A CASE OF NEIGHBORING LOCI WITH SIMILAR EFFECTS

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INTRODUCTION

IT HAS been assumed, more or less as a matter of course, that the hereditary unit in the chromosome which is not broken by crossing over—that is, the gene—also functions as a physiological unit in development. Such an assumption is indeed required by most of the evidence upon which rests the basic postulate of genetics—the occurrence of sharp segregation of alleles—since the segregating units are recognized by their phenotypic effects and the different allelic conditions of the unit of segregation generally retain a certain unity and relatedness in their effects on development.

There is, moreover, the fact which emerged when the first "maps" of the Drosophila chromosomes appeared, and which is generally true in linkage maps in other animals and plants, that gene loci are scattered through the chromosomes without regard to developmental effect. Loci which are so close as to be seldom separated by crossing over do not necessarily resemble each other in their effects, while those with chief effects on very different structures or functions may occur near together or far apart. The differential physiological effect of each allele appears to derive from its own locus, dependent, of course, on cooperating influences from other loci and from the environment. This statement remains generally true in spite of the proof that in a few cases the effect of a gene is changed when its position is changed. The observed position effects in Drosophila represent changes in the magnitude of an effect, like those associated with changes at the same locus; they do not alter the fact of qualitative dissimilarity between neighboring loci.

A third fact of interest is that what seems to be the same differential effect on development may be brought about by any one of several distinct loci in different parts of the chromosome complement. When these are widely separated, it can be supposed that each represents the same type of change in an element that is repeated in several parts of the chromatin. In fact the correlation between repeated chromosomes (polyploidy) and repeated loci (duplicate genes) receives its best interpretation from just such a supposition.

The question has seldom been asked "what meaning is to be attached to such mimic or duplicate genes when they are very near to each other in the chromosome and when their effects are not identical but only very similar?" Such questions present themselves when actual cases of this sort are found, and even though such cases are rare, they suggest hypotheses which call into

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question the universal correspondence between units of crossing over and units of developmental effect.

A preliminary description of such a case in the house mouse has recently been given (DUNN and CASPARI 1942). Five mutations, each affecting the tail and axial skeleton in similar ways, appeared to be located in the same chromosome, very near to each other. Three of them were at different loci separable by crossing over. Two possible explanations of these relationships were suggested. One was that the association of five similar mutations was due to chance —they happened to occur near to each other and happened to resemble each other. The other was that their similarity was due to their proximity—that is, that there was a unit of physiological effect within the chromosome of greater extent than a crossing over unit.

In order to test the first hypothesis (chance), two main questions must be answered. How near are the loci involved, and how many such loci are there in the house mouse? Preliminary studies of crossing over led to an estimate of the likelihood of association of the three loci being due to coincidence as $p = .000008$. The present paper will present the full breeding data obtained on this question and a revised probability estimate which leads to the rejection of the hypothesis of coincidence.

The investigation of the second hypothesis requires first an answer to the question "How alike are the developmental effects of the contiguous loci?" This can be obtained from a comparison of the adult phenotypes of the mutations involved and particularly of the embryological processes through which the phenotypes are reached. Although some preliminary results on these questions have been obtained during the breeding experiments reported herewith, the embryological and anatomical observations are as yet incomplete and will be published separately later.

DESCRIPTION OF THE MUTATIONS

Brachyury and taillessness

Of the five mutations dealt with, three have already been described in detail. These are Brachyury or dominant short tail (T) and the recessives t^0 and t^1, either of which when combined with T (Tt^0 or Tt^1) produce taillessness. The first was described by DOBROVOLSKAIA-ZAVADSKAIA (1934) and CHESLEY (1935), and some details on its expression have been added by GREEN (1936) and DUNN (1942). The other two, taken from lines discovered by DOBROVOLSKAIA and KOBOZIEFF, were studied by CHESLEY and DUNN (1936), DUNN and GLUECKSOHN-SCHOENHEIMER (1939) and GLUECKSOHN-SCHOENHEIMER (1938) as reviewed by DUNN (1941). The essential points are that T usually behaves as a dominant, its principal effect in heterozygous condition being to shorten the tail. Its expression is subject to marked influence by a number of genetic factors, some of which enhance its effect, $T +$ being nearly tailless; others of which tend to suppress the expression of T,$T +$ being nearly normal. The form of the tail is also subject to modification, ankyloses or fusions between neigh-

boring vertebrae, contortions, angular kinks and similar irregularities occurring with some frequency in different stocks.

The most constant feature is shortness of the tail, and this phenotype is reached as a result of the death and dissolution of the distal part of the tail which was fully present in the embryo. This degenerative change is connected with the absence of the notochord which stops short in the tail or, as in the tailless forms, at the end of the sacrum, the parts lacking notochord undergoing histolysis. Homozygotes (*TT*) lack normal notochord and allantois and regularly die at about 11 days after fertilization, while t^0t^0 embryos die during gastrulation without ever forming mesoderm. The effects of t^1t^1 are not known, since these embryos die before implantation.

Fused

The dominant mutation Fused was first described by REED (1937). He found it to have quite variable effects on the tail, many of these, such as shortness or absence, presence of asymmetrical fusions of vertebrae with thickenings, kinks and contortions, resembling the variations found in the Brachyury mutant. Unlike Brachy, however, it proved to be viable in the homozygous condition, since REED found a few adult homozygotes, and it produces certain effects not found in Brachyury—namely, frequent fusions of ribs and of thoracic and lumbar vertebrae and occasional bifurcations of the tail. Some animals heterozygous for Fused presented an entirely normal appearance and could be detected only by progeny tests. REED reported also that such phenotypically normal animals ("normal overlaps") were much more frequent in outcrosses of Fused females than of Fused males, a circumstance not observed with Brachy or with any other mutation in the mouse. Because of the absence of proved crossovers between Fused and Brachy, REED supposed that Fused might be a less extreme allele of Brachy.

In addition to these, we have noted other effects of Fused. The most striking of these is a type of choreic behavior like that of the Japanese waltzing mouse but more variable and chaotic. This is usually accompanied by deafness. These abnormalities are found frequently in both heterozygotes and homozygotes but have not become constant under inbreeding. Rarely in Fused stocks anaemic young are found at birth. Neither waltzing, deafness, nor anaemia have ever been found in our extensive experience with Brachyury.

In our experience the viability of Fused homozygotes is very poor, the young *Fu Fu* dying shortly after birth, most of them with pronounced urogenital and gut abnormalities which will be described elsewhere.

No descriptions of the embryological manifestations of Fused have been published beyond the statement of REED (1937) that the first observable abnormalities in Fused embryos are poor alignment of notochord and curves and angles of the neural folds and that its embryological effects resemble those of Brachyury. An embryological study of Fused now in progress suggests further differences between Fused and Brachyury.

Kink

In 1937 a mutation like Fused was found by MR. S. P. HOLMAN and was studied and described as "Kinky tail" by CASPARI and DAVID (1940). Its manifestations in heterozygotes have since been more carefully studied by CASPARI (unpublished manuscript). The phenotypic effects of Kink proved to be very similar to those of Fused: it is dominant, usually shows reduction in the number of tail vertebrae, with abnormal fusions and ankyloses between neighboring vertebrae which cause angular kinks or stiff segments and occasionally bifurcations of the tail. Like Fused also, Kink-tailed animals frequently show disturbed behavior of the choreic or waltzing type, and many of them are deaf. All three manifestations (Kink tail, disturbed behavior, and deafness) appear to be effects of the one mutation and have not been separated from it. All three are also variable and show different frequencies and degrees of expression in different inbred strains. The most reliable indication of *Ki* is the tail abnormality which is present in 95 percent of *Ki* heterozygotes. Occasional animals show no external signs of fused vertebrae, but when the skeletons of these are stained and cleared for study, all show abnormal or reduced vertebrae or fusions. Tail length varies from tailless (rare) to nearly normal length; the number of kinks and the form of the tail is likewise variable. Length and form of the kink tails tend to become more constant in a stock after inbreeding.

Deafness was found in 31 percent of 925 kink-tailed animals of five different inbred strains. The strain frequency varied from 5 to 57 percent. Disturbed behavior was noted in 24 percent of 1040 kink-tailed animals of the same inbred strains as above. The frequency differed in different strains and could be increased by selection, showing that other factors besides *Ki* are concerned. Nearly always disturbed behavior is accompanied by deafness, although an occasional animal with abnormal behavior fails to show the external tail abnormality. It is likely that behavior and deafness are influenced by some of the same modifying factors. Although the deafness and disturbed behavior which accompanies Fused have not been carefully studied, it is probable that they closely resemble those connected with Kink.

