study of the embryos from the mating semi-sterile × normal. A different frequency of abnormal types from properly controlled reciprocal crosses would be evidence favorable to the hypothesis; absence of such a difference would be inconclusive.

Hypothesis 8

In the formation of gametes 1 and 2 there is complete disjunction of the translocation chromosomes at the first maturation division; in the formation of gametes 3, 4, 5, and 6 there is disjunction along one axis only of the cross-shaped figure produced by pairing, with non-disjunction along the other axis. It is a not implausible assumption, especially if the non-disjunction axis is a long one and carries the centromeres, that the postponement of the separation of this axis and the unravelling of its chiasmata till the second maturation division would result regularly in the production of chromosome breaks. If this were the case, all zygotes produced by gametes 3, 4, 5, and 6 would be unbalanced. This hypothesis is in satisfactory accord with the facts. The best chance of confirming it would appear to be through cytological observations.

There is at the present time no basis for a choice between hypotheses 5, 6, 7, and 8. The possibility, of course, exists that none of these hypotheses is the correct one.

SUMMARY

From a group of radiation-induced translocations in mice, six were selected for intensive study. These were: $T(5;8)a$, $T(1;?)c$, $T(2;?)d$, $T\text{-}F_1111$, $T\text{-}F_1194$

and T-$R_1 8$. These were tested for linkage against the genes a, b, Ca, d, f Fu, ln, p, s, sh-2, v, W, wa-1 and wa-2, and in the case of $T(1;?)c$ against je, Re and Sp also. No linkages were found in the case of T-$F_1 111$, T-$F_1 194$, and T-$R_1 8$,, and these were therefore discarded. $T(5;8)a$ was found to involve chromosomes 5 and 8, the break being about one unit from a and 19 units from pa on chromosome 5, and 21 units from m and 29 units from b on chromosome 8. $T(1;?)c$ was found to involve chromosome 1, the break being very close to c, with crossing over somewhat reduced in the c-p segment on one side and the c-sh-1 segment on the other. Semi-sterility in the case of $T(2;?)d$ showed linkage with d.

By the use of linked genes it was possible to establish accurately the ratio of semi-sterile to normal from the mating semi-sterile $\times$ normal. The expected 1:1 ratio was closely approximated.

The fertility of the different translocation heterozygotes was tested by comparing the mean litter size from the mating semi-sterile $\times$ normal with that from comparable normal $\times$ normal matings. The values, expressed as percentages, ranged from 38.4 to 62.2 for females and from 41.3 to 68.9 for males. The differences from 50 percent are unquestionably significant in the case of four of the translocations. In the case of $T(5;8)a$, the fertility percentage is about 50 in the female and 61 in the male, the results being consistent in three entirely distinct crosses and unquestionably significant. Five of the other six translocations also show greater fertility in the male.

$T(5;8)a$ and $T(1;?)c$ are viable in the homozygous condition, though there is a suggestion of a reduced viability in the case of the latter. The other translocations were not tested.

A mating of $T(5;8)a$ heterozygote $\times$ heterozygote, with appropriate marker genes, showed that pa and b lie on opposite arms and that non-disjunction may occur along both axes but is most common on the axis which does not carry the marker genes. Since centromeres must lie on opposite arms, it can be stated that if the centromere lies on the pallid end of the pa-a chromosome, it must lie on the brown end of the b-m chromosome, and *vice versa*.

A mating of $T(1;?)c$ heterozygote $\times$ heterozygote with appropriate marker genes failed to produce either of the possible non-disjunction classes of young. The litter size was also unexpectedly small. Possible explanations for this are discussed.

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