STUDIES OF A SIZE CROSS IN MICE, II

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SEVERAL years ago GREEN made a cross between two races of mice of very different body size, the experiment being designed to throw light on the mechanism of size inheritance. His working hypothesis was that differences in body size are determined by genes borne in chromosomes, that such genes are numerous, and that accordingly it should be possible to demonstrate the existence of certain of them in the same chromosomes as contain coat color genes.

As a small parent race, he used *Mus bactrianus*; as a large race of nearly twice as great body size, he used LITTLE's dilute brown race of *Mus musculus*. The small race contained the three independently inherited dominant genes, agouti (A^w), black, and dilution. The large race contained the three recessive alleles, non-agouti, brown, and dilution. In F_2 and backcross populations it was found that larger body size was associated with each of the recessive segregates, non-agouti individuals being larger than agoutis, browns larger than blacks, and dilutes larger than intense individuals. This was regarded as supporting the hypothesis that in each of the three marked chromosomes derived from the larger parent there was present one or more genes for larger body size. On further investigation, however, GREEN concluded that the differences observed were sufficient to have unmistakable statistical significance only in the case of the chromosome containing the brown gene. This was the state of the question when we undertook its further investigation. We attempted a substantial repetition of GREEN's experiment with slightly different stocks available to us. As a small parent race we used at first not *Mus bactrianus* but a supposed domestic derivative of it, the race of black-and-white Japanese waltzing mouse studied by GATES (1926). Later, through the kindness of Drs. GREEN and LITTLE, we were able to employ also the same strain of *Mus bactrianus* which GREEN had employed. As the large parent race in both these crosses (which, for convenience, we may call Cross 1 and Cross 2, respectively), we used a derivative of LITTLE's dilute brown race in which two additional recessive gene mutations had been incorporated, namely pink-eye and short-ear.

The F_1 animals produced by both crosses were only a little smaller than animals of the larger parent race but were of remarkable vigor, fecundity, and longevity. Both were reciprocally backcrossed to LITTLE's dilute brown race of *M. musculus*. The F_1 animals from Cross 1 were also backcrossed to the actual *musculus* parent race, the pink-eyed short-eared dilute brown race of GATES. We may now proceed to the discussion of these backcross populations.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO D BR MALES

In a previous paper, we have discussed the mature body weight and the body length at age six months of this population consisting of 1,236 animals. We have shown, in confirmation of GREEN's earlier conclusion, that brown individuals are heavier and longer bodied than blacks, and that dilute individuals are heavier and longer-bodied than intense ones, though the difference is less in the case of dilution than in that of brown, as GREEN had previously observed. But we have been led to adopt an explanation for the manifest superior size of brown and of dilute segregates different from that of GREEN. Instead of invoking the action of size genes located in the same chromosomes as the brown and dilution genes, we assume that it is the physiological action on growth of the brown gene and the dilution gene themselves which produces the observed effects. To this question we shall return later. For a detailed statement of results as regards weight and body-length, the reader is referred to our previous publication. CASTLE, GATES and REED (1936).

At the time these mice were chloroformed and measured as to body length, their tail length was also measured by SUMNER's method, keeping the body under a uniform tension of 20 grams. We have only recently studied the data on tail length, which show one surprising and unexpected feature. The greater size of brown and of dilute individuals, as compared with black and intense individuals respectively, finds expression as expected in all three criteria studied, namely, (1) maximum weight at or prior to six months of age, (2) body length, and (3) tail length at age six months. Brown has regularly a greater influence than dilution on weight and body-length, as reported in our previous publication, but as regards tail-length their relations are reversed. Dilution has a greater influence than brown in elongating the tail. This is the unexpected feature of a study of tail length in the backcross population, and it finds support, as we shall see, in the backcross from Cross 2. The data on the variation in tail length of the backcross population are contained in table 1. The numbers there reported (637 females and 439 males) are smaller than for the body length studies reported in our previous paper because of occasional injury to the tail (particularly in the case of males caged together). A single domineering male will, by biting the tails of his cage mates, destroy the possibility of obtaining normal tail measurements for them. Nevertheless the available data for both sexes are entirely in harmony, in that they show that the tail length of blues is greater than that of browns, the order of size among the color classes as regards tail length being (1) black, (2) brown, (3) blue, and (4) dilute brown. Brown females as compared with blacks have an increased tail length of 1.5 percent, but dilute females as compared with intense have tails 2.3 percent longer. Also brown males

TABLE 1

Variation in tail length of four different color classes from matings of F_1 females from Cross 1 to d br males

