# BUFF, A NEW ALLELOMORPH OF WHITE EYE COLOR IN DROSOPHILA

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In the course of certain experiments to find out the frequency of the occurrence of primary non-disjunction in Drosophila (see BRIDGES 1916), I found a new eye color mutation, "buff" (July 28, 1915), which has proven to be a new allelomorph of white.

Eosin miniature females had been mated to wild males, giving, as expected in the $F_1$ generation, eosin miniature males and wild type females. But along with these two kinds of flies, there appeared also in one bottle two, and in another eight, females which were long-winged like their normal sisters, but whose eye color was lighter than that of standard eosin females and a little darker than that of eosin males.

These light-eyed females were put into a new bottle where they produced offspring, having evidently been fertilized by their eosin miniature brothers. Unfortunately little attention was paid to the nature of the offspring of the females. Apparently a majority of the sons were eosin with some lighter in eye color. Only one of these males was bred, a miniature male whose eye color was much lighter than eosin, being a pale cream or "buff."

The experiments which are to follow prove that the buff color of this male is a new allelomorph of white. One of the important theoretical questions with respect to a new member of a series of allelomorphs is its origin, whether directly from the wild by a single act of mutation, or indirectly from an already existing mutant member of the series (see MORGAN, STURTEVANT, MULLER and BRIDGES, 1915, Chap. 7). Because of the uncertainty with respect to the offspring of the original light females we cannot be entirely sure in this case whether buff arose from the wild type of the father or from the eosin of the mother. It seems more probable that the buff arose from the wild type of the father for on this view we may explain the light color of the mothers as eosin-buff compound, this light color being without explanation if the buff arose from the eosin.

The buff miniature male was mated to a wild female and gave in the $F_1$ generation only red daughters (195) and red sons (187) which shows buff to be recessive. About half the $F_2$ males but none of the $F_2$ females showed the "buff" color, which is thus shown to be due to a sex-linked gene (table 1). Not one of the sons was eosin in color; therefore if buff is eosin plus a modifier which reduces the color to a pale cream then this modifier must be very closely linked to eosin. There remained besides this possibility still two other possibilities: buff might be a simple allelomorph of white or might be in another locus altogether.

TABLE 1

*$P_1$ wild ♀♀ × buff miniature ♂♂. $F_1$ wild type ♀♀ × $F_1$ wild type ♂♂.*

| | Non-crossover ♂♂ | | Crossover ♂♂ | | Total males | Crossovers | Crossover value |
|---|---|---|---|---|---|---|---|
| $F_2$ wild type ♀♀ | Buff min. | wild type | Buff | min. | | | |
| 612 | 172 | 194 | 104 | 94 | 564 | 198 | 35.2 |

The amount of crossing over between buff and miniature (35.2 percent) is in agreement with the amount of crossing over between white and miniature, therefore the view that buff occupies the white locus seemed the more probable one. In subsequent generations (table 2) back-cross experiments were run which furnished in both males and females additional linkage data similar to that of the males of table 1.

TABLE 2

*Wild type ♀♀ heterozygous for buff and miniature back-crossed to buff miniature ♂♂.*

| Non-crossovers | | Crossovers | | | | Crossover |
|---|---|---|---|---|---|---|
| Buff min. | Wild type | Buff | Min. | Total | Crossovers | values |
| 512 | 524 | 284 | 302 | 1622 | 586 | 36.1 |

The buff females which appeared in these back-crosses were of identically the same color as the buff males. This is strong evidence against the modifier-view that buff is eosin plus a linked diluter; for of the great number of tested combinations of eosin with other eye colors (including specific diluters of eosin) there has not yet been found a single one in which eosin does not retain its bicolorism, that is, in which the females are not *darker* than the corresponding males.

Between the two remaining possibilities, namely that buff is a simple allelomorph of white, and that buff occupies a locus distinct from white, we are able to decide by finding more accurately the locus of buff. This was done by finding the amount of crossing over between buff and yellow (yellow is at the zero point of the X chromosomes). Yellow, buff and miniature were used in the same experiment so that the occurrence of the very small double crossover class might give conclusive evidence as to the location of the genes.

A buff miniature female was mated to a yellow male and yielded in the $F_1$ generation wild type females and buff miniature males. A few of these were inbred and gave (table 3) in the males the desired linkage information. The yellow buff miniature males were used to run two three-point back-crosses (tables 4 and 5).

TABLE 3

*$P_1$ buff miniature ♀ ♀ × yellow ♂ ♂. $F_1$ wild type ♀ × $F_1$ buff miniature ♂.*

| | $F_2$ females | | | | $F_2$ males | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Non-cross-overs | | Crossovers | | $\frac{y}{w^{bf}\quad m}$ | | $\frac{y \mid w^{bf}\quad m}{\mid}$ | | $\frac{y \qquad \mid m}{w^{bf} \mid}$ | | $\frac{y \mid w^{bf} \mid}{\mid \qquad \mid m}$ | | Total males |
| No. | Buff min. | Wild type | Buff | Min. | Yellow | Buff min. | Yellow buff min. | Wild type | Yellow min. | Buff | Yellow buff | Min. | |
| 1 | 71 | 58 | 36 | 35 | 57 | 60 | 2 | 3 | 31 | 43 | — | — | 196 |
| 2 | 41 | 39 | 26 | 37 | 43 | 29 | — | 1 | 20 | 19 | — | — | 112 |
| 3 | 39 | 50 | 24 | 22 | 33 | 34 | 1 | 1 | 22 | 17 | — | — | 108 |
| 4 | 62 | 64 | 28 | 40 | 43 | 65 | 3 | 1 | 20 | 28 | — | — | 160 |
| 5 | 43 | 52 | 19 | 19 | 39 | 46 | — | 1 | 25 | 24 | — | — | 135 |
| 6 | 34 | 39 | 31 | 22 | 35 | 31 | 1 | — | 28 | 22 | — | — | 117 |
| 7 | 28 | 48 | 21 | 19 | 33 | 39 | 1 | 1 | 24 | 23 | — | — | 121 |
| 8 | 27 | 27 | 14 | 13 | 27 | 24 | — | 2 | 13 | 8 | — | — | 74 |
| 9 | 44 | 38 | 23 | 30 | 33 | 40 | 1 | 3 | 18 | 24 | — | 1 | 120 |
| 10 | 49 | 62 | 27 | 20 | 39 | 45 | 2 | — | 27 | 28 | — | — | 141 |
| Totals | 438 | 477 | 249 | 257 | 382 | 413 | 11 | 13 | 228 | 236 | 0 | 1 | |
| | 915 | | 506 | | 795 | | 24 | | 464 | | 1 | | 1284 |

TABLE 4

*Wild type ♀ ♀ heterozygous for yellow, buff and miniature [$\frac{y}{w^{bf}\quad m}$] back-crossed to yellow buff miniature males.*

| | $\frac{y}{w^{bf}\quad m}$ | | $\frac{y \mid w^{bf}\quad m}{\mid}$ | | $\frac{y \qquad \mid m}{w^{bf} \mid}$ | | $\frac{y \mid w^{bf} \mid}{\mid \qquad \mid m}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Yellow | Buff min. | Yellow buff min. | Wild type | Yellow min. | Buff | Yellow buff | Min. | Total |
| 1 | 79 | 96 | 3 | 5 | 58 | 59 | — | — | 300 |
| 2 | 66 | 88 | — | 1 | 41 | 57 | — | — | 253 |
| 3 | 56 | 50 | — | 1 | 30 | 29 | — | — | 166 |
| 4 | 74 | 75 | 2 | 6 | 45 | 54 | 1 | — | 257 |
| 5 | 53 | 48 | 1 | 2 | 29 | 25 | — | — | 158 |
| | 328 | 357 | 6 | 15 | 203 | 224 | 1 | 0 | |
| Total | 685 | | 21 | | 427 | | 1 | | 1134 |

TABLE 5

*$P_1$ yellow buff miniature ♀ × wild ♂. $F_1$ wild type* [$\underline{y\ w^{bf}\ m}$] × *$F_1$ yellow buff min. ♂.*

| No. | $\underline{y\ w^{bf}\ m}$ |  | $\frac{y}{w^{bf}\ m}$ |  | $\frac{y\ w^{bf}}{m}$ |  | $\frac{y\quad m}{w^{bf}}$ |  | Total |
|---|---|---|---|---|---|---|---|---|---|
|  | Yellow buff min. | Wild type | Yellow | Buff min. | Yellow buff | Min. | Yellow min. | Buff |  |
| 1 | 32 | 37 | 3 | 3 | 27 | 24 | — | — |  |
|  | 42 | 29 | 1 | 1 | 16 | 19 | — | — |  |
| 2 | 39 | 38 | 1 | — | 17 | 17 | — | — |  |
|  | 32 | 38 | — | — | 24 | 40 | — | — |  |
| 3 | 55 | 56 | 3 | — | 19 | 16 | 1 | — |  |
|  | 48 | 45 | 1 | — | 29 | 26 | — | 1 |  |
| 4 | 31 | 43 | — | 1 | 24 | 17 | — | — |  |
|  | 33 | 24 | 1 | — | 11 | 22 | — | — |  |
| 5 | 45 | 47 | 1 | 2 | 29 | 25 | — | — |  |
|  | 60 | 51 | 2 | — | 16 | 28 | — | — |  |
| 6 | 45 | 53 | 1 | 3 | 18 | 24 | — | — |  |
|  | 36 | 35 | — | — | 22 | 21 | — | — |  |
| 7 | 35 | 27 | 1 | — | 14 | 16 | — | — |  |
|  | 28 | 27 | — | 2 | 13 | 17 | — | — |  |
| 8 | 22 | 31 | 3 | 1 | 13 | 23 | — | — |  |
|  | 30 | 33 | — | 1 | 11 | 11 | — | — |  |
| 9 | 25 | 44 | — | — | 18 | 18 | — | — |  |
|  | 25 | 29 | — | 1 | 9 | 14 | — | — |  |
| 10 | 29 | 36 | 1 | — | 19 | 15 | — | — |  |
|  | 31 | 32 | — | 2 | 15 | 19 | — | 1 |  |
| 11 | 38 | 36 | — | 3 | 22 | 20 | — | — |  |
|  | 52 | 31 | 1 | 1 | 24 | 17 | — | — |  |
| 12 | 41 | 53 | 2 | 3 | 25 | 23 | — | — |  |
|  | 50 | 46 | 2 | 1 | 24 | 33 | — | — |  |
| 13 | 53 | 66 | 2 | 2 | 26 | 26 | — | — |  |
|  | 40 | 42 | 1 | 2 | 21 | 21 | — | — |  |
| 14 | 71 | 82 | 2 | 1 | 43 | 59 | — | — |  |
|  | 71 | 53 | 2 | 1 | 33 | 37 | — | 1 |  |
| 15 | 34 | 33 | 1 | — | 16 | 21 | 1 | — |  |
|  | 36 | 37 | 2 | 1 | 15 | 15 | — | — |  |
| 16 | 53 | 54 | 1 | 3 | 29 | 40 | — | — |  |
|  | 55 | 51 | 1 | 2 | 27 | 33 | — | — |  |
| 17 | 38 | 51 | 1 | 2 | 24 | 21 | — | 1 |  |
|  | 34 | 40 | 1 | 1 | 21 | 26 | — | — |  |
| 18 | 38 | 30 | 1 | — | 31 | 19 | — | — |  |
|  | 38 | 43 | 2 | 2 | 19 | 20 | — | — |  |
| 19 | 33 | 31 | 1 | — | 15 | 22 | 1 | — |  |
|  | 43 | 37 | 2 | 1 | 15 | 15 | — | — |  |
| 20 | 31 | 34 | 1 | 1 | 19 | 30 | — | 1 |  |
|  | 38 | 45 | 1 | — | 13 | 25 | — | — |  |
|  | 1610 | 1650 | 46 | 44 | 826 | 935 | 3 | 5 |  |
| Totals | 3260 |  | 90 |  | 1761 |  | 8 |  | 5119 |

Table 6

*$P_1$ eosin ♀♀ × buff miniature ♂♂. $F_1$ eosin-buff ♀ × $F_1$ eosin ♂.*

| No. | $F_2$ Females | | $F_2$ males | | | | Total males | Crossover values |
|---|---|---|---|---|---|---|---|---|
| | | | Non-cross-over ♂ | | Crossover ♂ | | | |
| | Eosin | Eosin buff | Eosin | Buff min. | Eosin min. | Buff | | |
| 1 | 81 | 83 | 66 | 68 | 35 | 43 | 212 | 37 |
| 2 | 44 | 49 | 50 | 41 | 16 | 20 | 127 | 28 |
| 3 | 71 | 80 | 46 | 40 | 30 | 18 | 134 | 36 |
| 4 | 54 | 57 | 26 | 28 | 14 | 19 | 87 | 38 |
| Total | 261 | 269 | 188 | 177 | 95 | 100 | 560 | 35 |

Table 7

*$P_1$ white ♀♀ × buff miniature ♂♂. $F_1$ white-buff ♀ × $F_1$ white ♂.*

| No. | $F_2$ females | | $F_2$ males | | | | Total males | Crossover values |
|---|---|---|---|---|---|---|---|---|
| | | | Non-cross-over ♂ | | Crossover ♂ | | | |
| | White | White-buff | White | Buff min. | White min. | Buff | | |
| 1 | 53 | 47 | 29 | 25 | 14 | 14 | 82 | 34 |
| 2 | 65 | 64 | 50 | 35 | 22 | 35 | 142 | 40 |
| 3 | 45 | 43 | 24 | 32 | 11 | 15 | 82 | 32 |
| 4 | 52 | 49 | 25 | 31 | 13 | 13 | 82 | 32 |
| Totals | 215 | 203 | 128 | 123 | 60 | 77 | 388 | 35.3 |

Table 8

*$P_1$ cherry ♀♀ × buff miniature ♂♂. $F_1$ cherry-buff ♀ × $F_1$ cherry ♂.*

| No. | $F_2$ females | | $F_2$ males | | | | Total males | Crossover values |
|---|---|---|---|---|---|---|---|---|
| | | | Non-cross-over ♂ | | Crossover ♂ | | | |
| | Cherry | Cherry-buff | Cherry | Buff min. | Cherry min. | Buff | | |
| 1 | 66 | 65 | 38 | 31 | 20 | 20 | 109 | 34 |
| 2 | 66 | 76 | 34 | 31 | 10 | 18 | 93 | 30 |
| 3 | 74 | 75 | 51 | 51 | 35 | 28 | 165 | 38 |
| 4 | 69 | 65 | 44 | 63 | 30 | 29 | 166 | 38 |
| **Totals** | 275 | 281 | 167 | 176 | 95 | 95 | 533 | 35.6 |

By considering the smallest or double crossover classes of tables 3, 4 and 5, it is seen that all the data are consistent with the assumption that the gene for buff lies in the X chromosome between the genes for yellow and miniature, as does white. Furthermore the amount of cross-

TABLE 9

*Summary of linkage data.*

| Genes | Total | Crossovers | Crossover values | Crossover values by MORGAN and BRIDGES |
|---|---|---|---|---|
| Yellow buff | 7537 | 145 | 1.9 | 1.1 |
| Buff min. | 14811 | 5258 | 35.5 | 33.2 |
| Yellow min. | 7537 | 2787 | 36.9 | 34.3 |

ing over between yellow and buff is approximately the same as that between yellow and white or any of the other allelomorphs of white.

These linkage experiments limit our possibilities still further; for if buff is due to a gene having no relation to white, then this gene must still be very close to the locus for white, since the linkage relations of buff are practically identical with those of white. The next series of tests effectually proved that the gene for buff must occupy the same locus as white.

The other allelomorphs of white when crossed to each other give intermediates ("compounds") and just such compounds were obtained when a buff miniature male was mated to white, eosin and cherry. In each case the $F_1$ generation yielded females whose eye color was intermediate between the two eye colors which entered the combination. The white-buff compound was the lightest of the three and the cherry-buff the darkest. No red-eyed flies appeared.

The $F_2$ from the compounds furnished more compounds in the females, while the male counts showed that the amount of crossing over with miniature is the same for buff as for white, eosin or cherry (tables 6, 7 and 8).

In conclusion, (1) The data have shown that buff is due to a sex-linked gene whose linkage relations are the same as the linkage relations of white (table 9). (2) The occurrence of compounds effectually disposes of the possibility that buff is due to a gene having no other relation to white than a neighboring locus in the chromosome. (3) Against the modifier-of-eosin view we have urged the strong argument of the lack of bicolorism in buff. (4) The linkage experiments have furnished evidence that no modifier could be separated from eosin by crossing over; for in 14251 flies in which such a crossover might have appeared none was found (table 9). (5) We are led then to the conclusion that buff is a simple allelomorph of white. (6) From the evidence upon the origin of buff it seems more probable that buff arose from the wild than that it arose from the eosin.

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## LITERATURE CITED

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