THE POSITION OF *ru-2* AND *qv* WITH RESPECT TO THE *FLECKED* TRANSLOCATION IN THE MOUSE[1]

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THE *flecked* (*fd*) translocation [T(X;1)Ct], first described by CATTANACH (1961), is regarded as a 3-point nonreciprocal translocation in which a piece from the central region of linkage group I (L.G.I.) (EICHER 1967a; 1967b) has been moved to the middle of the X chromosome (X^{fd}) (CATTANACH 1966). Since the autosomal I piece translocated to the X^{fd} chromosome appears to have been inverted (EICHER 1967b; SLIZYNSKI 1967; and OHNO, as reported by CATTANACH and ISAACSON 1967), crossing over between the X^{fd} and I chromosomes would lead to the production of a dicentric chromosome and an acentric fragment and finally to unbalanced gametes. Therefore, the mapping of the two break points in L.G.I could not be accomplished using conventional methods. However, the position of the two break points was accomplished using a procedure analogous to deletion mapping methods but involving a comparison of the disomic and trisomic conditions of different genes (EICHER 1967a, 1967b; WOLFE 1967). In this way, the break points in L.G.I were shown to reside between two genes separated by 2 centimorgans (cM), *sh-1* and *Hbb*, *b.p.1* (break point 1), and between *p* and *pu*, separated by 22 cM, *b.p.2* (break point 2). Preliminary evidence seemed to indicate that the *qv* gene (quivering) was not transferred to the X^{fd} chromosome (EICHER 1967b) thus placing *b.p.2* between *qv* and *p*. (See Figure 1 for a map of L.G.I and gene designations).

LILLY (1966) reported a new gene in L.G.I, ruby-eye-2 (*ru-2*), which was closely linked to *p* but whose relative order with respect to *c* was not known. It was hoped that, if the gene order was *c–p–ru-2*, the location of *ru-2* with respect to the X^{fd} chromosome could more specifically pinpoint *b.p.2*.

In addition, the positions of *b.p.1* and *b.p.2* were estimated by means of another method.

MATERIALS AND METHODS

Flecked animals can exist in two common forms, Type I and Type II (CATTANACH 1961). Type I animals carry an X^{fd} chromosome, one normal autosome I, and a chromosome I (I^{Df}) carrying a deletion for the region translocated to the X chromosome. Type I animals possess the balanced form of the *fd* translocation. The other form, Type II, has an X^{fd} and two normal auto-

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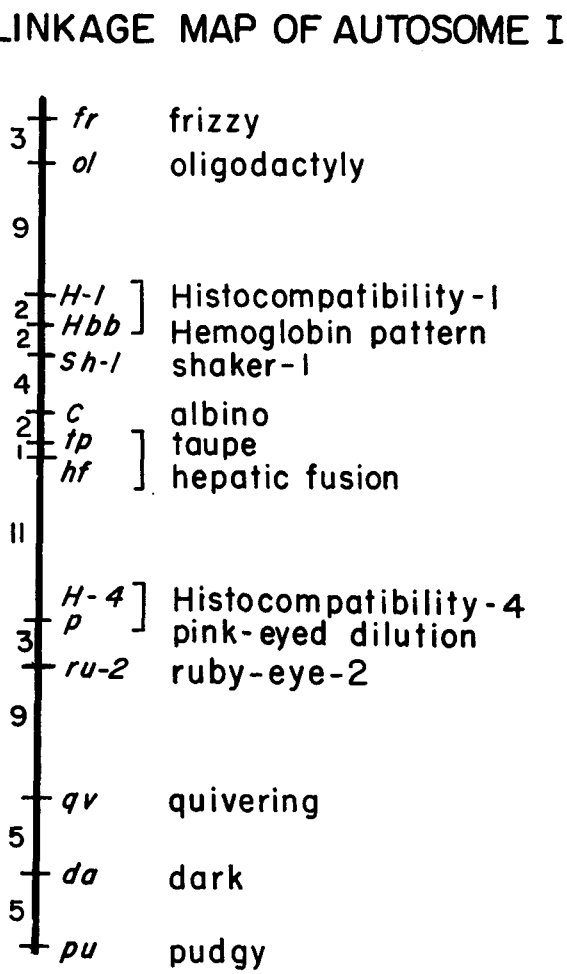

FIGURE 1.—Linkage map of chromosome I, modified after GREEN (1966). The brackets indicate uncertainty as to actual gene order. The numbers are centimorgans.

somes I. Type II animals carry the unbalanced form, being trisomic for the piece of autosome I carried in the X^{fd} chromosome. When crossed to normal animals, Type I mice have Type I, Type II, and normal offspring, whereas Type II mice have only Type II and normal progeny.

Type I and Type II females can be distinguished by their respective mosaic phenotypes as expressed when a mutant allele is carried in autosome(s) I and the wild-type allele is located in the X^{fd} chromosome. The situation seems to be an example of a variegation position-effect (CATTANACH 1961) as described in the reviews of Lewis (1950) and BAKER (1968) and to be specifically explained in the case of the *fd* translocation by the inactivation of autosomal genes brought into physical contact with X chromosomal material (LYON 1963; RUSSELL 1963). Type I and Type II males are normal in appearance (they only express the wild-type allele in the X^{fd} chromosome) but Type I males are usually sterile whereas Type II males are usually runts but fertile.

