APPARENT CHANGES WITH AGE IN CROSSING-OVER BETWEEN COLOUR AND SIZE GENES IN MICE.

By C. V. GREEN.

(*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.*)

Bridges (1929), summarising his own work and that of other investigators including Plough (1917) and Stern (1926), on the influence of age on crossing-over in *Drosophila melanogaster*, has shown that in certain portions of two autosomes, the age effect on crossing-over can be expressed by a W-shaped curve, exhibiting first a decrease, then an increase followed by a second decrease, etc., while the *X*-chromosome displays a lowering of crossing-over followed by a return to normal. No doubt remains that linkage is influenced by the age of the female in this insect, but in vertebrates the evidence has seemed less definite.

In the fowl, Haldane and Crew (1925) found that crossing-over between the sex-linked genes for barred feathers and silver hackles increased with age in the male to such an extent that linkage was nearly lost in the third year. Landauer (1933) on the other hand reported a slight tendency for crossing-over to decrease with age of mother between the autosomal genes for creeper and single comb.

Somewhat conflicting evidence has been reported for mammals. Although Castle and Wachter (1924) detected no influence of age on crossing-over in either sex in rats or mice, Crew and Koller (1932) and Bryden (1933) in cytological investigations on the mouse and rat respectively found that young males had definitely more chiasmata than older ones. On the hypothesis that chiasmata represent crossing-over, a strengthening of linkage with age is indicated.

The writer (1931, 1933, 1934) has reported linkage between certain size and colour genes in the back-cross generation of a mouse species cross (*Mus musculus* × *M. bactrianus*). The greater adult weight, for example, characteristic of the *musculus* parent was found associated with brown coat colour derived from the same parent. Similarly lesser weight and black coat colour, originating in *bactrianus*, tended to remain together in the back-cross hybrids. Indirect evidence points to an age effect, different in the two sexes, on crossing-over between the two types of genes.

Since plus genes making for larger size are found on the *musculus* chromosome with the gene for brown and/or minus genes for small size on the *bactrianus* chromosome with the gene for black, the mean weight of animals with the recessive colour exceeds the mean weight of those with the dominant allelomorph. There are, however, some large blacks because of crossing-over as well as phenotypic variation and occasional chance accumulations of plus genes on other chromosomes. Similarly, there are a certain number of small browns. Therefore, averages, rather than individuals, must be used to detect association between size and colour genes. If the ratio of brown to black weights (mean brown weight divided by mean black weight) is significantly greater than unity, linkage of the gene for brown coat colour with plus size genes is indicated.

In the production of the back-cross hybrids the inbred *musculus* parent is homozygous for the colour gene and presumably for the size genes carried on the same chromosome, so crossing-over between these two types of genes takes place only in the F_1 parent. If crossing-over increases or decreases with age in the heterozygous parent the mean brown-black ratio decreases or increases as the case may be.

During the past few years several back-cross populations have been produced. Of these, five are females and three males; of the eight, crossing-over takes place in the male parent in four and in the female in an equal number. Since the numbers in any one population are sometimes unfortunately small, each has been divided arbitrarily into two groups: young, in which the F_1 parent was 179 days or less in age, and old, in which that parent had attained 180 days or more. The adult weights of back-cross mice are 181st day weights, although in a very few instances the actual age was from one to three days greater. The data are summarised in Table I.

This table shows clearly that when the female parent was an F_1, the brown-black weight ratio is lower in offspring of older dams in all four populations, suggesting an increase with age in crossing-over between qualitative and quantitative genes. Conversely, when the male parent was the hybrid, in three cases out of four the ratio increased with age of the sire suggesting a decrease in crossing-over. The differences in most cases are slight and not significant taken separately, although the trend in the two classes of matings is obvious.

The several investigations involved were not all conducted in the same environment nor with the same diet, so differences in mean weights and variability resulted. In order to combine the diverse series to obtain the advantage of larger numbers several mathematically somewhat crude steps were necessary. In three of the five separate experiments both sexes were present. To reduce the male values to their female equivalents, the mean female weight in the three investigations was first divided by the mean male weight. The individual male figures were then multiplied by the resulting quotient. This reduced all mice to a female basis but left the differences in weight inherent in the different experiments. The mean of all females combined with the reduced males was computed and divided by the comparable means of each of the five experiments. The individual weights in each were then multiplied by the appropriate quotient. By this procedure, it seemed permissible to treat all weights

TABLE I.

Brown-black mean weight ratios.

Mating	Sex	Young			Old		
		Ratio	Min. age days	No. mice	Ratio	Max. age days	No. mice
$\frac{musculus}{bactrianus}$ ♀ × *musculus* ♂							
(Ann Arbor, 1928–9)	♂	1·06	93	35	1·02	289	34
(Ann Arbor, 1928–9)	♀	1·04	92	37	0·97	349	31
(Bar Harbor, 1933)	♀	1·09	60	84	1·00	247	41
$\frac{bactrianus}{musculus}$ ♀ × *musculus* ♂							
(Bar Harbor, 1933)	♀	1·05	59	72	1·00	281	46
musculus ♀ × $\frac{musculus}{bactrianus}$ ♂							
(Ann Arbor, 1928–9)	♂	1·04	87	21	1·06	455	42
(Ann Arbor, 1928–9)	♀	0·93	78	19	1·15	455	41
(Bar Harbor, 1930)	♂	1·05	77	143	1·15	256	20
(Bar Harbor, 1930)	♀	1·11	80	119	1·08	256	20

as though they belonged to a single experiment in one part of which the male parents and in the other the female were F_1 hybrids. The combined data are given below.