The Kink mutation is distinguishable from Fused by its lethal effect. Kink homozygotes regularly die at about the ninth day after fertilization, whereas most Fused homozygotes survive until birth. *Ki* nearly always manifests itself in heterozygotes, whereas *Fu* heterozygotes often show no external tail abnormalities; and *Ki* males and females have similar offspring, whereas Fused females have phenotypically Fused offspring with less than normal frequency (REED 1937). Finally, *Ki* was shown by crossing over tests to be distinct from *Fu*, although closely linked with it (DUNN and CASPARI 1942).

All these facts have been confirmed by the further observations reported in this paper. In our experience, Kink and Fused animals from unselected stocks are phenotypically indistinguishable. Either one can be distinguished from Brachy with certainty only when waltzing behavior is present, since this never appears as part of the Brachy phenotype. Experiments now in progress indi-

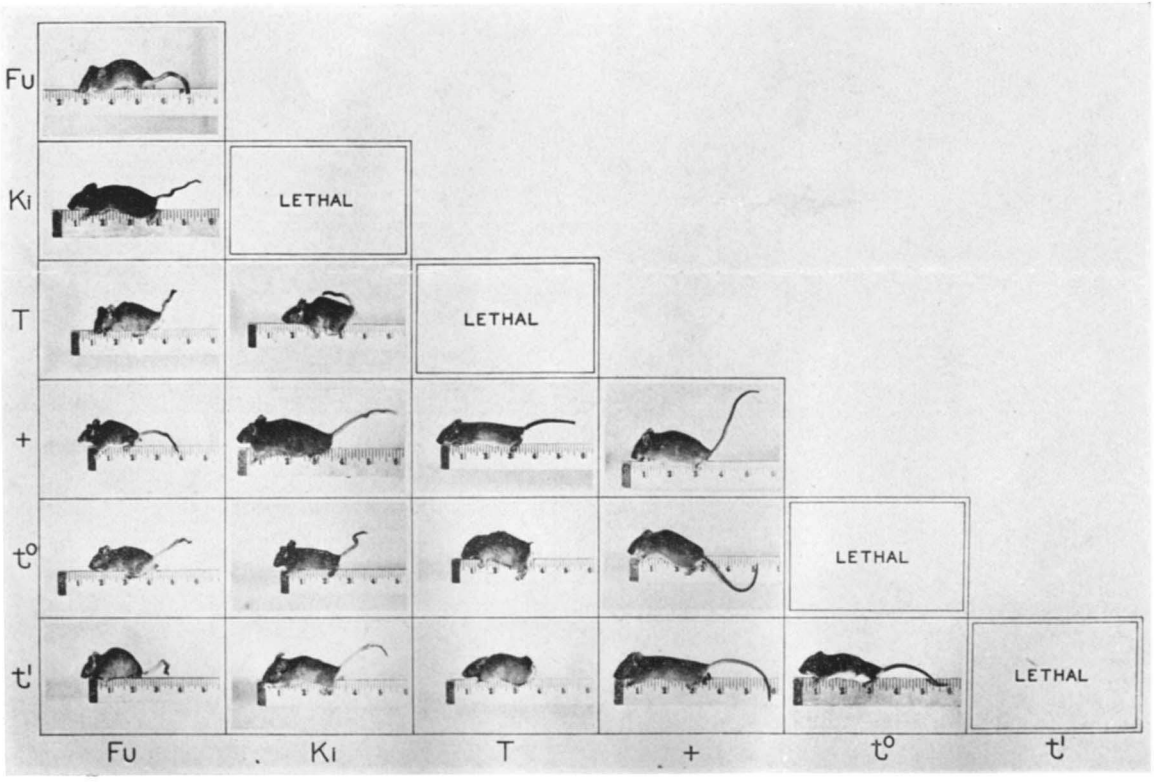

FIGURE 1.—Photographs of mice containing combinations of the neighboring mutations Fused (*Fu*), Kink (*Ki*), Brachy (*T*), t^0 and t^1. Gene received from father is shown at left, from mother below.

cate that partially isogenic stocks of *Ki*/+, *T*/+, and *Fu*/+ obtained by 16, 15, and 11 generations of backcrossing to a Bagg albino strain may show consistent differences in tail length, *T*/+ being shorter than *Ki*/+ or *Fu*/+, both of the latter being alike and of nearly normal length with few kinks.

EFFECTS OF THE MUTATIONS IN COMBINATION

When the individual mutations are combined with each other, an interesting set of relationships appears, as shown in figure 1. The specimens illustrated are typical or average, since Fused, Kink and Brachy are all quite variable and lines of these which are isogenic with each other have not been obtained.

Only one homozygous combination is viable—namely *Fu*/*Fu*, and this infrequently survives to reproduce. *Fu*/*Fu* usually has a shorter and more deformed tail than *Fu*/+, as REED noted, but some *Fu* homozygotes have tails like *Fu*/+.

The comparison of heterozygous combinations shows that the three dominants *Fu*, *Ki*, *T* do not influence each other appreciably. There is no marked cumulative effect of two mutations which have similar individual effects. It has been found, however, that in certain families derived from a crossover individual, *Ki T*/++, tailless animals occur. This probably depends on a special factor rather than on a cumulative effect of *Ki* and *T* when in the same chromosome, since *Ki T*/++ is often indistinguishable from *Ki*/*T* or *Ki*/+.

Neither *Ki* or *Fu* is markedly influenced by either of the recessives t^0 or t^1, whereas *T* is strikingly modified, *T*/t^0 and *T*/t^1 usually being entirely tailless. This difference is sufficient, in progeny tests, to distinguish *Ki* and *Fu* on the one hand from *T* on the other. We have found no difference in effect on the tail between t^0 and t^1 when combined with *T*. Although t^0/t^1 has a normal tail, these two mutations do interact to produce male sterility always, and head and eye abnormalities frequently. Such abnormal animals usually die as embryos (DUNN and GLUECKSOHN-SCHOENHEIMER 1943), but occasionally one survives, as for example, the animal in the lower right corner of figure 1 which has a microphthalmic left eye.

A few triple combinations have been observed and will be described in more detail elsewhere. *Ki T*/t^0 and *Ki T*/t^1 have a more extreme phenotype than *T*/t^0 or *T*/t^1 and show evidence of a cumulative effect in the greater frequency of malformations of the anus and urinary system. *Ki T*/*Fu* may be entirely tailless, but since *Ki T*/++ may also be tailless, no conclusions concerning cumulative effect can be drawn.

In respect of interaction effects, therefore, two groups of mutations can be recognized: (1) *Fu* and *Ki*—entirely indistinguishable from each other, except in the lethality of *Ki Ki*, and showing no marked interaction with each other or with the other three mutations; (2) *T*, t^0, and t^1 which mutually modify each other and do not share the waltzing, deafness, or duplicity (bifurcation) peculiarities of *Fu* or *Ki*.

All five mutations have this in common, that they alter the development of the spinal column in similar ways and give rise to similar phenotypes. The im-

portant question is thus raised what relation exists between their resemblances and differences and their locations in the chromosome.

RECOMBINATION FROM *Fu* +/+ *T*

In a first report we estimated that about two percent recombination occurred between *Fu* and *T*. This was based on the proof that four offspring (in a total of 400) from *Fu* +/+ *T* were crossovers of the ++ type, constituting half of the recombinations. These observations have been extended and confirmed.

The results of testing *Fu* +/+ *T* are shown in tables 1 and 2. The cross of *Fu* +/+ *T* males by normal females produces chiefly animals with Fused or Brachy tails. Two types of exceptions appeared. The first type was tailless and may represent either extreme expression of Fused or Brachy or possibly the crossover type *Fu T*/+ +. All of these died shortly after birth so that none could be tested. The second type was more frequent and had a normal tail. These could be either *Fu*/+ or *T*/+ which failed to manifest the mutant effect (normal overlap) or crossovers of the ++ type. These exceptions are

TABLE 1

Tests of Fu+/+T×++.

Fu+/+T PARENT	*Fu* OR *T*	TAILLESS	OFFSPRING NORMAL				CROSSOVERS	TOTAL
			TESTED *Fu+*	TESTED ++	NOT TESTED	TOTAL NORMAL		
Males	472	8	10	10	3	23	10	503
Females	111		10		16	26	—	137

TABLE 2

Tests of Fu+/+T ♂♂ by t^0t^1 ♀♀.

Fu (*Fu*/t^n)	TAILLESS *T*/t^n	NORMAL-TAILED TESTED *Fu*/t^n	TESTED +/t^n	NOT TESTED	TOTAL	CROSSOVERS	NON CROSSOVERS	TOTAL
74	75	1	4		5	4	150	154

fully viable and fertile and can be readily progeny-tested by mating to wild type (++). When this was done, ten of these exceptions proved to be *Fu*/+, while ten failed to produce mutant offspring in progenies of from 26 to 50, usually 35 (table 3). The latter are apparently ++ and represent half of the total crossovers. Their frequency should thus provide a good measure of the crossover value *Fu-T*. From *Fu* +/+ *T* males, the proved crossovers were ten out of 503 or two percent, leading to a crossing over estimate of four percent.

