# THE RELATION OF YELLOW COAT COLOR AND BLACK-EYED WHITE SPOTTING OF MICE IN INHERITANCE

C. C. LITTLE
*Harvard Medical School, Boston, Massachusetts*

[Received February 2, 1917]

CUÉNOT, STURTEVANT and MORGAN have all shown that a series of mutually allelomorphic forms of coat pattern exists in mice. There are four types of pattern in this series. These in order, from the most hypostatic to the most epistatic, are as follows: (1) *Non-agouti,* the ordinary "self" or "unticked" coat pattern. (2) *Agouti* or better *gray-bellied agouti,* the coat pattern seen in the ordinary wild house mouse. (3) *White-bellied agouti,* in which there is a decrease in brown and black pigmentation resulting in more yellow on the dorsal surface and white-tipped ventral hairs. (4) The final member of the series, the *yellow* coat pattern in which almost, if not all, the brown and black pigment of the coat has disappeared and is replaced by yellow.

It is easy to obtain mice homozygous for any of the three lower members of the series: non-agouti, gray-bellied agouti, and white-bellied agouti. No one, however, has yet obtained yellow mice which are homozygous. There is every reason to believe that the process of fertilization between two yellow-bearing gametes occurs (CASTLE and LITTLE 1910). The ratio of yellow to non-yellow young, however, makes it certain that the homozygous yellow zygotes do not reach a sufficiently advanced age to enable one to record them (CASTLE and LITTLE 1910, LITTLE 1911, DURHAM 1911, DUNN 1916). The size of litters when yellows are crossed *inter se* is also smaller than when yellows are crossed with any of the hypostatic types (CASTLE and LITTLE 1910, DURHAM 1911, DUNN 1916).

If we are to consider that allelomorphs occupy the same locus in the chromosome we are faced with a condition somewhat as follows. Four distinct stages occur in the restriction of brown and black pigment from the coat. The first three stages, though they cover a wide range of variation in extent of brown and black pigmentation, may all of them be obtained in a homozygous condition. The fourth step, however, apparently so affects the individual that it can exist only when balanced in the zygote by one of the three lower steps. When both gametes contain the factor for this advanced restriction, the zygote perishes early in its existence.

In a most interesting paper given at the recent New York meeting of the AMERICAN SOCIETY OF ZOOLOGISTS, KIRKHAM has reported the discovery of disintegrating embryos in mice, where the "homozygous yellow" embryos should be found. These disintegrating embryos occur in number corresponding sufficiently well with the Mendelian expectation to make the evidence concerning the fate of the homozygous yellow zygote conclusive.

It is also of fundamental importance to note that in this case, a color factor has shown that it may be an active force in morphogenesis long before the embryo has formed any pigment whatever. This indicates clearly that *the function of a genetic factor may be entirely different at different stages in ontogeny.*

A similar condition exists in the case of "black-eyed white" spotting in mice. Independent in inheritance of either self coat or the ordinary piebald spotting, the black-eyed white factor produces, when acting on "self" mice, an individual with a small number of white spots, occasionally irregular in outline, and when acting on piebald mice, a white individual with pigmented eyes. Such black-eyed whites, however, do not breed true, but give a ratio of approximately one piebald to two black-eyed whites, when crossed *inter se* (LITTLE 1915).

Since these two peculiar results in mice stand as entirely distinct from any others obtained in the study of color factors in rodents it seemed worth while to investigate what relation, if any, they bear to one another.

For the purposes of explanation we may construct the following diagrams to show the separate behavior of the "yellow" and the "black-eyed white" factors in heredity.

Let $Y$ equal the factor for "yellow" coat pattern, and $y$ equal that for non-yellow (non-agouti, gray-bellied agouti, or white-bellied agouti, as the case may be). A heterozygous yellow may be represented by the factors $Yy$. Such an animal forms gametes $Y$ and $y$. When two such yellows are crossed together, the following result is obtained:

Mating, $Yy \times Yy$
Gametes, $Y$ and $y$ $Y$ and $y$
Zygotes, 1 $YY$, homozygous form which fails to continue development.
2 $Yy$, heterozygous yellow.
1 $yy$, non-yellow.