Since the original translocation occurred in a CBA strain carrying the wild-type alleles for all

the mutant autosomal I genes considered in this report, *fd* mice will be assumed to carry only wild-type alleles on the X^{fd} chromosome.

The original strains and stocks are as given in EICHER 1967a. In addition, the quivering stock was kindly supplied by Dr. WALLACE MCNUTT, The University of Texas. The stock is maintained in heterozygous condition for *qv* since *qv/qv* mice are usually sterile. In addition, the stock is homozygous for *c* (albino) and is segregating for *b* (brown) and *s* (piebald spotting).

Dr. FRANK LILLY generously supplied the *ru-2* gene on two different backgrounds: 1. C57Bl/6–*ru-2* : *ru-2* first appeared as a mutation in the C57Bl/6 strain. We received F_7 mice. 2. BJR : a *p ru-2* stock originally produced by crossing C57Bl/6 × JK (*p/p*) animals to obtain F_1 animals designated BJ. After several generations of inbreeding, the BJ animals were crossed to C57Bl/6–*ru-2* animals. Homozygous *p ru-2* animals were extracted and called BJR (F2). We received F_7 mice.

The strain BALB/Ci (*b;c*) was kindly supplied by Professor ERNST CASPARI. The CBA/J-p^{2J} strain was obtained from the Jackson Laboratory. The origin of the JB stock homozygous for c^{ch} (chinchilla) and *p* has previously been described by EICHER 1967a and 1967b.

Because of the problems involved in each specific cross, the exact methods involving each separate gene will be considered in the RESULTS AND DISCUSSION section. The argument as to the location of a specific gene with respect to the X^{fd} or I^{Df} chromosome is given in EICHER 1967a and 1967b. The nomenclature used in this paper will be the same as that used in EICHER, 1967a, with the following exception: when a given gene is known to reside in the I^{Df} chromosome, it will be included in this chromosome. For example, instead of a Type I male being designated *fd*/Y; *pu*/Df (Df=deficiency), he will be represented by the symbolism *fd*/Y; *pu*/Df pu^+ since, as in the case of the pu^+ allele, the gene in question is located on the I^{Df} chromosome.

RESULTS AND DISCUSSION

The analysis of ru-2: *position in linkage group I:* The *ru-2* allele affects pigmentation, resulting in a grayish-brown color in the eumelanin region of the agouti hair and a dark red color of the eye. The pheomelanin part of the hair does not seem to be strongly affected. One might say the *ru-2* gene seems to be a "less intense" *p* gene. The effect of *ru-2* at the cellular level is not presently known.

Since the exact location of *ru-2* in L.G.I was not known, the determination of its position relative to *c* and *p* was undertaken using method A and B.

Method A: F_1 (♀BALB *c/c* × ♂BJR *p ru-2/p ru-2*) males were crossed to JB females (c^{ch} *p*/c^{ch} *p*). All +/*p* offspring were raised and tested for the presence of the *ru-2* allele by crossing them to C57 Bl/6–*ru-2* animals. It was required in the experiment that a minimum of 7 wild-type young be born to classify each animal as homozygous wild type for the *ru-2* gene. Two possible outcomes may be expected depending on the gene order: 1) *c–p–ru-2*, or 2) *c–ru-2 –p*. For the first order, in +/*p* animals proven to carry the *ru-2* allele, more c^{ch}/c are expected than $+/c^{ch}$ (wild type). For the second order, more $+/c^{ch}$ are expected to carry *ru-2* than c^{ch}/c. Although this cross permits the relative ordering of *c,p*, and *ru-2* and the determination of the *p–ru-2* map distance, it requires testing half of the F_1 offspring for *ru-2*.

Method B: F_1 (♀BALB *c/c* × ♂BJR *p ru-2/p ru-2*) animals were backcrossed to the BJR strain. All animals with nonpigmented eyes (*p ru-2/p ru-2*, *p ru-2/p* +, or *p ru-2*/+ *ru-2*) were raised and scored as to their pink-dilution ruby phenotype. All *p ru-2*/+ *ru-2* animals were then crossed to BALB animals to test for the presence of *c*. If the gene order is *c–p–ru-2*, +/*c* offspring would be the result

of a single crossover and +/+ offspring would be the result of a double crossover. If the order is *c–ru-2–p*, the reverse is expected. This cross allowed for the establishment of the order *c–p–ru-2* and the *p–ru-2* distance. Furthermore, it only involved the raising of animals resulting from crossing over between *p* and *ru-2*.

Of the offspring born using Method A to JB females × F1 males, 29 +/*p* females and 31 +/*p* males were tested for the presence of *ru-2*. Of these 60 animals, only one female (c^{ch} *p* +/*c* + *ru-2*) proved to carry *ru-2*. This suggested a gene order *c–p–ru-2* and a 1.7 cM distance for *p–ru-2*.

The results of method B are given in Table 1. Of the 9 known +/*p* animals, one female died before she could be genetically tested for the presence of *c*. The other 8 animals (4 females and 4 males) proved to be carrying the *c* allele. This establishes the gene order as *c–p–ru-2* and estimates the *p–ru-2* distance as 2.7 cM. It should be stated that equal viability of *p ru-2*, *p*, and *ru-2* animals was assumed in making the calculations.

LILLY (personal communication) obtained a 3.7 ± 1.2% recombination frequency (9/244) using the cross ♀F1(BJR × C57/Bl) × ♂BJR. He obtained a 2% value using the F_2 generation, averaging male and female values. LILLY's estimate of the *ru-2–p* distance and the estimate obtained in our laboratory are in good agreement.

In cross B, there appeared a few homozygous *p* animals which displayed a grayish shade of color in the eumelanin area of their hair compared to the tannish color displayed by the BJR animals. This difference was most pronounced in non-agouti animals. One of the grayish females was mated to a BJR male: her offspring showed a 1:1 segregation for the grayish to tannish color (4♀ 3♂: 4♀ 3♂). These 14 young were crossed to C57 Bl/6–*ru-2* mice to test their possible *p ru-2/p ru-2* versus *p ru-2/p* + genotype. The 7 grayish animals proved to be +/*ru-2* whereas the 7 "BJR"-like animals proved to be *p ru-2/p ru-2*. In addition two other grayish females and three grayish males from the original cross were shown to be of a genotype +/*ru-2*, whereas one tannish female and one tannish male were *ru-2/ru-2*. It is concluded that these grayish offspring are the reciprocal crossovers of the *p ru-2*/+ *ru-2* crossovers.

TABLE 1

Results of cross F_1 c + +/+ p ru-2 × + p ru-2/+ p ru-2 *to determine* p-ru-2 *map distance*

	+ *	At birth p &/or ru-2	Total	p(ru-2?)	At weaning ru-2	Total
Cross: ♀ F_1	271	250	521	216	7	223
Cross: ♂ F_1	122	129	251	116	2	118

% recombination† in ♀♀ $= \frac{7}{223-7} \times (100) = 3.24$

% recombination† in ♂♂ $= \frac{2}{118-2} \times (100) = 1.72$

% recombination† $= \frac{9}{341-9} \times (100) = 2.71$

* All killed at birth.
† Assuming equal numbers in reciprocal class.

Since the young in cross B were generally kept until 2 weeks of age, scored and sacrificed, the agouti young could not be successfully scored for the *p ru-2/p* + condition as the difference is seen in the eumelanin part of the hair. The number of *p ru-2/p* + animals were estimated for Table 1 from the reciprocal backcross: six were recovered by testcrosses. The phenotypic difference between *p ru-2/p* + and *p ru-2/p ru-2* is slight, but clear.

The analysis of ru-2*—position in the* fd *translocation*: Several different types of crosses involving *ru-2* and *fd* were carried out to establish the position of *ru-2* with respect to the *fd* translocation. Originally, females *fd*/+; *c/Df* were crossed to C57 Bl/6–*ru-2* males. Since no *ru-2* offspring were born (15 females, and 18 males), the *ru-2* gene had not been deleted but resided either on the X^{fd} or on the I^{Df} chromosome. Because of lack of mosaicism of the coat in the fifteen females raised (all were +/*a*), it was assumed that either 1) the *ru-2*+ allele was in the I^{Df} chromosome, or 2) *ru-2*/Df was lethal in cells where the X^{fd} chromosome was the inactive X chromosome, or 3) the *ru-2*/Df condition did not express the ruby phenotype. Four of the fifteen females were crossed to JB (c^{ch} *p*) males to determine their Type I, Type II or normal condition. Three of these proved to be Type I and the remaining female was Type II. The problem as to the position of *ru-2* was then attacked using two different crosses, a and b.

Type I females *fd*/+; *c*/Df were crossed to BJR males. The daughters expressing pink-eyed dilution spots were Type I *fd*/+; *p ru-2*/Df(*ru-2*+?). These Type I females were crossed to two different stocks of *ru-2* males.

Cross a: ♀ *fd*/+; *p ru-2*/Df (*ru-2*+?) × ♂ +/Y; *ru-2/ru-2* (C57 Bl/6–*ru-2*). It was of interest to observe whether a 1:2 or 2:1 ratio of ruby:+ eyes would result at birth. In crosses involving *c* or *p*, all *fd* animals express + eyes (the females generally having lighter eyes than the males), giving a theoretical ratio of 1 nonpigmented: 2 pigmented young. If the *ru-2*+ allele were located on the I^{Df} chromosome, a 2 nonpigmented: 1 pigmented ratio would be expected. In addition, a phenotypic difference between Type I and Type II female was possible.

The results of cross a are shown in Table 2. A 2 ruby:1+ ratio as shown by the pigmentation of the eye was observed at birth (167 ruby: 87 +), indicating that the *ru-2*+ allele is present in the I^{Df} chromosome. All the + animals scored as such at birth appeared completely wild-type at weaning. However, it was noted that several *ru-2* animals expressed a darker ruby eye color than had been previously observed in the C57 Bl/6–*ru-2* strain. Many females classified as *ru-2* at birth (some noted as having darker eyes) showed at weaning dark ruby eyes plus a mosaic coat consisting of a color intermediate between + and ruby (to be called intermediate) and of the ruby color. Six males expressed the dark ruby eyes and a whole-body coat of intermediate color. There were, in addition, nonmosaic-appearing ruby females and males.

The three phenotypically different types of females and the five intermediate males were tested for their Type I, Type II or normal (non-*fd*) condition as follows:

Females of the three phenotypes (ruby, mosaic and wild type) were crossed to BALB (*c*) males. Type I females would be expected to produce female offspring

TABLE 2

Offspring from cross: ♀ fd/+; p ru-2/*Df* (ru-2+?) × ♂ +/Y; ru-2/ru-2)

	♀	♂	Total
At birth:			
ru-2	96	71	167
+	43	44	87
Total	139	115	254
At weaning:			
ru-2	44	42	86
intermediate	0	6	6
mosaic	26	0	26
Subtotal	70	48	118
+	24	34	58
Total	94	82	176

in a ratio of 2 +: 1 mosaic for albino. Type II and non-*fd* females would not produce mosaic female offspring.

The mosaic and phenotypically normal ruby females were also crossed to JB (c^{ch} *p*) males. Type II females would be expected to produce female offspring in a ratio of 3 +: 1 mosaic for *p*. Non-*fd* females would produce offspring in a ratio of 1 +: 1 p. In addition, only Type II and non-*fd* females would be expected to carry the *p* allele, therefore producing non-*fd* p offspring (ratio at birth of 3 +: 1 p for Type II females and 1 +: 1 p for non-*fd* females). One further distinction which could be made from these crosses was that Type I females would have female offspring showing mosaicism for c^{ch} and *p*, not just *p*.

A total of 50 females were testcrossed: the results of the crosses involving these females are presented in Table 3.

It was clear that all tested wild-type females were Type I (17), the 19 mosaic females were Type II, and the 14 *ru-2* females were non-*fd*. One female (cross 1) died before a sufficient number of offspring were produced: in a total of 36 offspring, 20 were males, 8 females died before a classification for albino mosaicism could be made, and 8 females were wild type. From one litter which was isolated, *fd* females were born. Testes from six adult male offspring from the original female's last two litters were checked for size: 2 were normal, 1 was intermediate, and 3 were very small. Since Type I males generally have small testes, this strongly suggests three of the males were, in fact, Type I. Therefore, it is assumed that the original female was Type I.

Of the 5 intermediate-colored males testcrossed to CBA/J-p^{2J} or JB (c^{ch} *p*) females, 1 died before siring any young and 3 proved to be Type II males (they had *p* male offspring and their mosaic female offspring displayed the *p*/*p* (not *p*/Df) color in the mutant color patches. Only non-*fd* and Type II offspring from cross a males can carry the *p* allele. The remaining questionable male was not successfully tested; however, mitotic figures from bone marrow cells appeared to contain a large chromosome morphologically like the X^{fd} chromosome.

TABLE 3

Results of crosses to establish the fd *constitution of the young obtained in Table 2*

Cross	Number tested	Number proven	Comments
1. ♀ (*fd*/+; *ru-2*/Df)? × ♂ +/Y; *c*/*c* wild type	17	Type I = 15 1 died—no young 1 died—not sufficient young	15 of ♀ ♀ had ♀ offspring showing mosaicism: wild/albino
2. ♀ (*fd*/+; *p ru-2*/+ *ru-2*)? × ♂ +/Y; *c*/*c* mosaic: intermediate/ruby	7	not Type I = 7	59 + ♀ young—not mosaic
3. ♀ (+/+; *p ru-2*/+ *ru-2*)? × ♂ +/Y; *c*/*c* ruby	10	not Type I = 10	113 + ♀ young—not mosaic
4. ♀ (*fd*/+; *p ru-2*/+ *ru-2*)? × ♂ +/Y; c^{ch} *p*/c^{ch} *p* mosaic: intermediate/ruby	12 6 (from cross 2)	Type II = 18	All had mosaic: ♀ young = wild/p (non c^{ch})
5. ♀ (+/+; *p ru-2*/+ *ru-2*)? × ♂ +/Y; c^{ch} *p*/c^{ch} *p* ruby	4 3 (from cross 3)	not Type I and not Type II = 7	young born: + p ♀ 77 68 ♂ 51 51 75 of 77 ♀ + raised, not mosaic
Total females tested	50		

Since Type I females are known to give rise to three kinds of offspring when mated to chromosomally normal males, it was concluded that all the wild-type animals were Type I, the ruby animals were non-*fd*, and the intermediate-ruby mosaic females and intermediate males were Type II. This conclusion is supported by the data presented in Table 3.

Without the testcrosses of all three phenotypes, it would have been concluded that the *ru-2*+ allele was located in the I^{Df} chromosome. The phenotype of Type II females and males proves however, that *ru-2*+ is located in the X^{fd} chromosome. Since these crosses were made, a few Type I females *fd*/+; *ru-2*/Df, all *a*/*a*, showed some *slightly* different colored regions on an otherwise wild-type background. Of the 17 Type I females tested in cross I, Table 3, 9 were +/*a* and 7 were *a*/*a*. One of the *a*/*a* females was initially recorded as "possibly showing" color differences in some areas of her coat. The finding of mosaic areas in Type I ruby females is only possible if *ru-2*+ is located in the X^{fd} chromosome.

The data show clearly that the +/*ru-2*/*ru-2* condition is not wild type as has been found for the genes *c* and *p* but that the two recessive *ru-2* alleles do express themselves to some degree. Because of this fact, it seems even more puzzling that a theoretical *ru-2*/Df condition is not clearly expressed in mosaics. The problem may be due to relative viability of *ru-2*/Df compared to *ru-2*/*ru-2* cells. In Table 2, the number of Type I females to non-*fd* females at weaning was 24 to 44, respectively. The expected ratio is 1:1. The difference observed compared to expected is significant ($.025>P>.01$). Another possibility may be that the extent of inactivation of the autosomal region in the X^{fd} chromosome may be different in extent in Type I compared to Type II females.

Cross b: ♀ *fd*/+; *p ru-2*/Df (*ru-2*+?) × ♂ +/Y; *p ru-2*/*p ru-2* (BJR). In the offspring from this cross a phenotypic difference between Type I and Type II females would be visible by the expression of the *p* gene (mosaic spots of *p*/Df versus *p*/*p*, respectively.) Furthermore, the ratio of ruby:+ eyes would be informative (reasons as given in cross a). Here ruby indicates all light-eyed animals. In Table 4 are presented the results of the cross. At birth the ratio of ruby:+ eyes was 52:23, or a 2:1 ratio. However, all the dark-eyed animals were wild type at weaning for the ruby phenotype whereas the ruby-eyed animals were either homozygous *p ru-2* or some females showed a mosaicism of the fur for p ru-2 areas on an intermediate-colored background. No intermediate-colored males survived. However, from a cross of ♀ *fd*/+; *p ru-2*/ *p ru-2* × ♂ +/Y; *p ru-2*/*p ru-2* it is known that an *fd*/Y; *p ru-2*/*p ru-2* male expresses the intermediate phenotype.

Therefore, from cross b, six phenotypically different types of young can be obtained (the location of the *ru-2*+ allele in the X^{fd} chromosome will now be used to aid the reader to interpret the results (see Table 5)).

Three different types of female offspring are expected from a Type I female, if the gene in question is located in the X^{fd} chromosome. The results from cross b support the conclusion drawn from the previous cross.

In the Type II female offspring in cross b, there appeared to be a third color in the coat in addition to the *p*/*p ru-2*/*ru-2* spots present on the intermediate

TABLE 4

Cross: ♀ fd/+; p ru-2/*Df* (ru-2+?) × ♂ +/Y; p ru-2/p ru-2

	♀	♂	Total
Eye color at birth:			
+	13	10	23
light*	27	25	52
Total	40	35	75
Fur classified:†			
+	6	8	
mosaic	11	0	
intermediate	0	0	
p ru-2	8	13	

* Includes all *fd* II and non-*fd p ru-2* animals.
† Some young were killed before classification: the actual relative viabilities cannot be computed in this cross. However, in this cross no Type II *fd* ♂ survived.

background (the p areas are lighter than usual and are assumed to be homozygous *p ru-2*). The identification of these spots as to pink-dilution or ruby could not be made with certainty since it is not known how a *p/p* spot would be expressed on an intermediate background. The spots were relatively small. Unfortunately the *ru-2* gene causes difficulty in classification because there are differences between animals and within an animal as aging proceeds, and because *p* does interfere with *ru-2* identification. The *ru-2* gene is being combined with another gene where it is hoped this difficulty can be overcome.

TABLE 5

Cross involving ♀ fd/+; p ru-2/*Df* × ♂ +/Y; p ru-2/p ru-2

fd condition	Sex	Genotype	phenotype† Coat	Eyes
Type I	♀	*fd*/+; *p ru-2*/Df	Mosaic: +; p(ru-2)	dark (wild type)
Type II	♀	*fd*/+; *p ru-2/p ru-2*	Mosaic: intermediate; p/p(ru-2/ru-2)†	light red*
non-*fd*	♀	+/+; *p ru-2/p ru-2*	p/p; ru-2/ru-2	very light red
Type I	♂	*fd*/Y; *p ru-2*/Df	+	dark (wild type)
Type II	♂	*fd*/Y; *p ru-2/p ru-2*	intermediate	very dark red
non-*fd*	♂	+/Y; *p ru-2/p ru-2*	p/p; ru-2/ru-2	very light red

* In a ♀ *fd*/+; *p/p*, the eyes are lighter than wild type but not p. Mosaicism can be seen under a dissecting microscope in the iris and retina tissue. Females *fd*/+; *ru-2/ru-2* have an eye color definitely darker than non-*fd ru-2* animals. Mosaicism is also seen under the microscope. The interesting feature about the eye pigmentation in *fd*/+; *p ru-2/p ru-2* females is that the eye is a very light color similar to the color produced by the *p* allele. There is a definite interaction of *p* and *ru-2* to lighten the eye in Type II *fd* females beyond what either *p* or *ru-2* accomplish alone on the *fd* background.
† Mosaicism for both ruby and pink-dilution or just pink-dilution is hard to classify.

The analysis of quivering: Quivering animals show an instability of gait at approximately two weeks of age which increases with age. In general, animals were not scored for *qv* until weaning. Since *qv* cannot be maintained in homozygous condition, each animal from the *qv* stock had to be tested for the presence of *qv* before it could be used in crosses involving the *fd* translocation.

Females *fd*/+; c^{ch}/Df were crossed to known *c* +/*c qv* males. Daughters *fd*/+; c^{ch} +/*c qv*? and *fd*/+; *c qv*?/Df (qv^{+}?) were backcrossed to known *c* +/*c qv* males from the *qv* stock. There were a total of 16 Type II and 18 Type I females tested for the presence of *qv*: one half of these females would be expected to carry *qv*. Of the 16 Type II females, 9 proved to carry *qv*, 5 proved to be qv^{+} and 2 died before a sufficient number of offspring were classified. Of the 18 Type I females tested, 5 carried *qv*, 4 were qv^{+} and 9 died or were killed before a sufficient number of young were scored.

