

LINKAGE OF THE FACTORS FOR SHORT-EAR AND  
DENSITY IN THE HOUSE MOUSE  
(*MUS MUSCULUS*)

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INTRODUCTION

In 1921 Miss LYNCH reported a mutation in the house mouse in the form of "short-ears." The ears of this mutant are short, thick, and somewhat wrinkled. Correlated with this are modifications of the skull such as shortening of the muzzle, curvature of the frontal bones, and other structural variations.

Miss LYNCH reported this as a simple recessive mutation, the gene for which was located in an autosome. The author's observations are in harmony with Miss Lynch's, for he has obtained 397 short-eared individuals out of an F<sub>2</sub> population of 1613, where expectation is 403, a difference of only  $6 \pm 11.7$ .

This short-eared character, from a morphological standpoint, seems similar to that described by RITZMAN (1916) in sheep. In both cases the ears are short and thick, and both are due to a single Mendelizing factor. The two characters differ, however, in their inheritance. Short-ears in sheep is dominant, while in mice it is recessive.

In February, 1925, Miss Lynch very kindly sent to Doctor W. E. CASTLE of HARVARD UNIVERSITY six short-eared males. These were mated to normal mice, mostly pink-eyed, dilute browns. The F<sub>2</sub> from this out-cross gave a number of short-eared individuals, from which a strain of short-eared mice was established. These were inbred for three generations and proved constant for the short-ear character.

The author wishes to thank Doctor CASTLE for the gift of these short-eared mice, which constituted the original source of his stock. He also wishes to express his appreciation of kindly suggestions of this report by Doctor CASTLE.

During the winter and spring of 1925, the author, GATES (1926), tested this short-ear mutation as to linkage relationship with the waltzing factor. It showed itself to be inherited independently of this character. KEELER (1926) found it to be independent of the factor for rodless retina. Further than this, the author is not aware that the short-eared character has been tested for linkage with any other character aside from that reported herewith.

Since the most satisfactory method of testing linkage is a back-cross to a double recessive, and since the author had a strain of pink-eyed, dilute, brown, piebald waltzers, the short-ear was crossed to this strain in the hope of establishing a strain of pink-eyed, dilute, brown, piebald, short-eared waltzers, and then crossing these with the dense black, short-eared strain, in this manner testing the short-eared character for linkage with pink-eye, dilution, brown, piebald, and waltzing at one and the same time.

It was very quickly evident, however, that there was linkage between short-ear and density, for from the first, the dilute, short-eared class was lacking, although the other recombination classes showed up as expected.

With linkage between short-ear and density apparent, the cross involving the five point recessive was abandoned so as to eliminate the unequal growth conditions in litters containing both waltzers and non-waltzers. The parent races then selected were Doctor LITTLE's strain of dilute browns, and a pink-eyed, dense, short-eared strain. I am indebted to Doctor LITTLE for kindly sending me several pens of dilute mice which were mated with the short-eared race.

The  $F_1$  from this cross proved to be quite vigorous and hardy. The females were good mothers, seldom killing the young as was sometimes the case in the cross which involved several recessive characters including waltzing.

The character dilution involved in this experiment is that described and analyzed by Miss DURHAM (1908, 1911), LITTLE (1913), and others. It is that factor which dilutes the black color of mice to a maltese gray; and changes the chocolate brown color to a taupe. As summarized by the author, GATES (1926), this dilute factor has been shown to be inherited independently of the factors for agouti, brown, pink-eye, piebald, and waltzing. WACHTER (1927) reports it to be independent of black-eyed white.

#### METHODS

The mice were kept in wooden boxes with wire tops. Breeding pens were made up of not over four and usually only three females mated to

one male. Except in small litters, where there was no male, all mice of each generation were brother-sister mated.

Pregnant females were removed from the breeding pens and placed in separate boxes. The young were recorded at birth and the female left undisturbed for fourteen days. On the fourteenth day or after, the young were examined and their characters recorded.

While the characters of dilution and density are very early distinguishable (fourth to sixth day), those of short- and normal-ear cannot be determined before the young are from fourteen to sixteen days old. Occasionally, retarded growth may not enable one to distinguish the characters even at that age.

At three weeks or later, the young were weaned and their recorded characters checked. The mother was then returned to her pen. and the young mated or discarded.

All  $F_2$  young which resembled their  $F_1$  parents with respect to the two characters were mated *inter se* or with others like themselves. The short-eared individuals (which were all dense) were mated with the dilute and the dilute individuals with short-eared. The symbols used herewith are those adopted at New Haven, 1925, GATES (1926), namely, density— $D$ , dilution— $d$ , normal-ear— $S^e$ , and short-ear— $s^e$ .