The same males were tested by t^0/t^1 females (table 2). It has been shown

that t^0 and t^1 do not cross over, hence every gamete of such females carries either t^0 or t^1. The non-crossover progeny from such a cross should thus be Fu/t^n and T/t^n. The latter are tailless; the former generally Fused. Crossovers detectable would be $+/t^n$ with normal tails. Actually five out of 154 progeny were normal, and of these, four or 2.6 percent were crossovers producing, respectively, 43, 38, 51, and 45 normal progeny while one was an overlap. The results show that the frequencies of the two non-crossover gametes *Fu* and *T* are equal, since equal numbers of $Fu(Fu/t^n)$ and tailless (T/t^n) offspring were found.

From *Fu* +/+ *T* females, a high frequency of normal-tailed exceptions was obtained (26 out of 136), but upon progeny-testing ten of these, all proved to be *Fu*/+. The high frequency with which *Fu* failed to manifest itself in the prog-

TABLE 3

Progeny tests by ++ of exceptional normal-tailed offspring from Fu+/+T♂×++♀ which gave only normal progeny.

ANIMAL TESTED	NORMAL PROGENY
♀ 8256	26
♂ 8397	37
♂ 8151	37
♀ 8530	49
♂ 8544	33
♂ 9925	35
♀ 10137	42
♂ 10144	42
♂ 10145	50
♀ 10205	47

eny of *Fu* females was not unexpected, since REED (1937) had noted this fact; but failure to find a single crossover among ten tested was surprising. If crossing over in males and females is equally frequent, two crossovers would be expected in the sample examined. The deviation of the actual from the expected, in a total of 121, is not significant (p=.09); hence the result may be due to chance rather than to failure of crossing over in the female. Nevertheless, it seems safer to omit the offspring of *Fu* +/+ *T* females in reaching an estimate of the amount of crossing over between *Fu* and *T*. The totals are thus 14 detected crossovers out of 657, or 2.13 percent, representing a crossing over percentage of 4.26.

RECOMBINATION FROM *Ki* +/+ *T*

Our first estimate of about two percent of crossing over between the loci of *T* and *Ki* has not been greatly altered by additional evidence. The chief test cross (*Ki* +/+ *T* × ++) was carried out in both laboratories (Lafayette and Columbia) with stocks of different derivation. The method was to detect one type of crossover—that is, ++ (normal-tailed), since the other type expected (*Ki T*/+ +) could not ordinarily be distinguished from the non-cross-

over types *Ki* and *T*. In addition, *Ki* +/+ *T* males were tested by t^0/t^1 females. In this cross, the non-crossover gametes *Ki* and *T* lead to sharply different phenotypes, Ki/t^n having a Kinked tail, T/t^n being entirely tailless. This permits an estimate of the relative viabilities of the two non-crossover types .The data show these to be about equal. In all cases, suspected crossovers—that is, progeny with normal tails—were progeny-tested by mating with animals from normal stock, and only those which produced 30 or more (usually over 40) normal progeny were accepted as valid crossovers. This precaution is required by the fact that rarely *Ki*/+ fails to show the Kink phenotype and without progeny tests such normal-appearing animals would be counted as ++ crossovers.

TABLE 4

Tests of recombination in males and females of genotype Ki +/+ T.

Ki+/+*T* PARENT	*Ki* OR *T*	TAILLESS	NORMAL-TAILED				CROSSOVERS	TOTAL CLASSIFIED
			TESTED *Ki*+	TESTED ++	NOT TESTED	TOTAL NORMAL		
♂♂×++(C)	521	2	—	4	—	4	4	525*
♂♂×++(*L*)	326	5	2	6	2	10	6	334*
♀♀×++(C)	218	2	1	8	3	12	8	227*
♀♀×++(L)	304	9	3	8	2	13	8	315*
♂♂×t^0t^1 (C)	135	127	3	4†	2	9	4	269
Totals	504	145	9	30	9	48	30	1670*

* Untested normals and tailless could not be classified as crossovers or non-crossovers and have been omitted from the totals.

† Tested = $+/t^n$.

(C) = tested at Columbia; (L) = tested at Lafayette.

The complete data are shown in table 4. There were 30 proved crossovers in a total of 1670, or 1.8 ± .32 percent, leading to an estimate of 3.6 percent crossing over. This must be a minimum estimate, since out of the 48 normal-tailed suspected crossovers, the progeny tests of nine were not conclusive because of sterility, early death, or loss from other causes. Of those tested, about three-fourths (30 out of 39) turned out to be crossovers, while about a quarter proved to be normal overlaps. It is likely therefore that six or seven of the untested animals were also crossovers, which would raise the total number to about 36 out of 1670 and the estimated crossing over percentage to 4.3.

Crossing over occurred in both sexes. From heterozygous males the frequency of detected crossovers was 14/1128 or 1.24 ± .33 percent; from females 16/542 or 2.95 ± .73. The difference, 1.71 ± .8 percent, while barely significant, (p = .04) is in the same direction as sexual differences in crossing over previously reported for the house mouse.

The results obtained in the two laboratories are comparable. The crossovers detected at Lafayette from tests by ++ were 14/649 or 2.15 ± .57 percent; at Columbia from similar crosses these figures are 12/752 or 1.59 ± .46. The difference, .56 ± .60, is not significant.

RECOMBINATION FROM *Ki T*/+ +

From *Ki* +/+ *T* ♂♂ × ++ at Columbia, two tailless males were found (out of 527 test cross offspring). One of these was abnormal with spina bifida aperta. The other was vigorous and survived (♂ 8756). Suspecting that this might represent the *Ki T*/+ + recombination type, we tested this animal for *T* and for *Ki*. He was first mated to Brachy (*T* +) females which were dissected in the 10th–12th day of pregnancy. Typical *TT* abnormal embryos were found, proving that the father transmitted *T*. Normal females pregnant by the exceptional male yielded embryos with branched and forked tails typical of those found in Kink matings.

This male was then mated simultaneously to ++ and to t^0/t^1 females, and 81 and 95 offspring were recorded at birth, as shown in table 5.

TABLE 5

Offspring from tailless ♂ (from Ki/T×++) tested by normal (++) and by t^0/t_1 females.

MOTHERS	NORMAL	KINK	TAILLESS	TOTAL
+/+	33	24	24	81
t^0/t^1	52	5	38	95

The first mating, by normal, shows that about half the gametes (48) of the exceptional male transmitted an abnormal tail condition, either Kink or tailless. The appearance of the tail, the expression of waltzing behavior (frequent in Kink, absent in Brachy) and of spina bifida aperta among the progeny indicated that the factor *Ki* was being transmitted. The second experiment confirmed this and showed that *T* was also transmitted, since nearly all the abnormal-tailed offspring were tailless as in T/t^0 or T/t^1.

The results could be accounted for by assuming that the exceptional male was *Ki T*/+ +—that is, a crossover from *Ki* +/+ *T*. The five Kink animals from the cross with t^0/t^1 should therefore represent crossovers *Ki* +/+ t^n. Most of the Kink and tailless animals from the cross with normal should be *Ki T*/+ + like the father.

Progeny tests of 24 offspring from the cross *Ki T*/+ + ♂ × ++ ♀ (table 6) showed that one male (9525) was *Ki* +/+ +, two (9388 and 9227) were probably + *T*/+ +, while the rest were probably *Ki T*/+ +. The tests were made by mating each animal to both +/+ and t^0/t^1 or +/t^0 or +/t^1, as shown in table 6. Those which produced about equal numbers of normal and abnormal-tailed when bred to normal, and equal numbers of normal and tailless when bred to t^0/t^1 were assumed to be *Ki T*/+ +. Those which gave Kink and normal by normal mates and the same by t^0/t^1 were probably *Ki* +/+ +; while those which give Brachy and normal by normal, and normal and tailless by t^0/t^1 should be *T* +/+ +. The last result cannot be distinguished with certainty from the first (*Ki T*/+ + × t^0/t^1) but in large progenies the absence of abnormals, of waltzers, and of forked or Kinked tails among the progeny will

usually decide in favor of T +. The two diagnoses of T +/+ + are not certain, since they were based on tests of females which cannot be tested by t^0/t^1 since t^0/t^1 males are sterile. Both of these females when tested by normal gave equal numbers of offspring with normal and with short tails resembling Brachy; by $+/t^0$, each gave normal, tailless, and Brachy, with no signs of waltzing, Kinked tails, or other abnormalities. Both were thus probably T +/+ +.