Similarly we may let *W* equal the factor for "black-eyed white" spotting, and *w* its absence. A heterozygous black-eyed white mouse would have the formula *Ww*. If two such animals were crossed *inter se* the following result would be obtained:

Mating, *Ww* × *Ww*
Gametes, *W* and *w*    *W* and *w*
Zygotes, 1 *WW*, homozygous form which fails to develop.
2 *Ww*, heterozygous black-eyed whites.
1 *ww*, non-black-eyed white (ordinary piebald).

The interesting question to answer, if possible, is whether *Y* and *W* act in an identical manner and therefore are unable to exist in a single zygote. Further, if they are not identical, are they in any way related or are they entirely distinct physiologically and genetically.

All the "black-eyed white" mice used in the earlier experiments with this variety were non-yellow; i. e., gray-bellied agouti or non-agouti. This was shown by the fact that all of the piebald young produced by them were either gray-bellied agouti or non-agouti. We may then express the zygotic formula of the black-eyed whites as follows: *yyWw*, that is to say they were homozygous for "non-yellow" but heterozygous for the "black-eyed white" factor. The yellows used were entirely free from the "black-eyed white" factor and of course possessed the yellow factor in a heterozygous condition. Their formula would be *Yyww*. A cross between yellow and black-eyed white (non-yellow) mice would give the following result:

Yellow, *Yyww* × *yyWw*, black-eyed white (non-yellow)
Gametes, *Yw* and *yw*    *yW* and *yw*
$F_1$ zygotes, (a) *YyWw*, yellow.
(b) *Yyww*, yellow.
(c) *yyWw*, non-yellow.
(d) *yyww*, non-yellow.

If the lethal action of *Y* and *W* is the same, the *YyWw* individuals comprising class (a) of the $F_1$ generation should be non-viable. The *Yw* and *yW* gametes might meet in fertilization, just as do two *Y* or two *W* gametes, and the resulting zygote might perish before attaining sexual maturity as do the *YY* and *WW* zygotes. If this happened, class (a) of the $F_1$ generation would form but fail to develop and the resulting ratio of yellow to non-yellow young in $F_1$ would be one to two, not one to one. The actual numbers of $F_1$ young observed were, yellows 76, non-yellows 81. On a 1 : 1 ratio the Mendelian expectation would be 78.5 : 78.5. If a 1 : 2 ratio was the correct explanation the numbers expected are

52 yellow, 105 non-yellow. There is no doubt that the equality ratio is the one approximated. This being the case, it is certain that the action of *y* and *W* is not identical.

The next matter of interest is to determine whether *any* relationship between *Y* and *W* exists, or whether they are entirely distinct from one another.

One possibility is that no fusion between a *Yw* and a *yW* gamete is possible. There might be a selective fertilization. If this is the case, the *Yw* gametes would always be met in fertilization by *yw* gametes producing *Yyww* zygotes. The *yW* gametes will always fertilize or be fertilized by *yw* gametes producing *yyWw* zygotes. *The result would be that* $F_1$ *yellow animals when crossed inter se or with piebald mice of any color should never give black-eyed white young.* The $F_1$ ratio would probably approximate one yellow to one non-yellow and we should have to resort to a breeding test and an $F_2$ generation to determine whether or not there was actual selective fertilization. As a possible modification of this idea of selective fertilization one might imagine that the combination *YyWw rarely* did form but that would mean that an occasional yellow $F_1$ animal would carry the *W* factor and give rise to black-eyed white young, when suitably mated. The exact ratio of such yellows to the more common *Yyww* type would be determined by the degree of antagonism between *Yw* and *yw* gametes which had to be overcome before their union was possible.

The remaining hypothesis is that *Y* and *W* although they act alike in their elimination of a zygote containing a double dose of either of them, are physiologically and genetically entirely distinct. If such were the case, the $F_1$ generation would have an equality ratio of yellows to non-yellows and approximately an equal number of *each* color would or would not carry the *W* factor.