#### RESULTS

When two independent Mendelian characters enter a cross they tend to segregate in the  $F_2$  in the ratio of 9 showing both dominant characters, 3 showing one dominant and one recessive, 3 showing the other dominant and other recessive, and 1 showing both the recessive characters. Linkage will tend to disturb this ratio, and if it is quite strong, with crossing over very rare, it is possible that two cross over gametes will hardly ever unite, forming the double recessive. Complete linkage will eliminate the last class entirely and give a ratio in the other three classes of 2:1:1, in which 2 will show both dominant characters, 1 one of the recessives, and 1 the other recessive.

In this particular case the hybrid between a short-eared dense mouse and a normal-eared dilute is normal-eared and dense, as would be expected. When mated together these hybrids, if there is no linkage between short-ear and dilution, should theoretically produce four types of offspring, namely, normal-eared dense, normal-eared dilute, short-eared dense, and short-eared dilute in the ratio of 9:3:3:1.

Actually, however, these  $F_1$  double heterozygotes produced only three types, namely, normal-eared dense, short-eared dense, and normal-eared

dilute. These have, up to this time, occurred in the ratio of 504:234:236. Theoretically, with complete linkage, the expected ratio in a population of this size would be 487:243.5:243.5, a difference of  $17 \pm 10.53$ ,  $9.5 \pm 9.11$ , and  $7.5 \pm 9.11$  respectively.

The probable error of the first class is computed on the basis of a 1:1 ratio and the other two on a 3:1 ratio of the total population. Obviously, therefore, the deviation is so slight as to be well within the range of probability of a 2:1:1 ratio. It is to be noted, however, that the deviations from a 2:1:1 ratio are all *qualitatively* such as would be expected if linkage were *not quite* complete, that is if a small amount of crossing over actually occurred. Thus the first class is greater than expected and the other two classes are both less than expected. However, the variations in the numbers from time to time during the experiment would indicate that the deviations were due to random sampling.

The entire absence of the fourth class indicates very close or possibly complete linkage. It indicates that if crossing over occurs at all, it is extremely rare. It suggests as a bare possibility that crossing over may in this instance be restricted to one sex, as in *Drosophila*. But this suggestion, aside from facts reported below, is rendered highly improbable by the repeatedly verified fact that in mice crossing over occurs almost as freely in males as in females between the genes *c* (albinism) and *p* (pinkeye), (CASTLE 1925). The situation is similar as regards crossing over in rats, (CASTLE and WACHTER 1924), while in rabbits no significant difference is found between the sexes as regards frequency of crossing over, (CASTLE 1926).

A short-eared dilute individual would result only from a double cross over, that is, a cross over in both the gametes which united to form the zygote. The numbers are sufficiently large to make it obvious, therefore, that if crossing over is not restricted to one sex, it is so rare that there is little or no probability of two crossover gametes uniting.

From the results recorded above there is nothing to indicate that a crossover might not have taken place in one of the two gametes producing a zygote. In other words, the three phenotypes mentioned above might each possess a genetic constitution of a crossover in one of the two gametes, without altering its appearance. Thus the normal-eared dense individuals of the  $F_2$  generation might have a genetic formula of  $S^e s^e Dd$ ,  $S^e s^e DD$ ,  $S^e S^e Dd$ , or  $S^e S^e DD$ . A genetic constitution indicated by either the second or the third formula would mean that a crossover had taken place in one of the two constituent gametes but not in the other, while a genetic

constitution indicated by the last formula could result only from the union of two crossover gametes.

The genetic formula of each of these normal-eared dense individuals of the  $F_2$  generation could be determined by mating to similar sibs and noting the  $F_3$  generation, or by mating first to a short-eared and then to a dilute individual. The former mating being simpler, and usually involving less time, and fewer offspring, was the type of mating used to test the normal-eared dense individuals of the  $F_2$ . Such matings might be of ten possible combinations, namely:

$$\begin{array}{l}
 S^e s^e Dd \times S^e s^e Dd \\
 \quad " \quad \times S^e S^e Dd \\
 \quad " \quad \times S^e s^e DD \\
 \quad " \quad \times S^e S^e DD \\
 S^e S^e Dd \times S^e S^e Dd \\
 \quad " \quad \times S^e s^e DD \\
 \quad " \quad \times S^e S^e DD \\
 S^e s^e DD \times S^e s^e DD \\
 \quad " \quad \times S^e S^e DD \\
 S^e S^e DD \times S^e S^e DD
 \end{array}$$

A mating of the first combination, that is of double heterozygotes similar to the  $F_1$  generation, would alone produce the three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute. Any of the nine other possible matings would produce only one, or at most two classes of young, but never three. The normal-eared dense individuals of the  $F_2$  generation can thus, by mating *inter se*, be tested for crossovers in one or the other of the gametes which produced them.