The total tested offspring from the exceptional male by normal were thus 21 *Ki* T/+ +, one *Ki* +/+ +, two probably T +/+ +. This would indicate a very high proportion of crossovers, 3/24, but such results cannot be used as a measure of crossing over, because the non-crossover type *Ki* T has two abnormal mutations and is known to exhibit abnormalities such as imperforate anus and spina bifida aperta and to suffer a much heavier postnatal mortality than the crossovers in which the abnormal mutations are present singly.

From the cross of the exceptional male by t^0/t^1 many of the tailless offspring were abnormal at birth, while most of the others died before weaning. We were thus able to test only five tailless animals, all of which were probably *Ki* T/t^n. Two kink-tailed progeny (♂ 9714, ♀ 9213) proved to be *Ki* $+/t^n$ and were thus crossovers as assumed.

Most of the tailless animals from this cross contained three mutations *Ki* T/t^n and owe their abnormalities and poor viability to this condition. We made observations on 84 tailless offspring from *Ki* T/+ + × t^0/t^1. Of these only 24 (28 percent) lived to the age of one month; 47 (56 percent) died shortly after birth, and 13 (16 percent) showed imperforate ani at birth and were killed and dissected. All but two of the latter had anal and urogenital abnormalities which would have prevented their survival. Of the 84 born, no less than 34 had severe lesions of the spina bifida aperta type. It is obvious that *Ki* and T with either t^0 or t^1 tend to produce a very abnormal phenotype. Some *Ki* T/t^n animals survive and breed, however, and it has been possible to test ten such males for crossing over between *Ki* and T.

The above results demonstrate that the exceptional male had *Ki* and T on the same chromosome, and that more than half of his offspring received this chromosome. In a few cases, however, crossing over again restored the original combinations *Ki*/+ and T/+. The frequency of crossing over will be discussed below.

Tests of offspring from the exceptional male also showed that the presence of *Ki* and T on the same chromosome does not necessarily produce a more extreme phenotype than *Ki* +/+ T, as might have been suspected from the tailless condition of the original exception. Thus of 21 offspring shown to be *Ki* T/+ +, ten had Kink tails from $\frac{1}{8}$ to $\frac{3}{4}$ of the normal length, while 11 were tailless. The two classes probably intergraded. Five of these F_1 tailless males when bred to normal gave 81 normal, 73 Kink, and eight tailless; while four of the F_1 Kink-tailed males bred to similar normals gave 66 normal, 62 Kink, and nine tailless. Thus tailless and Kink-tailed males had similar progenies. Taillessness was so rare in the combination *Ki* +/+ T as to suggest that *Ki*

TABLE 6

Tests of exceptional male 8756 (Ki T/++) and of his descendants from crosses with normal (++).

TEST ANIMAL #	OFFSPRING FROM CROSS WITH ++			FROM CROSS WITH $+/t^m$			FROM CROSS WITH t^0/t^1			ASSUMED GENOTYPE
	NORMAL	KINK	TAIL-LESS	NORMAL	KINK	TAIL-LESS	NORMAL	KINK	TAIL-LESS	
♂ 8756 tailless	33	24	24	20	8	16	52	4^{+1}	38	*Ki T*/++
F1♂ 9121 "	14	11	—	—	—	—	16		9	*Ki T*/++
9172 Kink	31	19	—	12	2	6	37	2	35	"
9173 tailless		5	—	3	—	3	3		7	"
9174 "	18	20	—	15	5	14	17	1	21	"
9292 Kink	12	13	1	34	4	11	20	—	24	"
9293 tailless	13	13	3	7	—	8	3		2	"
9296 "	12	4	2	6	3	5	4		4	"
9386 "							5		4	"
9387 Kink	8	7	8	18	6	6	—		—	"
9418 "	10	16		15	8	9	32	2	29	"
9526 tailless	17	14	1	19	1	13	—	—	—	"
9527 "	7	6	2	8	2	6	5		8	•
	175	152	41	157	39	97	194	9	181	
F1♂ 9525 Kink	5	7					19	15	1	*Ki* +/++
F1♀ 9225 Kink*	1	6		8	1	5				*Ki T*/++
9227 "	8	6		13	10†	5				+*T*/++(?)
9228 "	10	8	2	2	1	2				
9229 "	10	8	1	11	5	3				
9230 "	11	8	3	4	1	4				
9294 tailless	1	2	5	2	3	2				
9295 "	2	2	4	4	1	2				
9321 Kink				3		4				*Ki T*/++
9388 Brachy	8	8		14	6†	14				+*T*/++(?)
9528 tailless	1		1*							
9417 Kink	3	2		10	1	6				

* Waltzing.

† Brachy in appearance.

and *T* do not interact cumulatively. Taillessness is much more common in the combination *Ki T*/+ +, but no conclusions concerning a possible position effect can be drawn from the data in hand, since all *Ki T* animals are descended from a single exceptional animal, whose tailless phenotype may have been due to other factors by which he differed from his *Ki* +/+ *T* ancestors. For the present, taillessness in *Ki T*/+ + may be assumed to be an extreme effect of *Ki* or of *T* when combined with other unknown factors.

The other tailless offspring from *Ki* +/+ *T* × ++ did not survive long enough to be tested. It is not known whether they were crossovers or extreme variants of *Ki*/+ or *T*/+.

FREQUENCY OF CROSSING OVER BETWEEN *Ki* AND *T*

From crosses of *Ki* +/+ *T* by ++ the frequency of crossing over was found to be about 3.6–4.3 percent. It is now possible to measure crossing over from *Ki T*/+ + ♂ by crossing with t^0/t^1 ♀. Crossing over does not occur between t^0 and t^1 in females (t^0/t^1 males are sterile). Hence the two regular (non-cross-

over) types from the above cross will be *Ki T*/t^0 or *Ki T*/t^1 (tailless) and ++/+t^0 or ++/+t^1 (normal), while one class of crossover (*Ki* +/t^0 or *Ki* +/t^1 can be readily detected as Kink-tailed, since *Ki* and t^0 do not interact. Another class of crossovers + *T*/+ t^0 or + *T*/+ t^1 is tailless and indistinguishable from a non-crossover class. Thus the Kink-tailed animals from this cross will represent half the crossovers.

The results of such crosses are shown in the last columns of table 6. About equal numbers of normal (194) and tailless (181) were produced, together with nine or 2.4 percent Kink-tailed. Three of the latter (♂ 9714, ♀ 10505, ♀ 10568) were tested by t^0/t^1 or +/t^0 and produced no tailless animals, hence lacked *T* and were thus crossovers + *Ki*/t^0 or + *Ki*/t^1. The detected crossovers represent half of the total, which may thus be estimated as 4.8 percent. This is probably an overestimate because one of the non-crossover classes (*Ki T*/t^n) has poorer viability than either crossover class *Ki*/t^n or *T*/t^n. It is somewhat higher than the 3.6–4.3 percent found for *Ki* +/+ *T*, but the difference is not significant.

RECOMBINATION FROM *Fu* +/+ *Ki*

It was shown in our first report that *Fu* and *Ki*, in spite of their great phenotypic similarity, were not mutations at the same locus, since at least one case of crossing over between *Fu* and *Ki* was found. Several other recombination individuals have now been tested and indicate that *Ki* and *Fu* are several crossover units apart.

The pertinent data are given in table 7. The normal offspring, which are

TABLE 7

Tests of recombination in crosses of Fu+/+Ki×++.

Fu+/+*Ki* PARENT	*Fu* OR *Ki*	TAIL-LESS	PROGENY NORMAL			TOTAL NORMAL	CROSS-OVERS	TOTAL
			TESTED *Fu* OR *Ki*	TESTED ++	NOT TESTED			
♂♂(L)*	176		8		1	9	—	185
♀♀(L)*	229	1	18	5	15	38	5	268
♂♂(C)†	25	—				—	—	25
♀♀(C)†	25	—	2			2	—	27
	455	1	28	5	16	49	5	505

* At Lafayette.

† At Columbia.

classed as ++ (recombinations), gave the following progenies when tested by normal mates: (a) 74 normals, (b) 62 normals, (c) 61 normals and one doubtful, (d) 54 normal and one doubtful (the doubtful cases having abnormal tail tips possibly from injury), and (e) 112 normals to three clearly ab-

normal. In evaluating these results, it must be mentioned that animals with slight tail abnormalities resembling Fused or Kink are found occasionally in the Bagg albino strain used in the test crosses. The three abnormals from animal (d) are possibly of this type, or they may represent the effects of other genetic factors. It seems unlikely that the exceptions are due to the presence of *Fu* or *Ki* in the test animals which produced a total of 289 normal and five abnormal or doubtful offspring. Probably the five animals tested (a–d) were valid recombinations. If so they represent .99 ± .44 of the total progeny 505 from *Fu/Ki* × ++. Since only half of the crossovers could be detected by the methods used, this would indicate a minimum crossing over value of 1.98 ± .62 percent.

We have tried to increase the data and to remove the ambiguity due to the occurrence of doubtful and abnormal young in the test crosses. Several attempts have failed because of the poor viability of *Fu/Ki* animals. In one experiment, all ten offspring of a cross of *Fu/Fu* × *Ki*/+ proved to be *Fu*/+. Where viable, *Fu/Ki* animals have usually shown poor fertility. For these reasons a reliable crossover value for the *Fu-Ki* interval has not been obtained.

THE ORDER OF THE LOCI *Ki*, *T*, *Fu*

To determine the relative locations of *Ki*, *T*, and *Fu*, matings were made between *Fu Fu* and *Ki T*/+ +. The offspring should consist of *Fu*/+ and *Fu/Ki T* in equal numbers. Actually there appeared seven normal-tailed, 32 Fused or Kink-tailed, and 12 tailless, two of the latter showing the abnormal urogenital syndrome. Twenty-four of these were tested by crossing with normal-tailed males and were found to be distributed as follows:

Tested	*Fu*/+	*Fu/Ki T*
5 normal	5	—
17 Fused	15	2
2 tailless	—	2

Most of those tested proved to be *Fu*/+; only four were *Fu/Ki T*. All except two of the tailless F_1's died before they could be tested. It is probable that they contained more of the *Ki T/Fu* type which apparently is less viable than *Fu*/+.

The *Ki T/Fu* animals were tested by normal. They gave 167 offspring with abnormal tails (*Fu*, *Ki*, or tailless) and 18 with normal tails. The latter are either +/+ crossovers or *Fu*/+ (overlaps). To separate these latter genotypes, the normal exceptions were tested individually by normal-tailed mates. Of nine thoroughly tested, six gave only normal offspring and were classed as crossovers, while three gave some *Fu* offspring and were thus *Fu*/+.

To account for these crossovers, it must probably be assumed that *Fu* does not lie between *Ki* and *T*, for if it did, all six crossovers (the only ones detected) could have arisen only by double crossing over. It is much more likely that they are single crossovers and that the loci occur in one of the following orders: (a) *Ki T Fu*, (b) *Fu Ki T*.

If the order is *Ki T Fu*, then the +++ progeny would represent half the crossovers between *T* and *Fu*, and the frequency of these, 3.24 percent, is somewhat higher than the proportion found in the offspring of *Fu* +/+ *T*, which is 2.13 percent, and thus might favor the order *Fu* – *Ki* – *T*. The present evidence, however, does not distinguish between the two possible orders. The linkage summaries based on detected crossovers only (half of those occurring) show that the "half intervals" *Ki* – *T* (1.91 ± .30) and *Fu* – *T* (1.81 ± .48) are about the same. The *Fu* – *T* estimate would probably be increased by the discovery of additional crossovers among the 19 untested exceptions, and *Ki* – *T* would be increased by a lesser amount from the nine untested exceptions, probably leaving the two not significantly different. *Fu* – *Ki* appears to be shorter (.99 ± .44), although its difference from the above is not statistically significant, and the estimate may be too low by reason of the 16 untested exceptions. If *Fu* – *Ki* could be shown to be significantly less than *Fu* – *T*, the order *Fu* – *Ki* – *T* would be indicated. As it is, no interval can be said to represent the sum of two component intervals.

TABLE 8

Summary of linkage data for Fu, Ki, and T.

COMBINATIONS TESTED	TOTAL PROGENY	TESTED++ CROSSOVERS	UNTESTED EXCEPTIONS	PERCENTAGE CROSSOVERS DETECTED	
Fu+/*T* ♂♂	657	14	3	2.13	1.81 ± .48
Fu+/+*T* ♀♀	137	—	16	0. —	
Ki+/+*T* ♂♂	1128	14	4	1.24 ± .33	1.91 ± .30
Ki+/+*T* ♀♀	542	16	5	2.95 ± .73	
Ki T/++♂♂	395	9	—	2.28 ± .75	
Fu+/+*Ki* ♂♂	209	—	1	0. —	.99 ± .44
Fu+/+*Ki* ♀♀	296	5	15	1.69	
Ki T/*Fu* ♂♂	185	6	9	3.41 ± 1.37	
Totals	3549	64	53	1.80 ± .22	

There are several reasons why the present data are not competent to answer questions depending on the accurate measurement of crossing over and why adequate data will be hard to get. First, in matings involving phenotypically indistinguishable dominants, the best that can be hoped is that one of the two crossover classes, that containing neither dominant, will be detected. Secondly, this class often resembles phenotypically one of the non-crossover classes when one of the latter fails to manifest the dominant effect. Crossovers can theoretically be separated from "overlaps" by progeny-testing, but some exceptions are always lost and remain unknown. Third, the viability and fertility of animals with one, two, or three such dominants are unequal and variable. Under these conditions it is not considered feasible to obtain an accurate map of this area.

The summary in table 8, however, shows one conclusive result of great im-

portance. This is that crossing over does occur regularly and frequently within each interval of the *Ki–T–Fu* area. In some 3500 observations from heterozygotes containing two or three different mutations, a minimum of two percent of all offspring were shown to have arisen from crossing over within the area.

ABSENCE OF RECOMBINATION FROM *Ki* +/+ t^0

Since *Ki* and *T* have been shown to be at separate loci, while *T* has shown no recombination with either t^1 or t^0, it is important to test whether any recombination occurs between *Ki* and t^0 or t^1. The simplest test is to make matings $Ki/t^1 \times Ki/t^1$ and $Ki/t^0 \times Ki/t^0$. Since *Ki*, t^0, and t^1 all act as early lethals, such matings should "balance," if no recombination occurs, the only surviving offspring being Ki/t^1 and Ki/t^0, respectively, whereas, if recombination occurs, exceptional gametes $Ki\ t^0$ and ++ should be formed. The latter when uniting with a regular gamete t^0 would produce a normal-tailed exception, so that half of the crossovers should be detectable. All exceptions must of course be progeny-tested in order to separate overlaps (normal-tailed animals in which *Ki* is present but unexpressed) from crossovers.

In order to produce animals for the tests, the Kink progeny from the cross $Ki/+ \times +/t^1$ were tested by mating with Brachy ($T/+$). Progeny which were $Ki/+$ produced no tailless offspring and were rejected; while those which produced normal, Kink, and tailless offspring were assumed to be *Ki* +/+ t^1. Similar crosses produced *Ki* +/+ t^0.

Table 9 gives the results of the testing of those Kink animals which produced tailless when bred to Brachy. The females in each case gave the ratios expected from the cross $Ki/t^1 \times T/+$—that is, one-quarter normal ($+/t^1$) one-half Kink tail ($Ki/+$, Ki/T); one-quarter tailless (T/t^1); the males in all cases gave too many offspring in those classes receiving t^1 from the father,—that is, normal ($+/t^1$) and tailless (T/t^1). This has been shown to occur wherever t^1 or t^0 is transmitted by males and occurs similarly in combinations of *Ki* with t^1 and t^0. Those animals shown by test to be *Ki* +/+ t^1 or *Ki* +/+ t^0 could not be distinguished phenotypically from $Ki/+$—that is, neither t^1 or t^0 has any detectable modifying effect on *Ki*.

Those animals shown to be *Ki* +/+ t^1 were bred together; similarly *Ki* +/+ t^0 males were crossed with *Ki* +/+ t^0 females, and balanced lines of each were set up and maintained for several generations. The results are shown in table 10. Out of 279 offspring, all except four were typical Kinks. Of the four exceptions, only one could be tested. This proved to be Ki/t^0—that is, an overlap. These data provide no evidence of crossing over between *Ki* and t^1 or t^0. The lines were difficult to maintain, since litter size was small (due to the death of two classes of embryos: *Ki Ki* and t^0t^0 or t^1t^1), and many animals suffered from the defects caused by *Ki*—that is, waltzing or deafness.

ABSENCE OF RECOMBINATION BETWEEN *Fu* AND t^0 AND *Fu* AND t^1

Animals of the constitution *Fu* +/+ t^0 were produced by mating *Fu/Fu*

TABLE 9

Tests to detect Ki+/+t^1 and Ki+/+t^0 animals.