Mating	Ratio (young F_1)	No. mice	Ratio (old F_1)	No. mice	Difference / Standard error
F_1 ♀ × *musculus* ♂	1·07 ± ·019	228	1·00 ± ·027	152	2·1
musculus ♀ × F_1 ♂	1·07 ± ·017	292	1·12 ± ·035	123	1·3

In the first type, in which crossing-over takes place in the homogametic sex, the ratio of mean brown weight to mean black weight decreases in old mice, with the difference 2·1 times its standard error. A difference of this magnitude with P less than 0·04 is probably significant. In the second type, with crossing-over occurring in the heterogametic sex, the ratio increases in old mice although the difference may be merely fortuitous since P equals roughly 0·19. For computing the standard error of a ratio of two means (m_1 and m_2) we are indebted to Prof. Sewall Wright for suggesting the following formula:

$$E\frac{m_1}{m_2}=\frac{m_1}{m_2}\sqrt{\left(\frac{Em_1}{m_1}\right)^2+\left(\frac{Em_2}{m_2}\right)^2}.$$

This formula assumes that the two values entering into it are uncorrelated, which assumption seems correct in the present problem.

Besides changes in crossing-over, there are at least two alternative explanations for the alterations in the brown-black ratios with age. Chance might be involved, although such a situation seems remote. That the potency of the size genes changes with age, but differently in the two sexes, appears equally unlikely, so although both alternatives may conceivably play a part, it seems plausible that our interpretation accounts for the major portion of the observed effects.

Since, in our data, crossing-over presumably increases in the homogametic and perhaps decreases in the heterogametic sex with age, it may be of interest to compare these results with those obtained in vertebrates by others when an age effect was indicated.

Investigator	Material	Sex	Crossing-over with age
Haldane and Crew (1925)	Fowl	Homogametic	Increased
Green (this paper)	Mouse	,,	,,
Landauer (1933)	Fowl	Heterogametic	Decreased
Bryden (1933)	Rat	,,	,,
Crew and Koller (1932)	Mouse	,,	,,
Green (this paper)	Mouse	,,	,,

These findings agree in that the age effect is to increase crossing-over in the homogametic and to decrease it in the heterogametic sex. Haldane (1922) has pointed out that when linkage is complete or strengthened such a condition occurs in the heterogametic sex. May it not be generally true, at least as far as vertebrates are concerned, that when age has an effect it decreases crossing-over in the heterogametic sex and increases it in the homogametic? With this possibility in mind, it may be well to review the results of several of our experiments on linkage in size inheritance. The errors given in this paper are all *standard* errors.

Female parents younger than 180 *days.*

Mating	Sex	Mean brown weight in gm.	No.	Mean black weight in gm.	No.	Difference / Standard error
$\frac{bactrianus}{musculus}$ ♀ × *musculus* ♂						
(Bar Harbor, 1933)	♀	23·0 ± 0·33	32	21·9 ± 0·41	40	2·1
$\frac{musculus}{bactrianus}$ ♀ × *musculus* ♂						
(Bar Harbor, 1933)	♀	23·2 ± 0·34	43	21·3 ± 0·36	41	3·8
(Ann Arbor, 1928–9)	♂	28·4 ± 1·04	19	26·9 ± 1·39	16	—
(Ann Arbor, 1928–9)	♀	23·8 ± 0·78	22	22·8 ± 1·18	15	—

Male parents of all ages.

Mating	Sex	Mean brown weight in gm.	No.	Mean black weight in gm.	No.	Difference / Standard error
musculus ♀ × $\frac{musculus}{bactrianus}$ ♂						
(Bar Harbor, 1930)	♂	22·5 ± 0·38	72	21·0 ± 0·33	81	3·0
(Bar Harbor, 1930)	♀	20·4 ± 0·37	78	18·3 ± 0·36	62	4·0
(Ann Arbor, 1928–9)	♂	27·6 ± 0·68	32	26·5 ± 0·61	33	1·2
(Ann Arbor, 1928–9)	♀	27·4 ± 1·10	33	25·1 ± 0·86	28	1·6

These tables show that brown weights exceed blacks, even if not significantly so, in all cases.

If genes for size and colour are located relatively far apart on a chromosome it becomes somewhat difficult to demonstrate linkage, especially when, as in our case (Green, 1932), the size genes located on that chromosome play a comparatively minor part. For the demonstration to be conclusive, it seems that conditions must be favourable. Just what constitutes such is still largely unknown. Weight as a criterion of general size is not fully satisfactory, although probably the best measure available, since too many factors may produce fluctuations. The most obvious are amount of food and water ingested and degree of obesity. While such conditions, not determined by heredity, should be non-selective and equally likely to affect blacks and browns, their magnitude may be such, when small numbers are involved, as to mask completely the effects of size genes or to accentuate genetic differences beyond all reason. The diet now in use, Purina Fox Chow and water, brings about better early growth, less juvenile mortality, and less tendency towards adult obesity—with resulting lessened variability in weight—than the mixed diet of grains, dog bread, milk and lettuce formerly employed. The diet, then, must be considered and kept constant in any single experiment.

The non-significant differences between blacks and browns in the early investigations may be partly the result of the great variability in weight, with consequent augmented standard errors, produced by an unsatisfactory diet, as well as of small numbers. Despite these deleterious conditions and the high subadult mortality, brown back-cross mice in every mating still averaged heavier than black when offspring of older F_1 dams were excluded. Since it appears probable that crossing-over increases with age of dam and possibly decreases with age of sire, the age span of the heterozygous parent should be taken into account.

SUMMARY.

Changes in the values of the brown-black mean weight ratios in the back-cross populations of a mouse species cross (large brown *Mus musculus* × small black *Mus bactrianus*) support the view that crossing-over between size and colour genes increases with age in the female (homogametic sex) and decreases with age in the male (heterogametic sex).

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