Such matings have given the following total numbers of individuals: 177 normal-eared dense, 90 short-eared dense, and 94 normal-eared dilute. These results are so close to the 2:1:1 expectation that there is small probability of a crossover having occurred, even if these individuals had not been further tested, for on the basis of a 2:1:1 ratio, the expectation is 180.5:90.25:90.25, a difference of  $3.5 \pm 6.41$ ,  $0.25 \pm 5.55$ ,  $3.75 \pm 5.55$ . Here it is to be noted that qualitatively the deviations are the reverse as would occur if crossing over were an occasional or rare event, for the double dominant class is *less* than expected and the single dominant class is *greater* than expected.

Furthermore, 54 males and 66 females of normal-eared dense animals of the  $F_2$  generation have been tested *individually* and have produced all three classes of young, showing themselves to have a genetic constitution similar to their parents of the  $F_1$  generation. In every instance, where there have been a sufficient number of young, the parents have proved to be double heterozygotes by producing the three types of young, indicating a condition of no crossing over.

Similarly, the normal-eared dense individuals of the  $F_3$  and  $F_4$  generations were tested by mating *inter se*. The results of these matings show a ratio of 146:69:74, the expectations (if linkage is complete) being 144.5:72.25:72.25 respectively, and the deviations from these expectations being  $1.5 \pm 5.73$ ,  $3.25 \pm 4.97$ , and  $1.75 \pm 4.97$  respectively. Here again the results follow closely the expectation of 2:1:1 and give no indication either qualitative or quantitative of crossing over. The matings in question have been made between 35 males and 41 females of the  $F_3$  and  $F_4$  generations and each mating has yielded in every instance the expected three classes of young.

Since there is little or no indication from the above results that a cross-over has occurred or that the normal-eared dense individuals are anything but double heterozygotes, we may combine these figures to obtain a total progeny population of 1624 for matings *inter se* of double heterozygotes, which will include 827 normal-eared dense, 393 short-eared dense and 404 normal-eared dilute, the respective expectancies being 812:406:406. Here the deviations from expectancy on the basis of 2:1:1 ratio are  $15 \pm 13.5$ ,  $13 \pm 11.7$ , and  $2 \pm 11.7$ . This is a very close approximation to expectancy and tends toward the same possible qualitative result shown by the  $F_2$  generation. But here again the results have varied from time to time and are not at all consistent. For instance, at an earlier stage in the experiment the numbers were 735:360:370 with expectation of 732.5:366.25:366.25, a deviation of  $2.5 \pm 12.8$ ,  $6.25 \pm 11.0$ , and  $3.75 \pm 11.0$ . This can hardly indicate anything but random sampling.

Futhermore, combining the normal-eared dense individuals of the  $F_2$ ,  $F_3$ , and  $F_4$  generations, which have been individually tested, we find a total of 224, both males and females. In no instance where the number of offspring has been sufficient has any individual of this number failed to produce all three types of young, showing that no crossing over has occurred in any of these double heterozygotes, and an indication that if crossing over occurs at all, it is a very rare event.

Each of the other two classes of the  $F_2$  generation might just as well be the result of the union of one crossover gamete with a non-crossover.

Under these conditions the short-eared dense individuals would have a formula of  $s^e s^e Dd$  and the normal-eared dilute  $S^e s^e dd$ , whereas the non-crossover formulae would be  $s^e s^e DD$  and  $S^e S^e dd$  respectively.

Both of these classes can be simultaneously tested for a crossover gamete by mating them with each other: short-eared dense with normal-eared dilute. Such matings, on the hypothesis of complete linkage, should produce only one type of young, normal-eared dense, similar in constitution to the  $F_1$ . If there should appear among the offspring a recessive individual, either short-eared or dilute, one or the other parent must have been produced by a gamete carrying a crossover. Thus,  $s^e s^e Dd$  (crossover)  $\times S^e S^e dd = S^e s^e Dd$  and  $S^e s^e dd$  (dilute) or  $S^e s^e dd$  (crossover)  $\times s^e s^e DD = S^e s^e Dd$  and  $s^e s^e Dd$  (short-eared). Furthermore, if this mating should produce three or four classes of young, it would indicate a crossover in one gamete of each parent, for  $S^e s^e dd \times s^e s^e Dd = S^e s^e Dd + S^e s^e dd + s^e s^e Dd + s^e s^e dd$ .

Up to date, 58 short-eared males and 66 short-eared females of the second, third, and fourth generations have been so tested, and in no case have the offspring been anything but normal-eared and dense. In some few instances the number of young has not been large enough to preclude the possibility of the missing class appearing among a larger population. But since expectation is half and half of each of the two possible classes in each case of a single crossover, the author has considered five offspring to constitute an adequate test. Likewise, 32 dilute males and 46 dilute females have been tested and all have proved to be homozygous for normal-ear as well as dilution. None has shown itself to have been produced by a crossover gamete.

Thus we find that both the short-eared and the dilute individuals of the  $F_2$ ,  $F_3$ , and  $F_4$  generations are homozygous for each of the two characters respectively, and therefore are like the original parental types.

Combining all these numbers we have a total of 426 individuals whose genetic constitution has been tested. Since each zygote is the product of two gametes, we may therefore conclude that 852 gametes have been tested with no case of a crossover, indicating a condition of very close or a possibility of complete linkage. Likewise, the further data afforded by the  $F_2$ ,  $F_3$ , and  $F_4$  generations tends to indicate rare or no crossing over.

Under such abnormal Mendelian ratios one must consider the possibility of lethal effects of a homozygous double recessive, therefore, at first, considerable attention was given to checking up all post-natal deaths. It was soon apparent, however, that there were no post-natal

lethal effects, for among the undepleted litters the same ratio was being obtained as among the general population. Less attention was then given to depletion of litters and the figures include both depleted and undepleted litters.

In *Drosophila*, MORGAN (1914) has shown that no crossing over occurs in the male. CASTLE (1925) has shown that in rats and mice crossing over is but very slightly greater in females than in males. This possibility was kept in mind in this case by testing both females and males of the  $F_2$  generation. The results showed that all normal-eared dense individuals, both *males* and *females*, were double heterozygotes like their parents. A similar condition was found to be true in the  $F_3$  and  $F_4$  generations. None of those tested proved otherwise.

Likewise, both male and female short-eared individuals of the  $F_2$ ,  $F_3$ , and  $F_4$  generations were tested by mating to dilutes and no crossing over was observed in either sex. The same mating in each case gave a test for a crossover in the opposite sex.

#### DISCUSSION

This instance of two well established and quite different Mendelian characters showing a relationship of extremely close or complete linkage brings to mind other possible cases of complete linkage, such as the often cited instance of the blue Andalusian fowl, which LIPPINCOTT (1918, 1921) analyzed as complete linkage and not partial dominance.

Reported instances of multiple allelomorphs may prove to be, and can easily be conceived to be cases of complete linkage. Agouti pattern and yellow color have long been considered allelomorphs (Cuenot 1911). But if complete linkage can be shown to exist in other cases, it is possible that agouti and yellow are completely linked characters and not allelomorphs.

Unlike the case reported by GOWEN and GOWEN (1922), there appears to be no factor, dominant or recessive, causing this linkage, for it behaves alike on all outcrosses and there is no instance of crossing over in the  $F_3$ ,  $F_4$ , or  $F_5$  generations, nor is there any indication of crossing over in gametes which go to form heterozygotes.

DANFORTH (1927) has recently called attention to the multiple effects of the gene yellow producing yellow color and obesity. Here again the interpretation may lie in close linkage of the gene yellow and a gene producing obesity, for the disposition to lay on fat is found in other than yellow mice, while yellow mice will nearly always do so under normal feeding conditions.

These two characters dilute and short-ear are so distinct, each of them having been bred extensively and independently for many years, that it is hardly conceivable that they are allelomorphs or that they are multiple effects of single genes, but, on the contrary, from the data here given, we are drawn to the conclusion that they are produced by two very closely linked factors. Although the number of tested gametes is quite large (852) caution should be observed in stating the degree of linkage. In rats, albinism and red-eye were at one time thought to be completely linked (IBSEN 1920), but later CASTLE and WACHTER (1924) found these to be closely linked with a very small percentage of crossing over. So here, it may be that in a larger population, cases of crossing over may occur. The present data, however, indicates that such cases would occur at least but rarely, and that the production of a short-eared dilute mouse from the present short-eared dense stock will at least involve large numbers.

#### SUMMARY

The data presented in this paper indicate that there is very close or possibly complete linkage between the gene short-ear and density and their allelomorphs normal-ear and dilution. No crossover has been observed in a single gamete out of a total number of 852. The  $F_2$  generation from this cross includes only three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute, in the ratio of 2:1:1; the normal-eared dense individuals of the  $F_2$ ,  $F_3$ , and  $F_4$  generations have produced only three phenotypes. The short-eared dense individuals of the  $F_2$ ,  $F_3$ , and  $F_4$  generations when mated with normal-eared dilute sibs produce only the one phenotype—normal-eared dense, showing no crossing over, but homozygosity in every case tested.

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