BRACHY ♀ (T+)×Ki+/+t^1 ♂				BRACHY ♀ ×Ki+/+t^0♂			
Ki/t^1 PARENT #	NORMAL	Ki	TAILLESS	Ki/t^0 PARENT #	NORMAL	Ki	TAILLESS
♂7624	27	3	29	♂7973	18	11	24
7625	45	17	49	8081	30	42	22
7693	24	17	39	8159	45	26	49
8033	6	—	2	8433	3	—	3
8186	3	4	2	8537	10	9	7
8315	4	—	9				
Total	**109**	**41**	**130**	Total	**106**	**88**	**105**
Exp.	70	140	70	Exp.	75	149	75
Ki+/+t^1♂×BRACHY ♂ T+				Ki+/+t^0♀ ×BRACHY ♂ T+			
♀7626	3	10	4	7970	5	8	2
7627	3	3	3	7971	5	7	4
7692	2	5	5	7972	6	6	2
8031	7	5	3		—	—	—
8032		4	3	Total	**16**	**21**	**8**
8038	2	4	3	Exp.	11	22	11
8087	2	3	4				
8088	7	9	2				
	—	—	—				
Total	**26**	**43**	**27**				
Exp.	24	48	24				

× +/t^0. The *Fu* offspring were tested by T/+ and those which gave tailless progeny (T/t^0) were selected and bred *inter se*. Similar experiments were carried out with t^1. Similar results were obtained with both experiments, and the results are combined in both discussion and tables.

The phenotypes *Fu* +/+ t^0 and *Fu* +/+ t^1 are indistinguishable from each other or from *Fu*/+. Neither t^0 nor t^1 acts as a strong modifier of *Fu*.

TABLE 10

Results of breeding "balanced" lines.
Ki+/+t^1 and Ki+/+t^0

PARENTS	OFFSPRING			RESULTS OF PROGENY TESTS
	KINK	NORMAL	AVERAGE LITTER SIZE	
Ki/t^0×Ki/t^0	107	2	3.3	1 n=Ki/t^0
Ki/t^1×Ki/t^1	172	2	3.6	

The results of the *inter-se* crosses of *Fu* +/+ t^0 × *Fu* +/+ t^0 (and of *Fu* +/+ t^1 × *Fu* +/+ t^1) are shown in table 11.

All except 36 of the progeny showed *Fu*. The normal-tailed exceptions may be either *Fu*/t^0 which fail to manifest *Fu*, or may arise from ++ recombination gametes—that is + +/+ t^0. To distinguish between these alternatives, 21 of the exceptions (12 ♂♂ 9 ♀♀) were tested by mating with *T*/+ (Brachy) and with normal (+ +). By normal mates, each exception produced *Fu* progeny, proving that an unexpressed *Fu* was present; by Brachy, each animal so tested produced tailless progeny (together with *Fu* and normal) indicating the presence of t^0 (or t^1) in the exceptional parent. All exceptions tested were thus shown to be *Fu* +/+ t^0—that is, they were normal overlaps and not recombinations.

TABLE 11

Results of matings of Fu+/+t⁰×Fu+/+t⁰ and Fu+/+t¹×Fu+/+t¹.

PARENTS	FU	TAILLESS	OFFSPRING NORMAL TAIL			TOTAL OFFSPRING	LITTER SIZE
			Tested *Fu*/t^0	Untested	Total exceptions		
Fu×*Fu*	472*	7	21	15	36	515	3.81

* Fourteen of these were recorded as normal-tailed at birth but as Fused at weaning.

It was necessary to progeny-test those *Fu* offspring of *Fu*/t^0 × *Fu*/t^0 matings which were to be used in further matings of *Fu*/t^0 × *Fu*/t^0 in order to eliminate the expected genotype *Fu Fu*. Out of 52 *Fu* tested by *T*/+ (Brachy), 44 proved to be *Fu*/t^0, and these only were used in further breeding; four proved to be *Fu Fu*, while in four cases the test was inconclusive. The number of *Fu Fu* detected is far below the expected proportion of one-third of the *Fu* offspring. There is other evidence that this is due to low viability of *Fu* homozygotes. Thus the litters from *Fu*/t^0 × *Fu*/t^0 were small (average 3.8), and abnormal animals were often found among the young at birth. One series of litters (from ♂ 10014) subjected to close scrutiny and dissection yielded four abnormals with imperforate anus, urethra or both, and four anaemics out of a total of 27 offspring dissected. Of the four homozygotes found, all had short abnormal tails. Of the seven tailless young from *Fu*/t^0 × *Fu*/t^0 none survived to be tested. The characteristics of *Fu* homozygotes will be discussed in another paper.

It may be concluded that no detectable recombinations of *Fu* with t^0 or t^1 have occurred in about 500 test progeny. Since only half of the crossover gametes (++) when combining with half of the non-crossovers (+ t^0) could be detected by the methods used, the test has only about 25 percent efficiency —that is, it contributes to the total tests of crossing over involving t^0 or t^1 about 125 negative observations.

EFFECT OF t^0 AND t^1 ON CROSSING OVER

The proof that crossing over occurs with some frequency in *Ki T*/+ + makes it possible to test whether t^0 and t^1 suppress crossing over in the interval *Ki–T*. Such tests can be made by mating *Ki T*/+ t^0 male or *Ki T*/+ t^1 male with t^0/t^1 females and comparing the results with those from *Ki T*/+ + × t^0/t^1.

Most of the tailless offspring from *Ki T*/+ + × t^0/t^1 may be expected to be *Ki T*/t^n, but a few of them may be + *T*/+ t^n (crossovers). In some cases another effect of *Ki* may be used to distinguish between these two genotypes. Many animals which carry *Ki* show the waltzing-deafness syndrome mentioned by CASPARI and DAVID (1940). From the tailless animals mentioned above, therefore, those may be assumed to be *Ki T*/t^n which show waltzing or which transmit it to their offspring. Ten tailless males met this test. Results obtained from these animals are shown in table 12.

TABLE 12

Tests of genotype and of recombination in Ki T/t^n *males.*

PARENTS		NORMAL	KINK	TAILLESS
MOTHER	FATHER			
normal (++)	*Ki T*/t^n*	175	54	12
normal (t^0t^1)	"	403	1?	432

* From *Ki T*/++×t^0t^1.

When crossed with normal females, such animals gave 175 normal and 66 Kink or tailless offspring. The excess of normal is due to the usual excess of t^n sperm formed by heterozygous males. The cross of *Ki T*/t^n males by t^0/t^1 females constitutes the crucial test. The expectation here is that non-crossovers will be *Ki T*/t^0 and *Ki T*/t^1 (tailless) and t^0/t^1 (normal-tailed), while one class of crossovers (*Ki* +/+ t^n) should be clearly detectable as Kink-tailed. The 836 offspring fell into the two non-crossover classes with one doubtful exception (table 12). About half were normal tailed (t^0/t^1) and half[3] were tailless (*Ki T*/t^n.

[3] The ratio in which these classes appear has little meaning, since it is known that *Ki T*/t^n males produce an excess of t^n sperm, and this should lead to an excess of normal-tailed progeny. The approximate ratio of *Ki T* to t^n sperm is given by the ratio of *Ki* to normal progeny in the cross *Ki T*/t^n × ++. This ratio was about 2.6 t^n:1 *Ki T* (175/66). From the cross *Ki T*/t^n×t^0t^1 we should thus expect offspring as follows:

SPERM	EGGS		TOTAL	EXPECTED		OBTAINED	
	t^0	t^1		NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
1 *Ki T*	1 tailless	1 tailless	2 tailless	363.2	43.5	432	51.7
2.6 t^0	dies	2.6 normal	2.6 normal tail	471.8	56.5	403	48.3

The actual result shows a considerable deficiency of normal-tailed offspring. This is undoubtedly

The one possible exception was an animal with a slightly shortened tail, like a Brachy, T +. When tested by tailless (Tt^0), it gave three normal and two tailless as it would if it were t^0t^1. Although this test is not adequate, it is probable that this was a phenotypic variant of the type t^0t^1 and not a valid crossover.

We can conclude that in the presence of either t^0 or t^1 crossing over between *Ki* and T probably does not occur. This confirms the conclusion drawn from experiments with Ki/t^0, Ki/t^1, Fu/t^0, and Fu/t^1 and shows that t^0 and t^1 suppress crossing over in the whole region which includes the loci *Ki*, *Fu*, and T. Since this region is probably at least eight crossover units in length, t^0 and t^1 are revealed as effective over a considerable stretch of chromosome and therefore are not point or gene mutations. A summary of data on the absence of crossing over in the presence of t^0 or t^1 is given in table 13.

TABLE 13

Data showing suppression of crossing over by t^0 and t^1.

COMBINATION TESTED	TOTAL PROGENY	TESTED CROSSOVERS	UNTESTED EXCEPTIONS	PERCENTAGE CROSSOVERS
KiT/t^0 or $t^1\times t^0t^1$	836	—	1	0.00
$Ki+/+t^n\times Ki+/+t^n$	279	—	3	0.00
$Fu+/+t^n\times Fu+/+t^n$	515	—	15	0.00
$T+/+t^n\times T+/+t^n$	4829	—	—	0.00
$t^0+/+t^1\times T/+$	2488	—	—	0.00

There is no evidence as yet as to what kind of change in the chromosome t^0 and t^1 represent. If they are gross chromosome changes, it is more likely that they are inversions than deficiencies, since the compound t^0/t^1 is viable and since both t^0 and t^1 cover the lethal effect of *Ki* and T (Ki/t^0, Ki/t^1, T/t^0 and T/t^1 are viable). In the combinations studied each has a greater effect on T than on either *Ki* or *Fu* since T/t^0 and T/t^1 are usually tailless, while Ki/t^0, Ki/t^1, Fu/t^0, and Fu/t^1 resemble $Ki/+$ and $Fu/+$. It might thus be supposed that T showed exaggeration when opposite t^0 or t^1. Too much stress cannot be placed on this, however, since in outcross lines of T/t^0 and T/t^1, animals resembling $T/+$ have been found; while $T/+$, which usually has a short-tail, may in certain combinations be entirely tailless. These facts show that factors other than T, t^0 and t^1 are involved in producing the tailless phenotype; and until these have been made identical or comparable in all stocks, arguments from the interaction effects of t^0 and t^1 will have little weight.

due to the heavy mortality suffered by t^0t^1 animals both before and after birth (DUNN and GLUECKSOHN-SCHOENHEIMER 1943). Both variables, sperm ratio and selective mortality of t^0t^1 embryos, differ quantitatively in different matings. Several *Ki* T/t^n males which were tested simultaneously by ++ and by t^0t^1 females regularly gave about 68 percent normal (+ t^n) to 32 percent *Ki* T offspring (actually 96:46) by ++ females and about 38 percent normal (t^0t^1) and 62 percent *Ki* T offspring by t^0t^1. With full viability of t^0t^1, the latter ratio should have been 51.5 percent normal. The deficiency of 13.5 percent represents the excess mortality of t^0t^1 over *Ki* T/t^n.

COMPARISON OF *Fu*, *Ki* AND *T*

As a result of the experiments described, we find that of the five mutations with similar effects, three (*Fu*, *T*, and *Ki*) are properties of distinct loci which can be readily separated by crossing over, while two (t^0 and t^1) are sectional changes which have not been separated or combined by crossing over. The question "how near together are the loci of these similar mutations" can thus refer only to the first three.

Because of difficulties in identifying recombination classes, only rough estimates of "distance" can be made. The most reliable is probably the interval *Ki–T*. Based only on progeny-tested exceptions, 39 crossovers in a total of 2065 offspring (with nine untested exceptions) were recognized, or 1.89 percent. If the nine untested exceptions were all crossovers, the maximum number would be 48 out of 2065 or 2.32. Since only one recombination class could be identified, the crossover percentage would be twice the percentage recognized, or from 3.78 (minimum) to 4.64 (maximum).

The *Fu* – *T* interval is similarly estimated to be about 4.26 to 5.16 percent. The data for *Fu–Ki* are the least numerous and reliable. The estimates for this interval vary from 1.98 to about twice this figure. The single crossovers detected in *Ki T* +/+ + *Fu* were probably in the *Fu*–*T* interval and do not increase our information on total distance. Although no single interval is equal to the sum of the remaining two, it is still likely that the maximum number of crossing over units between the most distant of the three loci is about eight, since individual intervals average about four or a little less.

DISCUSSION

The question to be asked therefore is: what is the likelihood that three similar mutations should occur by chance within an area of eight crossover units? To estimate this likelihood we need to know how many such loci there are in the mouse, and how many areas of eight crossover units. At present we have record of some ten loci at which have occurred mutations with effects on tail and axial skeleton similar to those of *Ki*, *T*, and *Fu*. The ten loci are: *T* (DUNN 1941); *Fu* (DUNN and CASPARI 1942); *Ki* (CASPARI and DAVID 1940); *Sd* (Short tail Danforth) (DUNN, GLUECKSOHN-SCHOENHEIMER, and BRYSON 1940); *st* (shaker short) (DUNN 1934); *sb* (stub) (DUNN and GLUECKSOHN-SCHOENHEIMER 1942); *tw* (twist) (DUNN and GLUECKSOHN-SCHOENHEIMER unpublished); *sc* (screw) (LAANES and MACDOWELL 1942); *fl* (flex) (HUNT et al. 1933); *pt* (pigtail) (CREW and AUERBACH 1941). If these loci are distributed at random among the 20 chromosomes of the mouse, the chance that any chromosome should have three of these loci is approximately $[(10!/3!7!)(1/20)^3(1-1/20)^7]20$. The chance for a group of four or more loci is negligibly small. We do not know the genetic lengths of mouse chromosomes, but if the average number of chiasmata is about two (CREW and KOLLER 1932), then the average map length per chromosome would be about 100, which would include about 12 segments of eight crossover units each. The chance that three similar loci should fall within one such segment is $(1/12)^2$; and the likelihood that all

three should fall in one chromosome and in one segment is the product of the above probabilities. This is about .001; or if we take the minimum length of the *Ki–Fu–T* areas as four crossover units, it is even less. In either case, it is highly improbable that the association is due to chance.

If these mutations are not distributed at random, then it is probable that their similarity is due to their nearness. There are three main ways in which such a condition might arise. All three mutations may represent replications or displacements of the same genetic material like the "repeats" discovered by BRIDGES (1935) in Drosophila; or partially overlapping deficiencies may have arisen in neighboring loci as MCCLINTOCK (1944) has found in maize; or an area of a chromosome may be differentiated in such a way that mutations occurring in it tend to affect the same parts of the body.

The only proved examples of mutations which owe their similarity to their derivation by replication of the same genic material are the cases of Bar and Double Bar (BRIDGES 1936; MULLER, PROKOFIEVA, and KOSSIKOV 1936) and the mimic mutations "Star" and "asteroid" in Drosophila (LEWIS 1945). Other possible cases of mimic mutations in Drosophila lying close to each other have been studied by GOTTSCHEWSKI (1936) and by OLIVER and GREEN (1944) in the case of mutations near the lozenge locus; while bithorax and bithoraxoid are so similar and so near (BRIDGES and BREHME 1944) that they, too, may represent mutations in repeated loci. In rabbits it may be significant that two phenotypically similar mutations Rex_1 and Rex_2 have been reported by CASTLE and NACHTSHEIM (1933) as closely linked.

The most complete cytogenetic analysis of such neighboring mimics is that described recently by LEWIS (1945) for the mutations at the Star and asteroid loci near the left end of the second chromosome of *D. melanogaster*. The analysis makes it highly probable that the mutations which have similar effects in making the compound eyes smaller and rougher than normal involve two loci, *S* and *ast*. These are separable by crossing over but are so close together that the map distance between them is of the order of 0.02 map unit. The two loci are located in the opposite halves of one chromosome element, which appears as a double band or doublet in the salivary gland chromosomes. The loci probably are repeats which had arisen from a single locus. Strong and characteristic position effects exist between the loci such that a mutant change at one of them, as *ast*, has a pronounced effect upon the manifestation of the other locus, such as *S*, depending upon whether the mutations are adjacent (*ast S*) or opposite (*ast/S*). LEWIS assumes that the position effect is due to the fact that the loci are repeats and extremely close together. He thus suggests that some groups of mutations which appear to act as multiple alleles may be resolved into adjacent repeated loci which, because of the common position effect, act as a developmental unit.

The series of mutations described in this paper might represent such a group of repeated loci. They were in fact originally regarded as allelic, and only when crossing over was proved to occur did it become evident that three loci were involved.

A repeat hypothesis applied to the present case would assume that an original locus $+^T$ may have been triplicated to become $+^T +^{T1} +^{T2}$. One locus may then have mutated to *T*, another to *Ki*, and a third to *Fu*. The mutations t^0 and t^1 which suppress crossing over in this area might be assumed to be rearrangements such as inversions involving these loci.

Since there is at present no cytological evidence of such repeated loci, this hypothesis cannot be decisively tested. However, three considerations make it improbable. In the first place, *T*, *Ki*, and *Fu* are probably not immediately adjacent, since a relatively large amount of crossing over occurs between each pair of loci. Second, both *Ki* and *T* act as early lethals—that is, the recessive lethal effect of *Ki* is not "covered" by the duplicated normal loci which are present. In our notation homozygous *Ki* would be $+^T Ki +^{T2}/+^T Ki +^{T2}$. If $+^T$ and $+^{T2}$ contain the same genetic material as $+^{T1}$ (that is, $+^{Ki}$), this should suffice for viability regardless of mutations at one of the duplicated loci. The third objection to the repeat hypothesis comes from our present knowledge of the developmental effects of *T*, *Ki*, and *Fu*. On the repeat hypothesis one should expect alterations in the duplicate loci to act in similar ways like the similar developmental effects of mutations at the same locus. Although evidence on this is not yet complete, unpublished observations of GLUECKSOHN-SCHOENHEIMER and DUNN make it highly probable that *Ki* and *T* differ markedly in their effects on early development.

As CHESLEY (1935) showed, *T* acts upon the development of the notochord and cells ancestral to it, *T T* embryos having no notochord; the effects of *T* on other axial structures are apparently due to the dependence of these other parts upon influences emanating from the notochord. In *Ki Ki* embryos, on the other hand, the notochord is probably normal, and the most important departure from normal is an over production of embryonic tissue which results in doubling of certain parts such as axis, heart and others. The similar phenotypes of *T* + and *Ki* + seem to be reached in different developmental ways. The effects of *Fu* are probably different from those of either *T* or *Ki*. It is also to be recalled that *T* has never been found to effect behavior or hearing, whereas both *Fu* and *Ki* do. There is thus less evidence of basic developmental similarity than we should expect if all three mutations arose from replicated loci.

The second possibility, that the five neighboring mutations may represent partially overlapping deficiencies, is suggested by the recent results of MCCLINTOCK (1944). Two recessive mutations affecting seedling color—namely, pale yellow (*pyd*) and white (*wd*)—were shown to be small deletions in adjacent segments such that *pyd/pyd* was pale yellow and inviable beyond the seedling stage, *wd/wd* was white and inviable, while *pyd/wd* was pale yellow rather than normal as expected, since in *wd* the material containing the normal allele of *pyd* has also been deleted. Another mutation, yellow green seedling yg^2 was found to be a property of the chromatin that was deleted in *wd*, since yg^2/pyd gave normal seedlings, whereas yg^2/wd gave yellow green. Two series of allelic relationships are thus established: I, green > *pyd* > *wd;* and II, green > yg^2 >

wd. One might expect *pyd* and yg^2 to interact as alleles, but this combination gives normal green color. This situation resembles the relations of the two lethals t^0 and t^1 since t^0/t^1 may be normal; while T/t^0 and T/t^1, although viable, each produce a mutant phenotype, tailless, which may represent an exaggeration of the effect of T. One might thus think of T as a deficiency including the loci at which mutations to t^0 and t^1 have occurred—that is, t^0 and t^1 behave as "pseudo-allelic" to T, in the sense in which BRIDGES (MORGAN, BRIDGES, and SCHULTZ 1938) used the term. The effects of t^0 and t^1, however, are not confined to single loci, since they prevent crossing over in the whole area studied. *Ki* could be represented as a deficiency within the area covered by t^0 and t^1, and possibly *Fu* also, although since *Fu Fu* may be viable, it could not lack much genic material.

MCCLINTOCK has described the methods by which such allelic or pseudo-allelic "mutations" occur in maize as a result of chromosome breakage which repeatedly induces mutants with the same small deficiencies and the same phenotypic changes. A similar mechanism may have operated to produce the five mutations described in this paper.

However, there are marked differences between the mutations encountered in mice and those studied by MCCLINTOCK in maize. The maize mutations were all recessive, whereas three of the mouse mutants are dominant, hence the phenotype in the latter cannot be due to a homozygous deficiency. In mice, moreover, all of the dominants are separable by crossing over, and thus properties of *different* areas of chromatin. Even though the mechanism responsible for the origin of the maize mutations should also have produced the several changes in the mouse, we should still be left without an explanation as to why different neighboring blocks of chromatin should produce similar but not identical phenotypes. Nor is this relationship clear in MCCLINTOCK's case. Pale yellow and white phenotypes seem to be due to losses of specific loci in adjacent chromomeres, but there is no indication whether these properties are associated with adjacent chromomeres by chance or by some factor resulting from propinquity. The type of change (loss or reduction of chlorophyll) is so frequent in plants that it may be the common expression of a great variety of changes, any one of which might have a good chance of occurring in any block of chromatin. Such seedling mutations may well be the phenotype toward which many small deficiencies converge, and thus their resemblance may be unconnected with their proximity. For these reasons it is doubtful if analogy with the maize mutations can shed much light on the main question at issue—namely, the relationship between nearness in the chromosome and phenotypic resemblance.

The third hypothesis assumes that the area in which these mutations occurred is so constituted that mutative changes in it are likely to affect the axial skeleton and related structures. This is not to say that among the chromosomes of the mouse there is a "tail area" or a "spinal area" where genes regulating these parts are located. Such relationships between genes and development apparently do not exist as a rule and are made improbable in special

cases by the epigenetic nature of development and the dependence of part upon part and gene upon gene. But it is conceivable and examples exist that mutative changes of a certain type are always accompanied by the same type of developmental change. One of the earliest examples of this was the mutant change "Minute bristles" in *Drosophila melanogaster*. Many mimic or duplicate "Minute" mutations had this in common, that each was lethal when homozygous and each involved the deletion of a small section of chromosome. In addition all of the Minutes which were tested for effect on rate of development were found to delay the growth of the larvae and the onset of pupation (SCHULTZ 1929; DUNN and MOSSIGE 1937; BREHME 1939). The areas in which Minute mutations have been recorded are too numerous and too scattered to make it at all likely that one or a few repeated loci are responsible. It is more probable that deletion of a certain quantity of genic material so disturbs development at the time when bristles are being determined as to regularly cause this process to fall below some normal level and to result in small bristles. The numerous mutants in maize in which the amount of chlorophyll is reduced (albino, luteus, virescent and pale types generally) perhaps also represent a convergent response of many different small deficiencies which upset the chlorophyll-forming mechanism in similar ways.

However, amount of genic material deleted cannot be the sole determinant of such phenotypes as "Minutes" and chlorophyll deficient mutants, since the individual mutants differ among themselves. Whether these differences are correlated with the extent of deficiency (in the case of Minutes) is not known; but it is evident that the phenotype depends also upon the kind of genic material which is changed, since not all deficiencies are accompanied by Minute or by chlorophyll effects. The kind of change in the chromosome by which the mutation occurs may also be important in producing the convergence, but of this little is known.

In the present case the mutations *T*, *Ki* are lethal and *Fu* is sub-lethal; all are dominant and all converge on a similar phenotype in respect of tail form. It might be assumed then that the area of chromosome in which they and t^0 and t^1 occur is by its structure such that any mutation in it is likely to be so extensive that it will disturb development at the time when the form of the tail and spinal column is being determined. Tail abnormalities, on this view, would be common responses to developmental disturbances, just as "Minute" mutations seem to be; the response would be determined by the "size" of the mutation, and changes in the section of chromosome in question would be more likely to reach the required "size" by reason of structural peculiarities. The form-dependence ("organizer") relationships between the parts of the developing axial structures (notochord, spinal column and cord) and between these and other systems such as the urogenital system and the gut probabaly rest on easily disturbed equilibria, since correlated abnormalities in these parts are common both from genetic and non-genetic causes (DUNN and GLUECKSOHN-SCHOENHEIMER 1944). Thus the responsiveness of the developing parts is probably of equal importance with the susceptibility to change of some particular segment

of chromatin. Since we have no cytogenetic evidence of the type of change involved, this hypothesis has not been tested. Evidence concerning the developmental relations of the effects of the five mutants discussed above will be published later.

SUMMARY

In the house mouse five mutations have been studied which produce similar effects causing shortness or absence of the tail or fusion of neighboring vertebrae, as well as certain other effects.

Breeding experiments involving some 5000 test progeny have shown that all five mutations are located near together in the same chromosome. The three dominants, *T*, *Fu*, and *Ki*, are separated from each other by distances of from two to five crossover units and are contained within a chromosome segment having a maximum length of about eight units. Two recessives, t^0 and t^1, appear to be sectional changes, since they suppress crossing over throughout this segment.

Three possible explanations of the relation between the contiguity of these mutations in the chromosome and the similarities in their developmental effects are considered. The first, that contiguity is due to chance, is rejected since the probability that three such similar mutations should chance to occur within one chromosome segment of eight units is only about 0.00007.

It is likewise considered unlikely that they represent "repeats" of one original locus, as in the case of Star and asteroid mutations of Drosophila (LEWIS 1945), since they are not immediately adjacent, and the lethal effect of a mutation at one locus is not "covered" by the other unmutated loci. Similar reasons indicate that they are probably not partially overlapping deficiencies like the group of neighboring mutations in maize described by MCCLINTOCK (1944).

It is suggested that the segment of chromosome in which these mutations occurred is so constituted that any change in it is likely to be extensive and to cause abnormalities in early developmental processes, the effects of which converge on the axial structures (spinal cord, notochord, etc.) which have "organizer" relationships with each other and with earlier processes.

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