	FEMALES			MALES		
	NO.	MEAN	S.D.	NO.	MEAN	S. D.
Black	151	75.5±.22	3.98±.15	103	78.7±.27	4.09±.19
Blue	170	77.5±.19	3.76±.14	116	80.1±.24	3.86±.17
Brown	159	76.9±.22	4.08±.15	121	79.0±.27	4.40±.19
Dil. Brown	157	78.5±.22	4.07±.15	99	81.7±.23	3.38±.17
Total	**637**	**77.2±.11**	**4.10±.08**	**439**	**79.8±.13**	**4.15±.09**
All Blacks	321	76.5±.15	3.96±.10	219	79.4±.18	4.03±.13
All Browns	316	77.7±.16	4.18±.12	220	80.2±.19	4.20±.13
All Intense	310	76.2±.15	4.07±.12	224	78.8±.18	4.10±.13
All Dilute	327	78.0±.15	3.93±.10	215	80.84±.17	3.74±.12
Brown minus Black		1.20±.22=5.4			.80±.26=3.0	
Dilute minus Intense		1.77±.21=8.4			1.98±.24=8.2	

as compared with blacks, have tails 1.2 percent longer, but dilute males as compared with intense have tails 2.6 percent longer. In both sexes dilution has an influence on tail length superior to that of brown, although in regard to body length and total weight the relation is reversed. Both mutant genes (brown and dilution) for which segregation is occurring in this backcross population have a positive (increasing) effect on general body size, as indicated in body weight, body length, and tail length. Their combined action is also additive or cumulative in all cases. But apart from general body size, there would seem to be also a special specific influence of dilution on tail length.

BACKCROSS OF F_1 MALES FROM CROSS 1 TO D BR FEMALES

We were able to obtain a smaller population from this backcross than from the reciprocal one already discussed because of the smaller number of mothers available. The number studied is approximately 100 of either sex, more exactly 99 females and 106 males. Comparative data on the segregation in this and the reciprocal backcross are contained in table 2.

In making this comparison we are interested in two questions: (1) will the chromosomes marked by brown and dilution here show the same accelerating influence on growth as in the reciprocal backcross, and (2) is there a significant difference in size between the progeny of F_1 mothers

TABLE 2

Comparison of the several classes of individuals produced by reciprocal backcrosses between F_1 animals and the "d br" race

	FROM ♀F_1×♂*d br*				FROM ♀*d br*×♂F_1				DIFFERENCE		
FEMALES	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	WEIGHT GMS.	BODY MM.	TAIL MM.
Black	337	21.91±.08	91.51±.12	76.5 ±.15	41	23.18±.29	93.51±.34	76.82±.51	1.27±.30	2.00±.36	.32±.53
Brown	328	22.61±.12	92.84±.12	77.7 ±.16	58	23.73±.25	94.71±.27	77.27±.36	1.12±.27	1.87±.29	−.53±.39
Intense	324	22.04±.09	91.79±.12	76.2 ±.15	49	22.75±.24	92.88±.23	75.72±.44	.71±.25	1.09±.26	−.48±.46
Dilute	351	22.45±.10	92.55±.12	78.0 ±.15	50	24.24±.28	95.52±.30	78.27±.39	1.79±.29	2.97±.32	.27±.41
Totals and means	**675**	**22.24±.10**	**92.17±.12**	**77.1 ±.15**	**99**	**23.93±.25**	**94.18±.25**	**77.0 ±.40**	**1.69±.27**	**2.01±.28**	**−.01±.42**
MALES											
Black	280	28.34±.13	95.77±.14	79.4 ±.18	62	28.28±.21	97.14±.22	78.85±.39	.15±.24	1.37±.27	−.55±.43
Brown	291	29.56±.12	97.73±.10	80.2 ±.19	44	29.63±.29	98.48±.29	79.71±.51	.07±.27	.65±.30	−.49±.54
Intense	278	28.67±.12	96.26±.14	78.8 ±.18	53	28.51±.25	97.21±.24	77.58±.47	−.49±.27	.95±.27	−.22±.50
Dilute	293	29.34±.13	97.21±.14	80.84±.17	53	29.17±.23	98.24±.27	80.64±.32	−.17±.26	1.03±.30	−.20±.36
Totals and means	**571**	**29.00±.12**	**96.74±.13**	**79.82±.17**	**106**	**28.95±.24**	**97.76±.25**	**79.25±.35**	**−.05±.26**	**1.02±.28**	**−.47±.39**

mated with d br males and the progeny of d br females mated with F_1 males? In other words, has the large race mother any superior influence on the size of her offspring? Table 2 contains the answer to the first question, an emphatic affirmative. Brown and dilution are found in heavier and longer-bodied individuals than their alleles in this as in the reciprocal backcross. