THE INHERITANCE AND LINKAGE RELATIONS OF A NEW RECESSIVE SPOTTING IN THE HOUSE MOUSE

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The chief types of spotting thus far described in the house mouse, *Mus musculus*, are ordinary recessive piebald spotting and dominant spotting. Dominant spotting has been shown to be lethal in the homozygous condition because of the anemia associated with it. The purpose of this paper is to describe a new recessive spotting and its linkage relations. Since this new spotting gene is very closely, if not inseparably, linked with the flexed-tail-anemic complex described by HUNT and PERMAR (1928), I shall first review the work that has been done on flexed-tail.

TAIL MUTATIONS IN THE HOUSE MOUSE

In 1910, PLATE described a kinky-tail mutation which appeared in the offspring of four albino mice bought from a fancier in Berlin. From somewhat meagre data he concluded that its inheritance did not follow Mendelian laws. His observations, however, suggest a possible linkage between kinky-tail and pink-eye, and the tail abnormality appeared more often among females than males. He does not mention any anemia associated with the kinky-tail character, although it is possible that it was present but overlooked.

Somewhat later BLANK (1916) made an extensive study of the histology of PLATE's kinky-tail material. He concluded that flexure of the tail is caused by a one-sided fusion of two neighboring tail vertebræ, bringing about an angular arrangement of these joints. He found that the degree of fusion varied greatly and that in extreme cases the whole cross diameter was fused. Bending was caused by a fusion on one side accompanied by continued growth of the epiphyseal groove on the other. The epiphysis on the fused side was broken through or dislodged toward the other side.

HUNT and PERMAR (1928) have described a similar mutation which they have called flexed-tail. This mutation arose in an albino stock secured originally from the BUSSEY INSTITUTION. They found that the character was recessive but gave a ratio of 6.9 normals to 1.0 flexed-tail in the F_2 . In a recent publication HUNT, MIXTER, and PERMAR (1933) obtained 40.98 ± 1.12 percent of flexed-tail mice in a backcross. Thinking that their failure to obtain the expected 50 percent of flexed animals might be due to a high postnatal death rate in this class, they tried using only those litters at the final classification (21–28 days) in which no deaths had occurred since birth. The use of these litters increased the percentage of flexed-tail

animals to 43.97 ± 1.44 percent. In an effort to do away with both a differential prenatal and postnatal death rate, undepleted litters of 7 or more animals were used. In these litters, the percentage of flexed-tail mice was 48.30 ± 1.72 , which is fairly close to the expected 50 percent. They used the same method on their F_2 data, and were able to increase the percentage of flexed mice from $16.17 \pm .68$ (all litters) to 18.66 ± 1.08 percent (undepleted litters of 7 or more). Even then, however, the deficiency of flexed-tail animals is over 6 percent (25 percent expected). Further on I shall present evidence to show that this apparent deficiency is due partly to normal overlaps.

HUNT, MIXTER, and PERMAR also mated together animals with the same type of flexed-tail to see if the various shapes and forms of the tail were controlled by genetic factors. They were forced to give this up because of sterility which resulted from inbreeding, but found a slight significant correlation between the tail form of parents and offspring as far as they went.

HUNT also noticed that flexed-tail mice are definitely anemic at birth, being lighter in color than their normal-tailed sibs. MIXTER and HUNT (1933) determined the number of erythrocytes and leucocytes, as well as the hemoglobin percentage, in flexed-tail mice and their normal sibs from backcrosses. The normal sibs, of course, would be heterozygous for flexed-tail. A homozygous normal line was also used for an additional control. Unfortunately, the animals used were classified on the basis of flexed-tail rather than upon their anemic appearance. According to data presented in this paper, the flexed-tail animals used were undoubtedly anemic, but the heterozygous control line may have contained a large number of anemics which were genotypically flexed but phenotypically normal-tail. Nevertheless, the comparison between the flexed-tail animals and those of the normal homozygous control line is undoubtedly valid. It was found that the flexed animals were, on the average, deficient at birth in hemoglobin and erythrocytes but became normal at about two weeks of age.

R. J. KAMENOFF, of COLUMBIA UNIVERSITY, gives me permission to state that in an embryological study, as yet unpublished, he has found that flexed-tail mice exhibit anemia as early in embryological development as the 14th day. In a study of the tail, he finds that the fusion between vertebrae in the flexed animals may be unilateral or more or less bilateral, the latter forming the stiff, straight type of joint found in some animals. In both the normals and flexed the mesenchyme differentiates into early or procartilage in the region of the centrum and the intervertebral disc. In normals, this procartilage differentiates into fibers in the intervertebral area and to cartilage in the centrum, while in the flexes, at the point of flexure, it forms cartilage instead of fibers.

HUNT states that occasionally he has obtained flexed-tail mice which are not anemic at birth. In my own crosses, I have observed one such animal, but unfortunately it died. It is very probable that anemia and flexed-tail are caused by two very closely linked genes, the rare flexed non-anemic animals being crossovers. It should be borne in mind, however, that these apparent crossovers may be flexed individuals that recover from the anemia before birth.

DANFORTH (1930) believes that flexed-tail is caused by persistence of the caudal intestine which throws the vertebræ out of alignment.

R. J. KAMENOFF (1932) exhibited slides of specimens obtained in an embryological study of the development of flexed-tail. He says: "This character is expressed as a unilateral fusion between successive tail vertebræ, as seen in the cleared specimens. In the fourteenth day of gestation the mesenchyme of the invertebral anlage fails to form fibrous tissue on one side of the bent notochord. The successive slides show this non-fibrous anlage forming a cartilaginous bridge between the adjoining cartilaginous vertebræ. This bridge's later ossifying prevents sub-epiphyseal growth on that side, thus accentuating the angle and pushing the nucleus pulposus to the lateral position seen in the slide of the adult."

DOBOVOLSKAIA-ZAVADSKAIA (1927a, 1927b, 1928a, 1928b) and DOBOVOLSKAIA-ZAVADSKAIA and KOBOZIEFF (1927a, 1927b, 1928, 1930a, 1930b) have described a dominant lethal mutation in the mouse which results in partial or complete absence of the tail, and in various flexures and constrictions of the tail remnant. In some cases a little coiled persistent filament is all that represents the tail. Certain vertebræ may be missing in the back as well as in the tail. It was shown that mice homozygous for this dominant gene die during embryonic life.

In a recent paper in which symbols for this anomaly are discussed, DOBOVOLSKAIA-ZAVADSKAIA (1933) attributes these tail defects to the action of one dominant lethal gene (*T*) in combination with various modifying genes. She states that various tail forms which emerged during breeding experiments in mice with this mutation were not only transmitted from parent to offspring, but could also be isolated by selection into separate lines.

In my own crosses, I have found that a very close fit to a 1:1 ratio is obtained when short-tailed mice are mated with normals, but the tail anomaly may vary tremendously in its expression, even among the offspring of a single cross.

An embryological study may result in a better understanding of the gene or genes causing this tail abnormality. I am working on the linkage relations of this character and hope to report on it in the near future.

Another occurrence of interest is the appearance of a dominant muta-

tion for flexed-tail noticed by Dr. KEELER in the mouse colony of the BUSSEY INSTITUTION. This type of flexed-tail is very similar in its phenotypic expression to recessive flexed, except that no anemia is associated with it. Some individuals show a shortening or thickening of the tail and look exactly like mice carrying the gene of brachyury. Preliminary data, however, indicate that this gene does not have a lethal effect when homozygous.

BELLYSPOT, FLEXED-TAIL AND ANEMIA

While trying to isolate various piebald patterns in Dr. HUNT's laboratory at MICHIGAN STATE COLLEGE, I noticed that many of his flexed-tail

FIGURE 1.—Bellyspot.

mice had a bellyspot about the size of a dime on the abdomen. In segregating populations, this character varies phenotypically from no spot at all (normal overlap) to a large spot covering most of the ventral surface and, in rare cases, extending up the sides in the form of a partial belt. Except for these rare cases in which the spot extends up the sides, bellyspot, as the name signifies, is limited to the ventral region. In this respect, it is similar to the Irish spotting factor in the Norway rat. Mice which are homozygous for bellyspot have white toes and may or may not have a spot on the tip of the tail. A mouse with a typical bellyspot is shown in figure 1.

Two flexed-tail bellyspot males from HUNT's stock were crossed with females from LITTLE's inbred black non-spotted strain. Sixty-seven F_1 mice were obtained, all of which showed no spotting (except on the tips of the toes). Bellyspot, therefore, behaves as a recessive. A large F_2 popu-

TABLE 1
F₂ flexed-tail bellyspot × normal self (coupling).

	NORMAL	BELLYSPOT NON-FLEXED	FLEXED NON-SPOT	BELLYSPOT FLEXED	TOTAL
Observed	494	15	1	70	580
Expected 9:3:3:1	326.25	108.75	108.75	36.25	
Dev.	167.75	-93.75	-107.75	33.75	
P.E.	8.06	6.34	6.43	3.93	
Dev./P.E.	20.81	14.76	17.00	8.59	

lation was obtained which is classified in table 1, the results indicating that bellyspot and flexed-tail are closely linked. The parental classes are greatly in excess, the deviations from a 9:3:3:1 ratio being many times greater than the respective probable errors for the four classes. Only 71 flexed animals appeared out of the expected number, 145, and only 85 bellyspot animals were recovered out of the expected number, 145. There are indications that the deficiency of flexed animals is due to normal overlaps rather than to high mortality and that bellyspot likewise gives a high percentage of normal overlaps, that is, fails to appear in the phenotype, though present in the genotype. The fact that 70 of the 71 phenotypically flexed mice also had a bellyspot indicates, in spite of normal overlaps, that these two characters are very closely linked. Applying the formula of FISHER and BALMUKAND (1928), this distribution indicates less than 2 percent of crossing over.

Some of the F_2 double recessives, bellyspot flexed animals, were backcrossed to the F_1 with the results shown in table 2.

TABLE 2
Backcross involving flexed-tail and bellyspot (coupling).

	NORMAL	BELLYSPOT NON-FLEXED	FLEXED NON-SPOT	BELLYSPOT FLEXED	TOTAL
Observed	382	45	9	149	585
Expected 1:1:1:1	146.25	146.25	146.25	146.25	
Dev.	235.75	-101.25	-137.25	2.75	
P.E.	7.06	7.06	7.06	7.06	
Dev./P.E.	33.39	14.34	19.44	.39	

Since this is a backcross, we should expect a 1:1:1:1 ratio, if there were no linkage. The bellyspot flexed class fits very closely to the expected number on the basis of no linkage. That this is merely an effect caused by normal overlapping is shown by the poor fit of the other classes and by the data on normal overlaps which follow. The other parental class is

present in great excess, while the two crossover classes are strikingly deficient. These other three classes indicate a strong linkage between flexed-tail and bellyspot. Other data which have since been obtained throw considerable light on these results. As the classification of the backcross young stands, it indicates a crossover percentage of about 9.

Thirty-five litters, with a total of 187 mice, were obtained by mating double recessives, bellyspot flexed animals, *inter se*. Of these 187 mice, 57 were normal (double overlaps), 26 were bellyspot non-flexed (overlaps for flexed), 33 were flexed non-spot (overlaps for spot), and 71 were bellyspot flexed, like their parents. All these mice were anemic at birth, which is, as I shall show later, sufficient evidence that they are homozygous for

FIGURE 2.—Flexed-tail.

flexed-tail and bellyspot, whether they show them phenotypically or not. We find from these data 30.5 percent double overlaps, 48.1 percent overlaps for bellyspot, and 44.4 percent overlaps for flexed. This proves that both bellyspot and flexed-tail give a high percentage of normal overlaps and indicates that in the F_2 and backcross data some of the individuals in the crossover classes (bellyspot non-flexed and flexed-non-spot) were overlaps and not true crossovers at all. If this is true, the linkage is even closer than the F_2 data indicate. Data from other crosses reveal more fully the relationship between these two characters.

Flexed-tail animals, except for possible rare exceptions, are anemic at birth. HUNT has reported several such exceptions. I have looked carefully for such animals in large F_2 and backcross populations and have found only one, which died soon after birth. If true crossovers do occur between

flexed-tail and anemia, they are very rare indeed. We may use anemia, then, as an index of whether or not a mouse is genotypically flexed-tail.

It has been noticed that bellyspot animals are always anemic. Bellyspot anemic mice were crossed with a non-spotted inbred strain, and the numbers of young indicated in table 3 were obtained.

TABLE 3
F₂ bellyspot anemic × *normal self*.

	NORMAL		ANEMIC		TOTAL
	SELF	BELLYSPOT	SELF	BELLYSPOT	
Observed	205	0	29	36	270

It will be noticed that there is a good 3:1 ratio of normal to anemic mice (205:65), but that only slightly more than half of the anemics bear a bellyspot. However, none of the normals are bellyspot. Hence there is no indication of crossing over between anemia and bellyspot, but rather a failure of bellyspot to express itself in 44.6 percent of the anemic mice. In other words, 44.6 percent of the anemics are overlaps for bellyspot. This is a close approximation to the percentage obtained when bellyspot animals are mated *inter se* (48.1 percent). That such unspotted (self) animals are genetically bellyspot is shown by evidence presently to be discussed. A close association of this kind between bellyspot and flexed-tail anemia is somewhat analogous to that existing between dominant spotting and its lethal anemia.

Further evidence of the close association of bellyspot, flexed-tail, and anemia is revealed by *inter se* matings of animals from the *F₂* supposed crossover class, bellyspot non-flexed. Three pairs of such mice were mated and produced 38 young, all of which were anemic, indicating that the parents were homozygous for anemia. (Animals recorded in tables 1 and 2 were not classified for anemia.) Eighteen of the young were bellyspot, while 20 were non-spot, which gives 53 percent of normal overlaps for bellyspot. Eleven animals were flexed-tail (ten of them also bellyspot). At first thought, this might appear like a 3:1 segregation for flexed-tail, but since flexed-tail animals are almost certain to be anemic, it is logical to conclude that the parents were overlaps for flexed and not true cross-overs.

Flexed non-spot mice were also mated *inter se*. All of them were either obtained from the so-called flexed non-spot crossover class in table 2, or resulted from *inter se* matings of that class. Seven such matings, involving seven females and two males, produced 57 young, all of which were anemic. Of the 47 young which survived, 15 were flexed non-spot like the parents, 13 were bellyspot, of which 7 were flexed and 6 non-flexed, and

19 showed neither bellyspot nor flexed-tail. Since all the young were anemic we may safely assume that the parents were also homozygous for anemia. Because of failure in all our crosses to find any bellyspot mice which were not anemic, it seems reasonable to conclude that the 13 bellyspot animals which appeared from these matings were not the result of simple segregation but were produced from parents which were overlaps for spotting (duplex for bellyspot, anemia, and flexed-tail). In this case, then, 30 percent of the young were bellyspot and so genetically homozygous for the bellyspot gene. The percentage of normal overlaps for flexed from this same cross was 53.

FIGURE 3.—Hydrocephalus.

We may conclude, then, that bellyspot, flexed-tail, and anemia are due to the same gene, or to very closely linked genes, for the following reasons:

1. Flexed-tail animals, in my observation, with one exception, have shown anemia.
2. Bellyspot mice likewise regularly exhibit anemia, or if not observed to be anemic, are shown by subsequent breeding tests to be homozygous for the character.
3. In segregating populations, no bellyspot mice have appeared which were not anemic, and only one non-anemic flexed-tail animal has been observed.
4. *Inter se* matings in the apparent crossover classes, flexed non-spot and bellyspot non-flexed, produced only anemic young.

INTERPRETATION OF F_2 AND BACKCROSS RESULTS (TABLES 1 AND 2) ON THE BASIS OF COMPLETE LINKAGE WITH NORMAL OVERLAPS

We may assume, from data already presented, that there is no crossing over between bellyspot and flexed-tail. If such is the case, the bellyspot non-flexed and flexed non-spot classes in tables 1 and 2 are not crossover classes but are merely overlaps which genotypically belong in the double recessive, bellyspot flexed class. If we add these classes together in table 1, we get a ratio of 494 to 86, which is a very poor fit to the expected 3:1 ratio. Data given later, on mortality among anemic mice, indicate that the poor fit is due to overlaps rather than to differential mortality. Considering flexed-tail alone, we have a ratio of 509 normal to 71 flexed-tail, where we should expect 435 normal to 145 flexed-tail. Approximately 74 of the normals are probably overlaps for flexed, this amounting to 51 percent normal overlaps for flexed. Of these flexed overlaps, 15 are doubtless found in the bellyspot non-flexed class. The remaining 59, we may assume, are to be found in the normal class.

For bellyspot we get a ratio of 495 non-spot to 85 bellyspot, where we should expect a ratio of 435 to 145. Therefore, 60 of the non-spotted class should be overlaps for bellyspot. This would indicate about 41.4 percent of overlaps for bellyspot. Fifty-nine of the 60 overlaps would be in the normal class, and one would be represented by the single flexed non-spot individual. Since, with both flexed-tail and bellyspot, 59 overlaps must be looked for in the normal class, these 59 will be double overlaps. The percentage of double overlaps, then, is about 40.7, and single overlaps for either bellyspot or flexed, 11.0 percent.

In the backcross we should expect a 1:1 ratio of normal to bellyspot flexed, if there were no normal overlaps. The ratio obtained was 382 normal to 203 which phenotypically are duplex for one or both recessive factors. Considering flexed-tail alone, a ratio was obtained of 427 non-flexed to 158 flexed, when theoretically we should expect 292.5 to 292.5. This gives us 134 overlaps for flexed, or 46 percent. Forty-five of them would constitute the bellyspot non-flexed class, and the remaining 89 would be expected to occur in the normal class.

With bellyspot, we get a ratio of 391 non-spot to 194 bellyspot, or a deviation from the expected 1:1 ratio of 98.5. This gives 33.7 percent normal overlaps for bellyspot. Eighty-nine of the 98.5 overlaps would be double overlaps. The double overlaps (overlaps for both flexed and bellyspot) would be 30.4 percent, and there are 18.4 percent single overlaps (for bellyspot or flexed).

Table 4 shows the percentage of double, single, and total overlaps obtained with bellyspot and flexed-tail from different types of crosses. A glance at the F_2 results on bellyspot (crosses 1 and 6) shows that from 40

TABLE 4
Percentage of overlaps obtained from various crosses.

CROSS	DOUBLE	SINGLE		TOTAL		TOTAL NUMBER MICE
		BELLYSPOT	FLEXED	BELLYSPOT	FLEXED	
1. F ₂ involving bellyspot and flexed	40.7	0.1	10.3	40.8	51.0	580
2. Backcross, bellyspot and flexed	30.4	3.1	15.4	33.5	45.8	585
3. Bellyspot flexed <i>inter se</i>	30.5	17.7	13.9	48.2	44.4	187
4. Bellyspot non-flexed <i>inter se</i>				30.8		26
5. Flexed non-spot <i>inter se</i>					53.2	47
6. F ₂ of bellyspot				44.6		193
Weighted average	34.8	3.8	13.0	39.3	48.0	

to 45 percent bellyspot overlaps may be expected in an F₂ population. The percentage may be expected to vary, depending upon the spotting modifiers present in the strains used. The inbred strains used were LITTLE's inbred non-agouti black strain in cross 1, and for cross 6, several strains were used. Overlaps of flexed-tail alone varied from 45 to 50 percent. Between three-fourths and four-fifths of all the overlaps obtained were double overlaps involving both bellyspot and flexed-tail.

Since the matings together of bellyspot flexed give 30 percent of double overlaps, we should expect to obtain a like percentage in a backcross population, which is the case if complete linkage is assumed. Since data given later indicate conclusively that mortality among flexed anemic animals is only 10 percent greater than among normals, serious distortion of the ratios by differential mortality is ruled out. Both crossover classes should appear in nearly equal numbers, if there were true crossing over, but in the F₂ they are in the ratio of 15:1, and in the backcross 45:9. Also the double recessive class in a backcross should be nearly equal in number to the normal class, but actually it is less than half as large. In view of the evidence presented, the only satisfactory explanation of the F₂ and backcross ratios is to assume complete or nearly complete linkage of bellyspot and flexed with a high percentage of normal overlaps. Such an assumption is in accord with the complete association of anemia with bellyspot and the probable complete association of anemia with flexed. In the latter case, crossing over, if it occurs, is very rare.

MORTALITY RATES IN CROSSES INVOLVING BELLYSPOT-FLEXED-ANEMIA

Bellyspot-flexed-anemic animals were crossed with an inbred non-spotted dilute brown strain. No spotting occurred in the F₁ mice. An F₂ population consisted of 644 animals and, in the case of the anemics, the

tip of the tail was snipped off soon after birth, so that they could be recognized later. The ratio of normal to anemics at this time is shown in table 5.

TABLE 5
F₂ from anemic × normal, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	477	167	644
Expected 3:1	483	161	
Dev.	-6	6; P.E. 7.41; Dev./P.E. 0.84	

Good agreement with a 3:1 ratio is found, the deviation being only 0.84 of the probable error. This shows that the prenatal mortality is no higher among anemics than among normal mice. These same litters were then examined after weaning (20 to 30 days after birth), and the numbers recorded in table 6 were found.

TABLE 6
Same litters as classified in table 5, when observed at 20 to 30 days of age.

	NORMAL	ANEMIC	TOTAL
Observed	430	131	561
Expected 3:1	420.75	140.25	
Dev.	9.25	-9.25; P.E. 6.92; Dev./P.E. 1.34	

The numbers obtained from the second classification still fit a 3:1 ratio fairly well. The Dev./P.E. is 1.43. The odds are only 1.63 to 1 against such a deviation being due to chance. A comparison of the first and second classifications would indicate that meanwhile 47, or 9.85 percent of the normals died, while 36, or 21.56 percent of the anemics died. Although the postnatal mortality is thus twice as high among anemics as among normals, the difference is not great enough to affect the ratios seriously in a population of four or five hundred animals.

Additional F₂ data involving anemia, obtained from other crosses, may be combined with the data recorded in table 5, giving the result shown in table 7. In all cases, the classification for anemia was made at birth. The

TABLE 7
F₂ from anemic × normal, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	1240	405	1645
Expected 3:1	1233.75	411.25	
Dev.	6.25	-6.25; P.E. 11.85; Dev./P.E. 0.53	

very close fit to a 3:1 ratio indicates that this type of anemia behaves genetically as a simple Mendelian recessive. This conclusion is supported further by backcross results shown in table 8.

TABLE 8
Backcross involving anemia, classified at birth.

	NORMAL	ANEMIC	TOTAL
Observed	62	56	118
Expected 1:1	59	59	
Dev.	3	-3; P.E. 3.66; Dev./P.E. 0.82	

HUNT, MIXTER, and PERMAR (1933), although they mention the probability of normal overlaps occurring in their backcross and F_2 data, emphasize mainly the high prenatal and postnatal death rate in the flexed-tail classes and endeavor to correct for this by using only large undepleted litters. My results indicate that the prenatal death rate is no higher among flexed than among normal mice, and that the postnatal death rate, although higher in the flexed group, is not enough higher seriously to affect the final count.

The distribution of anemic mice in the F_2 and backcross classes of the data presented by HUNT, MIXTER, and PERMAR indicates the presence of normal overlaps for flexed. In a backcross, they obtain at birth only 3 flexed animals which are not anemic out of a total of 347 flexed-tail mice. If these 3 animals represent crossovers between flexed-tail and anemia, the other (complementary) crossover class to be expected would be normal-tail anemic, and this should be approximately as large as the first crossover class. Even if crossing over does take place, some discrepancy might exist between the two crossover classes because of the difficulty of classifying flexed-tail at birth. We find, however (table 2), that there are 78 normal-tail anemics and 28 with an intermediate or doubtful tail. My data indicate that flexed-tail and anemia are very closely, if not inseparably, linked. All these facts tend to show that the anemics in both the normal-tail and doubtful tail classes are really overlaps for flexed-tail. If we assume that they are, and put them in the flexed-tail class, we find that the percentage of flexed-tail animals at birth is 47 and the percentage of normal overlaps is about 23.4.

HUNT, MIXTER, and PERMAR, by using undepleted litters of 7 or more mice, obtained 18.66 ± 1.08 percent of flexed in F_2 , where 25 percent would be expected. Thus the flexed class shows a shortage of over 6 percent, even when large undepleted litters are used. If we take the anemics in the normal-tail class and half of the mice in the classes in which the condition of

the blood was uncertain and include them in the flexed class, the percentage of flexed animals is raised to 22.2 percent. Even then the ratio differs significantly from the expected 3:1 proportion. If we include all those animals whose blood condition was uncertain in the flexed class, the percentage is raised to 23.2. The ratio deviates, then, from a 3:1 ratio by only 25.2 ± 11.24 .

In my experiments, no difficulty has been experienced in detecting anemic segregates, provided the litters were classified the day they were born. Also, I found no evidence of a higher prenatal death rate among flexed mice than among normals, and the postnatal death rate was only 10 percent higher among anemics (flexed) than among normals. My matings of flexed $\times$ flexed gave many normal overlaps, while HUNT obtained scarcely any. Possibly our strains differ in the number of factors present that modify the action of the flexed-tail gene. In view of my results showing the close relationship between flexed-tail and anemia, it is logical to conclude that part of the shortage of flexed-tail mice in HUNT's tables is due to normal overlaps. Also, part of the shortage, as he has shown by using large undepleted litters, is due to a higher mortality in the flexed class.

OTHER TYPES OF SPOTTING IN THE HOUSE MOUSE

The discovery by SNELL (1931) that piebald spotting is linked with hairless has greatly simplified the study of new types of spotting in the mouse. Uncertain results, due to difficulty of classification, are usually obtained when two types of spotting enter into the same cross. Now, however, new spottings can be differentiated from piebald by crossing with a self hairless strain. This method is being employed by Dr. C. E. KEELER of the BUSSEY INSTITUTION in the analysis of several new spottings. One of these is *Akhissar spotting* (KEELER 1933b). This type of spotting arose from a male mouse with a small bellyspot captured by KEELER in a grain shop at Akhissar, Turkey. When the Akhissar spotted strain was crossed with a self strain, there were obtained 7 self young and two having white-tipped tails. In the F_2 many selfs appeared, in addition to mice showing all grades of spotting from white-tipped tails to belted. Akhissar spotting was crossed with self hairless, and the F_2 data indicate that it is a form of recessive piebald. This is a very good example of a piebald strain developed in the laboratory by selection of a wild sport.

Bar Harbor bellyspot. LITTLE's inbred non-agouti chocolate mice frequently exhibit a small spot on the belly. J. M. MURRAY informs me that this type of spotting appears to be genetically different from dominant and piebald spotting. My results show that it is not identical with the bellyspot in my stock.

Mutation to white-bellied agouti. Four mutations to white-bellied agouti

have been reported by LITTLE (1916), and five mutations to black and tan have also been reported, one by PINCUS (1929) and four by SNELL (1931).

In my own crosses one mutation to white-bellied agouti has been encountered as follows. A flexed-tail dark belly agouti animal, which carried non-agouti, was crossed with a black non-agouti from KEELER's black rodless strain. All the F_1 animals were either black non-agouti or black agouti with dark belly. In this particular case two F_1 males (one was agouti and one non-agouti) were mated in a stock cage with several F_1 females. The females, when pregnant, were isolated. One of the agouti F_1 females had a litter of nine wild-type young, one of which had a white belly. This mutant was mated with a black non-agouti male and produced 7 white belly agouti young and 12 dark belly black non-agouti. All three F_1 animals were tested further, but no more white-bellied young appeared in the F_2 . This indicates that only one, or possibly a few, of the germ cells of one of the F_1 animals mutated to the white-bellied condition.

LINKAGE TESTS

Linkage tests were made for the purpose of finding out whether the gene (or closely associated genes) causing bellyspot, flexed, and anemia are linked with any of the other known genes of mice.

Test for linkage between bellyspot-anemia and hairless

Since bellyspot is a recessive spotting, it was deemed important to determine whether or not it is an allele of the piebald gene, also recessive and at times phenotypically similar. But hairless is known to be linked rather closely with piebald, with only about 9 percent of crossing over. Accordingly, if hairless were shown to be independent of anemia, it would follow that piebald also must be independent of anemia, a fact which it would be difficult to establish by a direct cross between anemia and piebald, since it would be impossible to discriminate with certainty between true piebald and bellyspot, both of which would be expected to occur in F_2 .

Crosses were made with a hairless strain which was dominant spotted (Ww). Since my tests with Ww (presented on pages 26 to 30) show that bellyspot is not linked with that gene, I considered it valid to disregard all spotting in this cross, and base my conclusions on the relationship between anemia and hairless. Of course WW anemics will be present at birth in F_2 , but their early death will take place before the time of classification. Those anemics that live will be of the type associated with bellyspot and flexed-tail. Since piebald is linked with hairless with about 9 percent crossing over, if bellyspot actually were a form of the piebald gene, we should expect in F_2 only about two anemic mice in 64 to be hairless. The cross was made as follows:

P₁ *Ww hrhr FF* × *ww HrHr ff*
 F₁ *Ww HrHr Ff spotted*
ww Hrhr Ff self

An F₂ population was obtained by mating the spotted F₁'s (*Ww Hrhr Ff*) *inter se*. The results are as follows (disregarding *Ww*).

TABLE 9
F₂ involving hairless and bellyspot anemia (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>Hr</i>	<i>hr</i>	<i>Hr</i>	<i>hr</i>	
Observed	67	24	16	4	111
Expected 9:3:3:1	62.4	20.8	20.8	7.0	
Dev.	4.6	3.2	-4.8	-3.0	
P.E.	3.53	2.77	2.77	1.72	
Dev./P.E.	1.30	1.16	1.74	1.74	

Since four out of 20 anemics obtained were hairless, where we should expect none if anemia were closely linked with hairless, we may conclude that the bellyspot-anemia complex is independent of the piebald gene. The deficiency of the recombination double recessive class is only 1.74 times its probable error and may be attributed to chance or to a slightly higher mortality among anemic hairless mice.

In earlier data from this same cross, in which the F₂ classification was based on animals phenotypically flexed rather than upon the more reliable anemia counts, the distribution in table 10 was obtained.

TABLE 10
F₂ involving hairless and flexed-tail (repulsion).

	NORMAL		FLEXED		TOTAL
	<i>Hr</i>	<i>hr</i>	<i>Hr</i>	<i>hr</i>	
Observed	109	60	12	6	187
Expected 3:1			13.5	4.5	
Dev.			-1.5	1.5; P.E. 1.24; Dev./P.E. 1.21	

Although the expected number of flexed-tail mice did not appear, probably because of normal overlaps, 6 out of 18 which did appear were hairless. This is in close agreement with expectation, showing that flexed and hairless recombine freely. But since flexed is due to the same gene as bellyspot (or to one closely linked with it), and since hairless is closely linked with piebald, it follows that bellyspot and piebald must also be independent of each other (not linked).

Further evidence of the independence of bellyspot and piebald was obtained by crossing some of my bellyspot females with a selected strain of piebald produced by Dr. GREGORY PINCUS, which is phenotypically similar to my bellyspot race. Indeed, the two kinds of bellyspot look exactly alike, but that of PINCUS depends for its expression upon the presence of *ss* (the piebald gene). All the F_1 animals resulting from this cross were non-spotted, which in itself is strong evidence that the two spottings are genetically different. If the two are independent, we should expect in F_2 a ratio of 9 normal (non-spot):3 Pincus bellyspot:4 anemic (Clark bellyspot), and of these last, one would carry Pincus bellyspot in addition. The numbers recorded in table 11 were obtained.

TABLE 11
F₂ involving anemia (bellyspot) and a piebald bellyspot.

	NORMAL	SPOTTED NON-ANEMIC	ANEMIC	TOTAL
Observed	110	36	44	190
Expected 9:3:4	107	35.6	47.5	
Dev.	3.0	0.4	-3.5	
P.E.	4.61	3.63	4.03	
Dev./P.E.	0.65	0.01	0.87	

As can be seen, very good agreement with a 9:3:4 ratio was obtained, which is added proof that bellyspot (anemia) is not controlled by the piebald gene. Many of the F_2 spotted animals had dorsal spotting of some kind in addition to the ventral spot characterizing the grandparents. Some of them had a complete belt, isolated dorsal spots, a white nose, or a combination of all three. Some of these were anemics and some were non-anemics. Only eleven mice would be expected to be homozygous for both kinds of spotting, and doubtless some of the anemic individuals with dorsal spotting belonged to this class. The others were probably mice carrying a double dose of one spotting and a single dose of the other.

*Tests for linkage between anemia (bellyspot) and
dominant spotting, W*

Bellyspot resembles dominant spotting (*Ww*) in that each has an anemia associated with it. But in the case of the former, the spotting is recessive, and the accompanying anemia is not lethal. It was thought barely possible that bellyspot, because of the anemia accompanying it, might be a recessive allele of dominant spotting. Since it is recessive to normal, we should hardly expect it to be allelic for physiological reasons.

If bellyspot were allelic, we should expect the following results from crosses with Ww . Let w' be used as the symbol for bellyspot, assumed, for the time being, to be an allele of W .

$$P_1 Ww \times w'w'$$

$$F_1 Ww' \text{ spotted, and } ww' \text{ non-spotted}$$

Mating the spotted F_2 *inter se* ($Ww' \times Ww'$), we should expect in F_1 : 1 WW (which dies); 2 Ww' (spotted non-anemic); 1 $w'w'$ (bellyspot anemic). All the mice which were not anemic would have dominant spotting.

If bellyspot and dominant spotting are *not* allelic, we should expect very different results. Let bellyspot be represented by ff . We should then have:

$$P_1 WwFF \times wwff$$

$$F_1 WwFf \text{ (spotted) and } wwFf \text{ (non-spotted)}$$

Mating together the spotted F_2 ($WwFf$), we should expect the following types in F_2 :

$$\begin{array}{l} 4 \left\{ \begin{array}{lll} 1 & WW & FF \text{ dies} \\ 2 & WW & Ff \text{ " } \\ 1 & WW & ff \text{ " } \end{array} \right. \\ 6 \left\{ \begin{array}{llll} 2 & Ww & FF & \text{non-anemic dominant spotting} \\ 4 & Ww & Ff & \text{" " " " } \end{array} \right. \\ 3 \left\{ \begin{array}{llll} 1 & ww & FF & \text{non-anemic selfs} \\ 2 & ww & Ff & \text{" " " } \end{array} \right. \\ 3 \left\{ \begin{array}{llll} 1 & ww & ff & \text{anemic (bellyspot)} \\ 2 & Ww & ff & \text{" " } \end{array} \right. \end{array}$$

If the two spottings are independent, we should accordingly expect a ratio of 3 non-anemic selfs to 6 non-anemic spotted to 3 anemic, or a 1:2:1 ratio. In case dominant spotting and anemic bellyspot are alleles, we should not expect the non-anemic self class to be produced, since all the non-anemics would be spotted. Moreover, if the two were not allelic but linked, we should expect this first class to be smaller than indicated above.

Some bellyspot-flexed-anemic mice were crossed with a dominant spotted strain, and the F_1 spotted animals were mated *inter se*. The character of the F_2 generation is shown in table 12.

Fairly good agreement is observed with a 1:2:1 ratio which indicates that the two types of spotting are neither allelic nor linked as explained above. Since this is a repulsion cross, if bellyspot and dominant spotting were linked, more than two-thirds of the non-anemics would be spotted; in fact, the closer the linkage, the nearer the results would tend to be like those outlined in the hypothetical case of allelism on the previous page.

TABLE 12
F₂ involving dominant spotting and bellyspot-anemia (repulsion).

	NON-ANEMIC SELF	NON-ANEMIC SPOTTED	ANEMIC BELLYSPT	TOTAL
Observed	10	25	16	51
Expected 1:2:1	12.7	25.5	12.7	
Dev.	-2.7	-0.5	3.3	
P.E.	2.09	4.72	2.09	
Dev./P.E.	1.29	0.11	1.58	

Further evidence of the free assortment of these two types of spotting is obtained when we mate the spotted F_1 ($WwFf$) with non-spotted F_1 ($wwFf$) animals from the same P_1 cross. In a repulsion cross of this kind, if these two characters were linked, we should expect to obtain very few, if any, anemic animals which have a face blaze characteristic of this particular strain of dominant spotting. In case linkage does not exist, we should expect the following types to be produced in F_2 .

$$\begin{array}{l}
 3 \left\{ \begin{array}{l} 1 \text{ } ww \text{ } FF \text{ non-anemic self} \\ 2 \text{ } ww \text{ } Ff \text{ " " "} \end{array} \right. \\
 3 \left\{ \begin{array}{l} 1 \text{ } Ww \text{ } FF \text{ non-anemic-spotted} \\ 2 \text{ } Ww \text{ } Ff \text{ " " "} \end{array} \right. \\
 1 \text{ } Ww \text{ } ff \text{ anemic dominant spotted} \\
 1 \text{ } ww \text{ } ff \text{ anemic bellyspot (or overlap for bellyspot)}
 \end{array}$$

In other words, a 3:3:1:1 ratio would be expected on the basis of no linkage. The observed result is shown in table 13.

TABLE 13
F₂ involving dominant spotting and bellyspot-anemia, obtained by crossing spotted F_1 with non-spotted F_1 (repulsion).

	NON-ANEMIC		ANEMIC		
	SELF	SPOTTED	DOMINANT SPOTTED	BELLYSPT	TOTAL
Observed	23	28	6	9	66
Expected 3:3:1:1	24.75	24.75	8.25	8.25	
Dev.	-1.75	4.25	-2.25	0.75	
P.E.	2.96	2.96	1.81	1.81	
Dev./P.E.	0.59	1.10	1.24	0.41	

The close agreement in table 13 between observed and expected numbers indicates that bellyspot and dominant spotting are not linked. Belly-

spot, then, is a new recessive spotting factor in mice, since it shows free assortment with both dominant and piebald spottings.

If bellyspot is a new recessive spotting, it was thought desirable to test its linkage relations with other characters. Because of failure to obtain crossovers between bellyspot and anemia, the use of anemia as an indicator for presence of the bellyspot gene was continued. For the P_1 crosses, however, mice were used which phenotypically showed bellyspot. Since all bellyspot animals which appeared in the F_2 were always anemic, this method was proved to be accurate.

Linkage relations were tested between bellyspot-anemia and the following characters:

1. *Sex*. If anemia were a sex-linked character, we should expect, from the cross of anemic male with normal female, to obtain only normals in F_1 , and in F_2 half the males should be anemic but all the females normal. In reality, of the 63 anemic mice produced in F_2 , 31 were males and 32 females. This result rules out the possibility of sex linkage for the gene concerned in anemia. Further, if the character complex were sex-linked, all the F_1 males would be flexed-anemic when a flexed-tail ♀ was crossed with a normal ♂. But no flexed-tail males appeared in such crosses, which is additional evidence that the bellyspot-flexed-anemic complex is not sex-linked.

2. *Pink-eye*. Since PLATE's data indicated possible linkage of his kinky-tail mutation with pink-eye, this linkage test was of special interest. HUNT et al (1933) has shown, however, that flexed-tail is not linked with albinism. The data of table 14 confirm his results, since pink-eye and albinism are closely linked with each other.

TABLE 14
F₂ involving pink-eye and bellyspot (coupling).

	NORMAL		ANEMIC		TOTAL
	P	p	P	p	
Observed	132	40	42	14	228
Expected 9:3:3:1	128.2	42.8	42.8	14.2	
Dev.	3.8	-2.8	-0.8	-0.2	
P.E.	5.05	3.98	3.98	2.47	
Dev./P.E.	0.75	0.70	0.20	0.08	

3. *Roberts' pink-eye (p_2)*. ROBERTS (1931) reported a new pink-eyed mutation captured in the wild. He states that the shade of pink in the eye was indistinguishable from that in the eye of the common pink-eyed variety, but that the coat color, while plainly agouti, was lighter than that of a pink-eyed black agouti.

This gene dilutes both the yellow and black (or brown) in agouti individuals. A mouse duplex for this gene and for extreme dilution ($c^d c^d p_2 p_2$) has very little color, pigment being eliminated from the ears.

ROBERTS (1933, unpublished observations) finds that his pink-eye is linked with agouti, the crossover percentage being about 21.

The F_2 results from a cross of Roberts' pink-eye and anemia are shown in table 15. Since the number of animals recovered in the two crossover classes is very close to the number expected, it may be concluded that Roberts' pink-eye and bellyspot anemia are not linked. Since Roberts' pink-eye and agouti are linked, it follows that anemia is not linked with agouti.

TABLE 15
F₂ involving Roberts' pink-eye and anemia (repulsion).

	NORMAL		ANEMIC		TOTAL
	P_1	p_2	P_1	p_2	
Observed	60	23	14	8	105
Expected 9:3:3:1	59.1	19.7	19.7	5.5	
Dev.	0.9	3.3	-5.7	1.4	
P.E.	3.43	2.70	2.07	1.67	
Dev./P.E.	0.26	1.22	2.11	0.84	

4. *Agouti*. For tests with agouti, the agouti allele, tan belly, was used. Since agouti is linked with Roberts' pink-eye, a test with one of these two characters is sufficient. At the time these data were obtained, however, the fact that agouti and Roberts' pink-eye were linked was unknown. In this cross, bellyspot-anemic animals were crossed with an inbred short-ear dilute brown-and-tan Bussey strain, and an F_2 population was obtained. The F_2 results are shown in table 16.

TABLE 16
F₂ involving anemia and tan belly (repulsion).

	NORMAL		ANEMIC		TOTAL
	a^t	a	a^t	a	
Observed	167	80	57	17	321
Expected 9:3:3:1	180.6	60.2	60.2	20.0	
Dev.	-13.6	19.8	-3.2	-3.0	
P.E.	5.99	4.72	4.72	2.93	
Dev./P.E.	2.27	4.19	0.68	1.02	

It will be noticed that the deviation in the second class is 4.19 times its probable error. Since this is a crossover class and the deviation is positive,

it does not indicate linkage. Moreover, the double recessive parental type (anemic non-agouti) did not appear in excess in F_2 . We may conclude, then, that bellyspot anemia and agouti are not linked, thus corroborating the results obtained with Roberts' pink-eye.

5. *Dilution* (blue). The dilution used in this test came from the same inbred strain that was used in the previous cross. The F_2 results with anemia are shown in table 17. A very good fit to a 9:3:3:1 ratio was obtained, which indicates that bellyspot-anemia and blue dilution are located on different chromosomes. The largest deviation is in the double recessive class and is well within the limits of chance variation. It is quite possible that animals which have both dilution and anemia are more likely to die before time of classification.

TABLE 17
F₂ involving anemia and dilution (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>D</i>	<i>d</i>	<i>D</i>	<i>d</i>	
Observed	307	105	105	26	543
Expected 9:3:3:1	305	102	102	34	
Dev.	2.0	3.0	3.0	-8.0	
P.E.	7.80	6.13	6.13	3.80	
Dev./P.E.	0.26	0.49	0.49	2.11	

6. *Chocolate*. The data given in table 18, derived from the same cross indicate that anemia and chocolate are not linked. This time we have two classes which show a significant deviation from the expected numbers. If there were linkage, however, we should expect a large plus deviation in

TABLE 18
F₂ involving anemia and chocolate (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>B</i>	<i>b</i>	<i>B</i>	<i>b</i>	
Observed	244	42	78	21	385
Expected 9:3:3:1	216.6	72.2	72.2	24.1	
Dev.	27.4	-30.2	5.8	-3.1	
P.E.	6.57	5.17	5.17	3.20	
Dev./P.E.	4.17	5.84	1.12	0.97	

the parental classes and a large minus one in the crossover classes. This, however, is not the case. One of the large deviations is a positive one in the crossover class, normal black, and the other is a minus deviation in the parental class, normal brown. The other two classes, one parental and the

other crossover, show very good agreement with the expected numbers. We may conclude, then, that anemia and chocolate show independent segregation.

7. *Naked*. A male, heterozygous for naked, was crossed with an anemic-bellyspot female, and several of the F₁ naked males were crossed with bellyspot-anemic females. The results of this backcross are tabulated in table 19. Very close agreement with the expected 1:1:1:1 ratio was secured, indicating that the genes for naked and anemia are located on different chromosomes.

TABLE 19
Backcross involving anemia and naked (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>Nk</i>	<i>nk</i>	<i>Nk</i>	<i>nk</i>	
Observed	20	17	18	17	72
Expected 1:1:1:1	18	18	18	18	
Dev.	2	-1	0	-1	
P.E.	2.48	2.48	2.48	2.48	
Dev./P.E.	0.81	0.40	0.00	0.40	

8. *Rodless retina*. Two mice possessing the retinal eye defect, rodless, were secured from Dr. CLYDE E. KEELER and crossed with anemic animals. On the basis of free segregation, one would expect in the F₂ a ratio of 9 normal:3 non-anemic rodless:3 anemic non-rodless:1 anemic rodless. Or, considering the anemics alone (last two classes), we should expect one anemic out of four to be rodless. Since this is a repulsion cross, if these two characters were linked, less than one-fourth of the anemics should be rodless; and the stronger the linkage, the greater this deficiency would be. For this reason, I considered it sufficient to section the eyes of the anemics only and discard the non-anemics. The data secured by following this procedure are shown in table 20, and they show clearly that bellyspot-anemia and rodless are not linked.

TABLE 20
F₂ involving anemia and rodless retina (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>R</i>	<i>r</i>	<i>R</i>	<i>r</i>	
Observed	Discarded		27	6	33
Expected 3:1			24.75	8.25	
Dev.			2.25	-2.25; P.E. 1.68; Dev./P.E. 1.34	

9. *Waltzing*. An F_2 ratio obtained from a repulsion cross involving waltzing and anemia is shown in table 21. It will be noticed that the observed crossover classes fit very closely to the numbers expected on the basis of no linkage. Obviously, the genes for waltzing and bellyspot-anemia are borne on different chromosomes.

TABLE 21
F₂ involving anemia and waltzing (repulsion).

	NORMAL		ANEMIC		TOTAL
	V	v	V	v	
Observed	46	9	13	5	73
Expected 9:3:3:1	41.1	13.7	13.7	4.6	
Dev.	4.9	-4.7	-0.7	0.4	
P.E.	2.86	2.25	2.25	1.39	
Dev./P.E.	1.71	2.09	0.31	0.30	

10. *Brachyury* (short-tail). Brachyury mice obtained from Dr. HUNT were crossed with bellyspot anemic animals, and F_1 short-tailed individuals were crossed with anemics. The backcross data are shown in table 22. Although the first class shows rather a large deviation from the expected number, the other three observed classes agree very well with the calculated numbers. The data indicate clearly that brachyury and bellyspot-anemia are not linked.

TABLE 22
Backcross involving anemia and brachyury (repulsion).

	NORMAL		ANEMIC		TOTAL
	Br	br	Br	br	
Observed	65	48	53	49	215
Expected 1:1:1:1	54	54	54	54	
Dev.	12	-6	-1	-5	
P.E.	4.29	4.29	4.29	4.29	
Dev./P.E.	2.80	1.40	0.23	1.16	

11. *Leaden*. Several leaden bellyspot-anemic mice, obtained from Dr. JOSEPH MURRAY, were crossed with normal mice, and the F_1 animals were backcrossed with the leaden anemic parents. The results secured from this backcross are shown in table 23.

The two parental classes (non-anemic non-leaden and anemic leaden) deviate considerably from the calculated numbers, but these deviations are only 2.25 times their respective probable errors, and one is a plus, while the other is a minus, deviation. The crossover classes deviate still

TABLE 23

Backcross involving anemia and leaden (coupling).

	NORMAL		ANEMIC		TOTAL
	L	l	L	l	
Observed	45	43	31	29	148
Expected 1:1:1:1	37	37	37	37	
Dev.	8	6	-6	-8	
P.E.	3.55	3.55	3.55	3.55	
Dev./P.E.	2.55	1.70	1.70	2.25	

less from their expected numbers. Leaden and anemia, then, are independent characters, and are located on different chromosomes.

12. *Hydrocephalus*. Hydrocephalus is a new recessive mutation in the mouse (CLARK 1932, ZIMMERMANN 1933). Mice with this defect have a dome-shaped skull, caused by pressure of the cerebrospinal fluid within the ventricles of the fore-brain. Serial sections of the brain of newborn and older hydrocephalic animals show that this hydrocephalus is of the obstructive or internal type, produced by a congenital occlusion of the Aqueduct of Sylvius (results as yet unpublished). The cerebrospinal fluid which normally reaches the subarachnoid space is trapped in the fore-brain, and its accumulation there causes great distention of the lateral and third ventricles.

Hydrocephalus, in some cases, can be detected at birth but often can not be recognized with certainty until about the 15th day of postnatal life. Mice homozygous for this gene rarely live to sexual maturity.

Preliminary results seemed to indicate that this character was either linked with flexed-tail anemia or caused by the same gene. It is now certain that these two characters are not caused by the same gene. Linkage tests are in progress and will be reported at a later date.

13. *Dwarf*. Three mice heterozygous for dwarf (kindly supplied by Dr. E. C. MACDOWELL) were mated with bellyspot-anemic mice. The F_1 females were tested for dwarf by mating them with one or the other of two males from some of my other crosses which were known to be heterozygous for anemia and proved also to be heterozygous for dwarf. It was found that the pedigree of these two males traced back to the dwarf stock of Dr. SNELL. Dwarf animals appeared when they were mated with female sibs of the same ancestry. The F_2 results are shown in table 24.

The fit of the observed to the expected ratio, although it might be closer, shows clearly that anemia and dwarf are independent. Both parental classes (dwarf non-anemic and non-dwarf anemic) have fairly large deviations, but one of them is a plus deviation, while the other is minus. In

TABLE 24
F₂ involving anemia and dwarf (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>Dw</i>	<i>dw</i>	<i>Dw</i>	<i>dw</i>	
Observed	51	24	12	9	96
Expected 9:3:3:1	54	18	18	6	
Dev.	-3	6	-6	3	
P.E.	3.28	2.58	2.58	1.60	
Dev./P.E.	0.91	2.33	2.33	1.87	

each case the deviation is less than three times its probable error. The two crossover classes agree very well with the calculated numbers.

14. *Zavadskaia shaker* (DOBROVOLSKAIA-ZAVADSKAIA 1928b). This mutation arose among the descendants of X-rayed mice, and behaves as a Mendelian recessive. Animals with the defect exhibit constant nervous shakings of the head.

Before testing this character with bellyspot, it was considered desirable to demonstrate that it is not identical with either ordinary shaker or waltzing. When Zavadskaia shaker was crossed with waltzing, normal animals were produced in the F_1 , proving that the two characters are different. In addition, Zavadskaia shaker mice, homozygous for chinchilla, were crossed with normal, fully colored mice. The F_1 were mated *inter se*, with the results shown in table 25. Since ordinary shaker is linked with

TABLE 25
F₂ involving Zavadskaia shaker and chinchilla (coupling).

	NORMAL		ZAV. SHAKER		TOTAL
	<i>C</i>	<i>c^h</i>	<i>C</i>	<i>c^h</i>	
Observed	21	8	12	2	43
Expected 9:3:3:1	24.2	8.1	8.1	2.7	
Dev.	-3.2	-0.1	3.9	-0.7	
P.E.	2.19	1.73	1.73	1.07	
Dev./P.E.	1.46	0.06	2.25	0.65	

chinchilla (albino series) with only 2 percent crossing over, this new shaker, if it were ordinary shaker, would also show linkage with chinchilla, but a good fit to a 9:3:3:1 ratio was obtained with no indication of linkage. After these results were obtained, DUNN informed me that he has crossed Zavadskaia shaker with both ordinary shaker and waltzing and finds that it differs genetically from both of them.

Preliminary results indicate that the Zavadskaia shaker character is not linked with bellyspot. The F₂ data are shown in table 26.

TABLE 26
F₂ involving anemia and Zavadskaia shaker (repulsion).

	NORMAL		ANEMIC		TOTAL
	<i>Sh</i> ₂	<i>sh</i> ₂	<i>Sh</i> ₂	<i>sh</i> ₂	
Observed	28	11	5	4	48
Expected 9:3:3:1	27	9	9	3	
Dev.	1	2	-4	1	
P.E.	2.32	1.82	1.82	1.13	
Dev./P.E.	0.43	1.98	2.20	0.88	

MUTANT CHARACTERS OF THE MOUSE

For the convenience of those interested in linkage studies in the mouse, I shall list the mutant characters of mice, indicating whether they are independent or linked, incompletely tested for linkage, extinct, or unfitted for linkage studies.

INDEPENDENT CHARACTERS OR		10. Waltzing
LINKED GROUPS		11. Dwarf
Map distance		12. Naked
1. Agouti series 0.0		Note: Leaden has not been tested for linkage with rodless or silver.
Roberts' pink-eye		
21.0 (ROBERTS)		
2. Chocolate		CHARACTERS NOT COMPLETELY TESTED FOR LINKAGE
3. Albino series 0.0		
Shaker 2.5 (GATES)		
Pink-eye 18.0 (HALDANE et al.)		13. Zavadskaia shaker
4. Short-ear 0.0		14. Dominant flexed-tail
Dilution 0.034 (SNELL)		15. Short-tail (brachyury)
5. Leaden		16. Hare-lip and cleft-palate
6. Rodless 0.0		17. Absence of <i>Corpus callosum</i>
Silver 12.0 (KEELER)		18. Myencephalic blebs
7. Piebald 0.0		19. Hydrocephalus
Hairless 8.0 (SNELL)		20. Hound-ear
8. Dominant spotting		21. Circling
9. Bellyspot		22. Waved
Recessive flexed-tail		
Anemia		

CHARACTERS DESCRIBED BUT EXTINCT	CHARACTERS WITH UNCERTAIN IN- HERITANCE PROBABLY NOT SUITED FOR LINKAGE STUDIES
23. Angora	
24. Twisted nose	
25. Inter-frontal	28. Ectromelus
26. Parted frontals	29. Polydactylism
27. Hyperglycæmia?	30. Wildness

As can be seen from the above list, there are twelve characters, or groups of characters, which have been shown to be independent of each other. Leaden, although it has not been tested with rodless or silver, was provisionally included in this list. Thus, there is the possibility that only eleven chromosomes are accounted for, since leaden may be carried on the same chromosome as rodless and silver.

The second group includes ten characters which have been only partially tested for linkage or not tested at all. Undoubtedly some of these characters will prove to be linked with each other or with characters of the first group.

The third group contains five characters which have been described but are now extinct.

The fourth group includes three characters which have an uncertain inheritance, and are probably not suited for linkage studies, although opinion might differ on that point.

CONCLUSIONS

Bellyspot, a new spotting character in the mouse, is shown to be recessive, independent of piebald and dominant spotting, and very closely linked with the recessive flexed-tail-anemia complex reported by HUNT and PERMAR.

It is demonstrated that both bellyspot and flexed-tail give high percentages of normal overlaps, while anemia, the third member of the complex, behaves as a typical Mendelian recessive without normal overlaps.

Postnatal mortality is shown to be 10 percent higher among anemic mice than among normals during the first few weeks of life. No evidence was found to indicate that anemic-bellyspot mice have a higher prenatal death rate than normals.

One mutation to white belly was found.

Data are presented proving that bellyspot is not linked with piebald, dominant spotting, sex, pink-eye, Roberts' pink-eye, agouti, dilution, chocolate, naked, rodless, waltzing, brachyury, leaden, dwarf, or Zavad-skaia shaker.

The bellyspot-flexed-anemic complex, therefore, is independent of the

eleven independent characters or linked groups in mice. With the completion of these tests, the number of mutually independent characters or linked groups is increased from eleven to twelve by the addition of belly-spot-anemia.

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