Of the five Type I females proven to carry *qv*, all were completely normal. It was concluded that qv^{+} was either in the X^{fd} chromosome or the I^{Df} chromosome: qv^{+} had not been deleted during the production of the original translocation.

All offspring from females proven to carry *qv* are considered in Tables 6 and 7. There were many litters in which no young survived. The quivering stock is not a good breeding stock: in three years only a few proven heterozygous males were recovered for use in these experiments.

The data from the five Type II females proven to be *fd*/+; c^{ch} +/*c qv* are presented in Table 6. Of the 33 *fd* females which survived to be classified, 10 were quivering. Only three *fd* males survived: one was quivering. From these crosses, it was concluded that Type II *fd* animals could express the full quivering phenotype, making it most unlikely that qv^{+} was translocated to the X^{fd} chromosome unless a +/*qv*/*qv* constitution, as in the case of the *fd* male, equalled *qv*/*qv* in phenotype. In the case of *sh-1* (EICHER 1967a; 1967b), *fd*/+; *sh-1*/*sh-1* females sometimes expressed a reduced shaker-1 phenotype: a few abnormal head movements were observed and some females became deaf. Females *fd*/+; *sh-1*/Df never expressed abnormal head movements but some did become deaf.

In Table 7 the data from the Type I females are presented. Half of the non-*fd* animals are expected to be quivering: there were 16 out of 26, the difference being nonsignificant (P = .0792). In this cross, Type I females could not be distinguished from Type II females by the phenotype of the mosaic areas expressed in the coat since a *c*/Df area is phenotypically the same as a *c*/*c* area. Here again, the number of female *fd* quivering young is high (8 qv: 7 +). It is interesting that the three *fd* quivering males recovered were not runts, therefore presumably Type I males. These could be produced as a result of crossing over between *qv* on autosome I and qv^{+} on autosome I^{Df}. Unfortunately, these animals could not be genetically tested.

The frequency of crossing over between *c* and *qv* in Type II *fd* females is estimated to be in agreement with the published map distance (Table 6) (GREEN 1966). Assuming a 26% frequency of recombination between *c* and *qv* and a 25% frequency of *qv*/*qv* for non-*fd* animals, the results for the 80 non-*fd* animals

TABLE 6

Cross involving ♀ fd/+; c^{ch}+/c qv × ♂ +/Y; c +/c qv

	non-*fd**			*fd**		
	+	qv	Total	+	qv	Total
♀ cc	17	5	22	8	4	12
♀ $c^{ch}c$	18	4	22	15	6	21
♂ cc	10	3	13	2	1	3
♂ $c^{ch}c$	19	4	23			
Total	64	16	80	25	11	36

* Died before classification for *qv* and/or *c* could be made: 1 ♀ and 2 ♂ *cc* and 8 ♀ ♀ and 14 ♂ ♂ *fd* and/or $c^{ch}c$.

from Table 6 are not significantly different than the expected values (χ^2= 4.94; .25>P>.10). Unfortunately, since only a few heterozygous *qv* animals were available for breeding, a direct estimate of the *c–qv* distance in the absence of the X^{fd} chromosome was not attempted.

It is concluded that the qv^+ allele has not been translocated to the X^{fd} chromosome but is located between *b.p. 2* and *pu*, therefore on autosome I^{Df}.

The *s* allele was segregating in the *qv* experiment. In *fd*/+; c^{ch}/c females which were *s/s*, the pigmented areas composed of + *versus* $c^{ch}c$ color were sharply delineated, whereas the non-*s* females showed much intermixing of the two types of hairs giving the borders a diffuse nature. One of the suggestions presented by SILVERS (1961) to account for the absence of melanocytes in some white-spotting genes was inhibition of migration of pigment precursor cells from the neural crest. The sudden distinct borders seen in *fd* females on a *s/s* background would suggest that the *s* mutation may prevent not only migration of melanocytes from the neural crest but also (the implied) extensive lateral migration.

The effect of a spotting gene has been noted by CHASE (1939) in the guinea pig and by RUSSELL (1964) on T(X;A) female mice. Both comment on the in-

TABLE 7

Cross involving ♀ fd/+; c qv/*Df*(qv+?) × ♂ +/Y; c +/c qv

	+	qv	Total	Died
♀ cc	4	9	13	1
♂ cc	6	7	13	5
♀ *fd* (I and II)	7	8	15	12
♂ *fd* (I and II)	12	3 not runts	15	8
Total	29	27	56	26

	observed	expected
At birth *fd*:non-*fd*	= 50:32	2:1 (58:24)
At weaning *fd*:non-*fd*	= 30:26	2:1

creased clarity of border distinction between two differently colored regions in the presence of a spotting gene.

Determination of the break points in L.G.I. by crossing over frequencies between chromosome I^{Df} and chromosome I: Since the break point at the *fr* end of L.G.I. (*b.p.1*) was known to lie between *Hbb* and *sh-1*, a distance of 2 cM, its location had been closely localized. In the previous section it had been concluded that *b.p.2* lies between *ru-2* and *qv* in the *pu* end of L.G.I. It was attempted to confirm these locations using a different method.

In order to determine the length of the deletion in autosome I^{Df} and therefore the length of autosomal I material inserted into the X^{fd} chromosome, the frequency of recombination between the fr^+ allele in chromosome I^{Df} and the *fr* allele in chromosome I, and between the pu^+ allele in chromosome I^{Df} and the *pu* allele in chromosome I was investigated in Type I females. The total frequency of crossovers would be equal to the length of autosome I^{Df}, assuming no inhibition of recombination.

Two crosses were set up:

a) ♀ *fd*/+; *fr sh-1* c^{ch} *p*/fr^+ Df × ♂ +/Y; *fr sh-1* c^{ch} *p*/*fr sh-1* c^{ch} *p* (FS-A^W)

b) ♀ *fd*/+; c^{ch} *p pu*/Df pu^+ × ♂ +/Y; c^{ch} *p pu*/c^{ch} *p pu*

All animals which received the X^{fd} chromosome would have pigmented eyes at birth. All non-*fd* animals would have nonpigmented eyes. In cross a, the frizzy condition could be scored at day 2 after birth, the chinchilla coat condition by 7 days, and the shaker-1 condition at 3–4 weeks. The Type I versus Type II condition was scored at weaning [(c^{ch} *p*/Df versus c^{ch} *p*/c^{ch}*p* mosaic spots respectively)].

All non-*fd* mice are expected to show 40 chromosomes (except for rare X0 and XXY mice). While it is known that the non-*fd* I/I^{Df} condition is lethal (CATTANACH 1961), no evidence is available for the non-*fd* I/I/I^{Df} condition. Some phenotypically +/*pu* or +/*fr* non-*fd* animals might be trisomics. Therefore, chromosome counts were made on all non-*fd* mice which were supposed crossovers (*pu*$^+$ or *fr*$^+$).

Distance from fd *to* b.p.1*:* The data are given in Table 8. Out of a total of 232 mice born, 27 died before their frizzy phenotype could be classified. After one generation of crossing into the FS-A^W strain, the viability of Type II animals is greatly reduced. In addition, homozygous *fr* Type II animals have an even greater reduction in viability; this is especially noted in Type II males. For this reason, Type II animals will not be used to estimate recombination frequencies.

Differentiation of Type I *versus* Type II females using chinchilla-pink-eye dilution mosaic spots is not as clear on a *b* background as on a b^+ background (FS-A^W is *b*/*b*). The identification of Type I and Type II males is based on body size; errors may occur with this method. For these reasons, non-*fd* animals allow for the best estimate of the *fr*–*b.p.1* distance. Type I females can be used to support the conclusion drawn from non-*fd* animals.

The recombination frequency between *fr* and *b.p.1* is 22.2% (20 fr^+ out of 90). All non-*fd* +/*fr* mice expressed *sh-1*. All showed 40 chromosomes in cells from

TABLE 8*

Recombination between fr *in autosome I and its allele* fr+ *in autosome* I^{Df}
Cross: ♀ *fd*/+; *fr sh-1* c^{ch} *p*/*fr*+ Df × ♂ +/Y; *fr sh-1* c^{ch} *p*/*fr sh-1* c^{ch} *p*

	+	*fr*	Stillborn : *fr* condition not known
♂ *fd*/Y Type I (not runt)	48	1	14
♂ *fd*/Y Type II (runt)	0	6	
♀ *fd*/+ Type I	33	8	7
♀ *fd*/+ Type II	3	16	
♀ non-*fd*	7	38	1
♂ non-*fd*	13	32	5
Total	104	101	27

Grand total 232

Recombination for non-*fd* offspring = 22.2%
Recombination for Type I female offspring = 19.6%

* Taken from EICHER (1967b).

bone marrow preparations. Using Type I females, the same *fr–b.p.1* distance is estimated to be 19.6 cM. The difference is not significant ($.95 > P > .90$). Combining the two estimates, a distance of 21.3 cM is obtained.

The published map distance for *fr–sh-1* is 16 cM (Figure 1). In EICHER (1967a) a value of 20.9 cM was obtained for the distance of *fr–sh-1* in Type II animals. It is seen that the two values obtained in our laboratory are in good agreement with each other.

It is concluded that there is no inhibition of recombination between *fr* and *b.p.1*.

Distance from b.p.2 *to* pu: The data are given in Table 9. Out of a total of 231 animals born, 41 *fd* animals died before their condition as to Type I or Type II could be established. The viability of Type II animals is low in these crosses and, in combination with *pu*, is greatly reduced. The problem of distinguishing Type I from Type II males is the same as in the *fr–b.p.1* estimate. For these reasons, the *b.p.2–pu* distance will not be estimated using Type II females or *fd* males. The *b* allele was not segregating in these crosses so that the classification of *fd* females as to Type I *versus* Type II was not impaired.

Of the 87 non-*fd* animals born, 7 were +/*pu* (crossovers), giving a frequency of recombination of 8%. Six of these showed 40 chromosomes present in metaphase preparations from bone marrow cells (the other non-*fd* +/*pu* animal was stillborn). Of the 50 Type I females, 6 were *pu*/*pu* (12% recombination). The values obtained for non-*fd* mice compared to Type I females are not significantly different. In combination, the non-*fd* animals and Type I females give a 9 cM distance from *b.p.2–pu*.

GREEN (1969) has also estimated the *b.p.2–pu* distance using the cross ♀ *fd*/+; c^{ch} p^{+} *pu*/Df pu^{+} × ♂ +/Y; c^{ch} *p pu*/c^{ch} *p pu*. Here the Type I and Type II females can be distinguished: Type I females will show c^{ch} *p*/Df mosaicism

TABLE 9*

Recombination between pu *in autosome I and its allele* pu+ *in autosome* I^{Df}.
Cross: ♀ *fd*/+; c^{ch} *p pu*/Df *pu*+ × ♂ +/Y; c^{ch} *p pu*/c^{ch} *p pu*

	+	pu	Stillborn or died early
♂ *fd* Type I (not runt)	40	1	17 { 6 +
♂ *fd* Type II (runt)	2	2	11 pu
♀ *fd*/+ Type I	44	6	24 { 11 +
♀ *fd*/+ Type II	3	5	13 pu
♂ non-*fd*	3	37	9 pu
♀ non-*fd*	3	28	7 { 1 +
			6 pu
Total	95	79	57

Grand total 231

Recombination for non-*fd* offspring = 8%
Recombination for Type I female offspring = 12%

* Taken from EICHER (1967b).

in their coats instead of c^{ch}/c^{ch}, as is the case for the Type II females. The non-*fd* and *fd* mice would not be clearly distinguished from each other at birth.

If one considers the percentage of recombination between *b.p.2–pu* using GREEN's data (Table 10), one obtains 17% (30/177 non-*fd* young); 8% (7/88 Type I females); and 30% (15/50 Type II females). Data using *fd* males are not meaningful because of viability and classification problems. Since the viability of pudgy at birth is so low (note in non-*fd* animals in Table 9, 18.8% of the pudgy young born were stillborn or died early) and the young could not be scored before the appearance of fur, this would increase the frequency of recombination for non-*fd* and *fd* Type II females (pu class = noncrossover class). If one combines values from Type I and non-*fd* females, the percentage of crossing over is 16.7 (29/174) which can be compared to a value of 11.1% using comparable data from Table 9 (9/81, therefore, not counting stillborns). These two values are not significantly different.

If one compares the homogeneity between non-*fd* male and female values using GREEN's data, $P < .005$. The female non-*fd* *versus* Type I female values for GREEN's data are also significantly different ($P < .005$). These same comparisons of homogeneity using data given in Table 9 are not significantly different. The difference is significant between GREEN's data and those given in Table 9 comparing non-*fd* females (stillborns not included) ($.05 > P > .025$). Ignoring all the Type II males in GREEN's data, the Type II and non-*fd* female classes appear to show an excess of crossing over. The reason is unknown.

According to the literature, the distance *qv* to *pu* is 10 cM (Figure 1). Since the data presented in this report indicate that *b.p.2* is located between *ru-2* and *qv*, either *b.p.2* is very close to *qv*, or there is inhibition of recombination between

TABLE 10

GREEN's *data* for recombination between* b.p.2 *and* pu.
Cross: ♀ *fd*/+; c^{ch} p+ *pu*/Df *pu*+ × ♂ +/Y; c^{ch} *p pu*/c^{ch} *p pu*

	+	pu	Total
♂ *fd* Type I	89	7	96
♂ *fd* Type II	6	1	7
♀ *fd* Type I	81	7	88
♀ *fd* Type II	15	35	50
♂ non-*fd*	8	83	91
♀ non-*fd*	22	64	86

* Taken from Mouse News Letter (1969) **40**: 29.

b.p.2 and *pu* as a result of the deletion present in autosome I^{Df}. Since no marker genes are known between *ru-2* and *qv*, no decision can be made to distinguish between these two possibilities.

Genetic length of the piece of linkage group I inserted in to the X^{fd} *chromosome:* The known genetic length of linkage group I is 56 cM (Figure 1). Of the 56 units, 21–23 (*sh-1–ru-2*) are known to be located within the X^{fd} chromosome, if *b.p.2* is close to *ru-2*. If *b.p.2* is close to *qv*, the genetic length would be between 30–32 units. Thus one can postulate that the percentage of the genetic length which has been translocated is roughly between 40–60% of the total length. Of course, this estimate assumes that *fr* and *pu* are the extreme genes located on linkage group I. But it appears well established that a considerable portion of the total known genetic length of L.G.I has been translocated to the X^{fd} chromosome.

In a study involving DNA replication, EICHER (1967b) noted that *fd/fd;* I/I^{Df} females seemed to have an extra small chromosome compared with *fd*/0; I/I females. It was postulated that this was the I^{Df} chromosome. It measured approximately 2 μ in length. This might indicate that chromosome I is 3.5–6.5 μ in length.

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SUMMARY

The gene *ru-2* resides 3 centimorgans from *p* in linkage group I, the order being *c–p–ru-2*. By the expression of various dosages of the *ru-2* and *qv* genes in Type I and Type II animals of the *fd* translocation, the *ru-2*+ allele has been shown to be located in the X^{fd} chromosome whereas the *qv*+ allele resides in autosome I^{Df}. The various phenotypes observed using the *ru-2* gene with forms of the *fd* translocation are discussed. By using crossover frequencies between *fr* and *pu* on autosome I with *fr*+ and *pu*+ on autosome I^{Df}, an estimate of the length of autosome I^{Df} has been made. Approximately 50% of the known linkage group I has been shown to be inserted into the X chromosome in the *fd* translocation.

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