Before turning to the experimental evidence on which the tests of these hypotheses are based, it may be profitable to review very briefly the main facts of the genetic behavior of black-eyed white mice. When black-eyed whites are crossed with self-colored, non-yellow mice, two sorts of $F_1$ animals are obtained. These I have described and figured in a previous paper (LITTLE 1915). I have called them type A and type B. Type A has a distinct spotted appearance. The head and hind quarters are commonly pigmented, but the trunk is usually unpigmented to a considerable degree. The spots of pigment in this position of the body are inclined to be small and irregular in outline in contrast to the large and regular type of pigment patch seen in piebald mice and guinea-pigs. Type B is either entirely without white or else possesses a small amount of white on the ventral surface. Mice of this type are indistinguishable from the heterozygotes obtained when self-colored and piebald mice are crossed.

The type A animals all of them carry the $W$ factor and produce a certain number of black-eyed white young when crossed *inter se* or with piebald animals. The type B animals never give black-eyed white young when crossed *inter se* or with piebalds. The actual numbers which I have obtained are as follows. These figures are a combination of those already reported (LITTLE 1915) with additional data from new crosses between self-colored dilute brown and black-eyed white (non-yellow) mice.

*Black-eyed white × self-colored:*

$F_1$ generation: Type A, 91, type B, 98.

*Type A × piebald:*

| | | | |
|---|---|---|---|
| Observed: | Self-colored, 64; | type A, or spotted, 96; | black-eyed white, 42. |
| *Expected:* | *50.5* | *101.0* | *50.5* |

*Type B × piebald:*

| | | |
|---|---|---|
| Observed: | Self-colored or type B, 94; | piebald, 101. |
| *Expected:* | *97.5* | *97.5* |

If we represent the factor for black-eyed white spotting by $W$ and its absence by $w$, and the factor for self coat by $S$ and its allelomorph for piebald coat by $s^p$, we may represent the above-mentioned crosses as follows:

Mating: Black-eyed white $s^ps^pWw \times SSww$ self-colored (non-black-eyed white)

Gametes: $s^pW$ $Sw$
$s^pw$ $Sw$

$F_1$ zygotes $Ss^pWw$, type A.
$Ss^pww$, type B.

Type A × piebald

$Ss^pWw \times s^ps^pww$

$SW$, $Sw$, $s^pW$, $s^pw$ } $s^pw$ gametes

Zygotes { $Ss^pWw$, type A
$Ss^pww$, self or type B.
$s^ps^pWw$, black-eyed white.
$s^ps^pww$, piebald.

Type B × piebald

$Ss^pww \times s^ps^pww$

$Sw$, $s^pw$ } $s^pw$ gametes

Zygotes $Ss^pww$, type B
$s^ps^pww$, piebald.

### EXPERIMENTAL EVIDENCE FROM CROSSES BETWEEN YELLOWS AND BLACK-EYED WHITE MICE

In making these matings reciprocal crosses were made and gave identical results. Generally speaking it may be said at the outset that the $F_1$ generation animals possessed much less white than the $F_1$ animals in either of the former crosses between black-eyed white and self-colored mice. If the $F_1$ animals are classified into type A and type B mice according to previous methods the yellow $\times$ black-eyed white matings gave only 63 type A to 94 type B instead of the expected equality ratio of 78.5 to 78.5. If we analyze this generation more closely we find that among the non-yellow animals of the $F_1$ generation, there is an almost absolute equality of type A and type B animals. The exact figures are forty-one non-yellow type A and forty non-yellow type B. The yellow $F_1$ animals, however, give a decided preponderance of animals with no dorsal white, which are classed as type B. The actual figures in the yellows are twenty-two of type A and fifty-four of type B. Superficially the evidence appears to favor the idea of selective fertilization. A study of the $F_2$ generation, however, brings out certain extremely interesting facts which make any such assumption unnecessary.

If the type B animals obtained in the $F_1$ generation of the yellow—black-eyed white cross be tabulated according to the approximate percentage of the ventral surface which is unpigmented it will be noticed that many of the *yellow* type B animals have a distinctly greater amount of unpigmented ventral surface than do the type B non-yellows obtained in the same crosses.

| Generation | | Percentage of ventral surface white | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 | 41-45 | 46-50 | 51-55 | 56-60 | — | — | — | 76-80 | **Total** |
| **$F_1$ yellow** | Type B | 29 | 8 | 8 | 2 | 3 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 54 |
| **$F_1$ non-yellow** | Type B | 31 | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 |

Thus thirty-nine of the forty or 97.5 percent of the non-yellow type B animals have less than 6 percent of the ventral surface white while only one has just 6 percent. On the other hand among the yellow type B mice seventeen or 31.4 percent have 6 percent or more of the ventral surface white. Nine of these or 16.6 percent of the total yellows have a degree of whiteness not recorded in type B animals of any previous cross. Eleven of the seventeen yellow animals showing 6 percent or more ventral white have been tested by crossing *and have all shown that they are really type A individuals, not type B as they had been* *classified.* If we remove these from the latter category and place them under type A where they really belong, we have the following result:

| | Before correction | After correction | *Expected* |
|---|---|---|---|
| Yellow type A | 22 | 33 | *39* |
| Yellow type B | 54 | 43 | *39* |
| Non-yellow type A | 41 | 41 | *39* |
| Non-yellow type B | 40 | 40 | *39* |

There is every reason to believe that if the other six yellows of a similar degree of whiteness to those tested had also been bred, a further addition to the yellow type A class and subtraction from the yellow type B class would be made, thus bringing the figures even closer to the expected results.

The corrected figures suggest that the proper hypothesis is one of complete independence between the factors for yellow and black-eyed white.

### TEST MATIGS OF $F_1$ ANIMALS

Non-yellow type A animals of the $F_1$ generation were tested by crossing them with piebald mice. The details of such a cross have already been worked out above. The expectation is one piebald to one type A, to one self-colored or type B, to one black-eyed white. Classing the first two named types together, since at times no distinguishing breeding tests were made, we should expect two spotted, to one self-colored or type B, to one black-eyed white. The actual result was:

| | Observed | *Expected* |
|---|---|---|
| Spotted | 22 | *22* |
| Self or type B | 10 | *11* |
| Black-eyed white | 12 | *11* |
| Total | 44 | *44* |

The agreement between observation and expectation is striking. Yellow type A animals, among which the eleven tested mice first classed as type B are included, have been crossed with piebald non-yellows and have given a total of 165 young. The classes of young expected in this cross are exactly the same as in the previous cross with the exception that there is an equal chance of an animal being yellow or non-yellow in color. The ratio expected then, is one self yellow or type B, to one black-eyed white (yellow), to two spotted yellows, to one non-yellow self or type B, to one black-eyed white (non-yellow) to two non-yellow spotted. The actual results follow:

| | Observed | *Expected* |
|---|---|---|
| Yellow spotted | 35 | *42* |
| Non-yellow spotted | 46 | *42* |
| Yellow self or type B | 31 | *21* |
| Non-yellow self or type B | 21 | *21* |
| Yellow black-eyed white | 4 | *21* |
| Non-yellow black-eyed white | 28 | *21* |
| Total | 165 | *168* |

If we tabulate this generation in a slightly different way we find that the expected figures are approximated more closely in the non-yellows than in the yellows.

| | Yellow | | Non-yellow | |
|---|---|---|---|---|
| | Observed | *Expected* | Observed | *Expected* |
| Spotted | 31 | *34* | 46 | *48* |
| Self or type B | 35 | *17* | 21 | *24* |
| Black-eyed white | 4 | *17* | 28 | *24* |
| Total | 70 | *68* | 95 | *96* |

It is possible by a close examination of the yellow animals to reclassify certain animals on the basis of comparison with the $F_1$ generation and of direct breeding tests. Thus there are among the thirty-one yellows of the self or "type B" class, eleven which are identical in appearance with the $F_1$ "type B" yellows that upon breeding turned out to be type A. While all eleven have not been tested by breeding, several have, and seem to justify classifying these eleven animals under the spotted category as being type A in genetic constitution. After this change the figures read:

| | Before change | After change | *Expected* |
|---|---|---|---|
| Yellow spotted | 35 | 46 | *34* |
| Yellow self or type B | 31 | 20 | *17* |
| Yellow, black-eyed white | 4 | 4 | *17* |

The discrepancy between the observation and expectation in the yellow black-eyed white class is interesting but I believe only apparent. If the yellow and non-yellow spotted animals are tabulated according to the percentage of the dorsal surface which is pigmented it will be noticed that there are fourteen yellows that have less pigment than all but two of the non-yellow spotted mice. Ten of them have less than thirty percent of the dorsal surface pigmented and eight have twenty percent or less. Ordinarily non-yellow black-eyed whites have, as I have shown in my earlier paper, rarely more than 5 percent of the dorsal surface pigmented. Since, however, certain of the $F_1$ and $F_2$ type A yellows have distinctly more pigment than the corresponding variety of non-yellows, it seemed probable that the same might hold true in the case of yellow and non-yellow black-eyed whites. In order to test this question, several of the lightly pigmented spotted yellows were bred to non-yellow piebald mice. The uniform result when yellows with twenty percent or less of dorsal pigmentation are crossed with piebalds was the production of a nearly equal number of black-eyed white[1] and piebald young. This proves that they are genetically black-eyed white mice and are not, therefore, to be included in the "spotted" column. We may then properly remove the yellows with twenty percent or less dorsal pigmentation from the spotted class and place them in the class of yellow black-eyed whites as follows:

| Generation | Percentage of dorsal surface pigmented | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 | 71-80 | 81-90 | 91-99 | Total |
| Yellow spotted $F_2$ | 8 | 2 | 1 | 3 | 1 | 3 | 3 | 7 | 8 | 36 |
| Non-yellow spotted $F_2$ | 1 | 0 | 1 | 0 | 4 | 10 | 6 | 11 | 9 | 42 |

| | Before change | After change | *Expected* |
|---|---|---|---|
| Yellow spotted | 46 | 38 | *34* |
| Yellow self or type B | 20 | 20 | *17* |
| Yellow black-eyed white | 4 | 12 | *17* |
| Total | 70 | 70 | *68* |

Undoubtedly the separation of yellows at the twenty percent mark is arbitrary and it is certain from breeding tests of lightly spotted yellows of other more advanced generations that animals with as much

[1] Among the black-eyed whites are classed yellows resembling their parents, i. e., with 5-20 percent of the dorsal surface pigmented.

as forty percent of the dorsal surface yellow may breed like black-eyed whites. This means that if breeding tests had been made of the two yellows with between twenty and thirty percent dorsal pigmentation both of them would in all probability have joined the class of black-eyed whites and brought the figures even closer to the expectation.

We may now combine the yellows and non-yellows in one category, according to whether they are spotted, black-eyed white, or belong to the self type B class. When this is done the following figures are obtained:

| | Observed | *Expected* |
|---|---|---|
| Total spotted | 84 | *82* |
| Total self type B | 41 | *41* |
| Total black-eyed white | 40 | *41* |
| Grand total | 165 | *164* |

Breeding tests of yellow type B animals crossed with piebald non-yellows show that, as expected, they give four classes of young,—yellow and non-yellow self or type B and piebald yellows and non-yellows in approximately equal numbers.

| | Observed | *Expected* |
|---|---|---|
| Yellow self type B | 36 | *35* |
| Non-yellow self type B | 37 | *35* |
| Yellow piebald | 30 | *35* |
| Non-yellow piebald | 38 | *35* |
| Total | 141 | *140* |

Non-yellow type B animals crossed with piebald give essentially similar results, the yellow classes are, of course, lacking.

| | Observed | *Expected* |
|---|---|---|
| Non-yellow self type B | 43 | *36* |
| Non-yellow piebald | 29 | *36* |

Breeding tests of the back-cross animals produced in the above crosses have shown that all of the expected genetic classes of young occur. In view of this fact it appears fairly certain that the test of the relationship of the yellow and black-eyed white factors has been satisfactorily made.

### SIZE OF LITTERS

CASTLE and LITTLE (1910) have called attention to the fact that litters from yellow × yellow matings are smaller on the average than litters from yellow × non-yellow matings. If the given explanation of the relationship of the yellow and black-eyed white factors is correct the litters produced by crossing together two yellow type A mice should be distinctly smaller than the litters produced when a yellow type A mouse is crossed with a piebald non-yellow. The reason for this will become apparent if we examine the types of zygotes formed when two yellow type A animals are intercrossed.

Mating: Yellow type A *YyWw* × *YyWw* yellow type A

Gametes: *YW YW*
*Yw Yw*
*yW yW*
*yw yw*

Zygotes as shown by the checker-board method.

| | | | |
|---|---|---|---|
| 1 *YW* *YW* | 2 *YW* *Yw* | 3 *YW* *yW* | 4 *YW* *yw* |
| 5 *Yw* *YW* | 6 *Yw* *Yw* | 7 *Yw* *yW* | 8 *Yw* *yw* |
| 9 *yW* *YW* | 10 *yW* *Yw* | 11 *yW* *yW* | 12 *yW* *yw* |
| 13 *yw* *YW* | 14 *yw* *Yw* | 15 *yw* *yW* | 16 *yw* *yw* |

Any zygote containing two "doses" of either the *Y* factor or the *W* factor is eliminated. This would remove zygotes Nos. 1, 2, 3, 5, 6, 9, and 11. Only nine of the sixteen original combinations can continue development and this would result in cutting the litters to nearly one-half their expected size. What actually happens may be seen from the following figures. Ten litters from yellow type A parents crossed *inter se* gave a total of thirty young or an average of three per litter. On the other hand nine litters of young produced by crossing yellow type A mice with non-yellow piebalds produced forty-five young or an average of five per litter. The figures though not extensive bear out the explanation of the relationship of the two factors as outlined above.

### INCREASE OF PIGMENTATION IN YELLOW MICE

The fact that yellow type A and black-eyed whites potentially yellow have considerably more pigment than the corresponding non-yellow varieties in the $F_1$ and $F_2$ generation suggests that the increase may be due to a darkening factor which coming in apparently with the *Y*-bearing gamete of the yellow race shows a marked tendency to remain coupled with yellow coat color in inheritance. Experiments have been started to determine whether the increased pigmentation of yellow animals is due to interaction of factors or to linkage of a darkening modifier as suggested above.

## CONCLUSIONS

(1) The factors for yellow coat color and for black-eyed white spotting in mice are both physiologically and genetically distinct and independent from one another.

(2) Certain apparent indications of selective fertilization and abnormal Mendelian ratios are found not to hold after breeding tests of the animals in question have been made.

(3) Yellow $F_1$ animals of "type A" are distinctly more heavily pigmented than non-yellow animals of the same type and generation.

(4) "Black-eyed whites," potentially yellow, have from 6-20 percent more dorsal pigmentation than do the corresponding black-eyed whites potentially non-yellow.

(5) Until further evidence is collected, this increase in pigmentation may be considered as due either (a) to a modifying factor linked with yellow or (b) to an interaction between the factor *Y* and the factor *W* of a purely somatic nature.

(6) Litters produced from yellow "type A" parents crossed *inter se* are distinctly smaller than litters in which only one parent is of this variety. This reduction is probably due to the formation and subsequent death of zygotes having a double representation of either the *Y* or *W* factor or of both.

## LITERATURE CITED

Castle, W. E., and Little, C. C., 1910 On a modified Mendelian ratio among yellow mice. Science N. S. **32**: 868-870.

Cuénot, L., 1911 Les déterminants de la couleur, chez les souris étude comparative. Archiv Zoöl. Exp. et Gén. **8**: 40-56.

Dunn, L. C., 1916 The genetic behavior of mice of the color varieties "black and tan" and "red." Am. Nat. **50**: 664-675.

Durham, F. M., 1911 Further experiments on the inheritance of coat color in mice. Jour. Genetics **1**: 159-178.

Little, C. C., 1911 The "dilute" forms of yellow mice. Science N. S. **33**: 896-897.

1915 The inheritance of black-eyed white spotting in mice. Am. Nat. **49**: 727-740.

Morgan, T. H., 1914 Multiple allelomorphs in mice. Am. Nat. **48**: 449-458.

Sturtevant, A. H., 1912 Is there association between the yellow and agouti factors in mice? Am. Nat. **46**: 368-371.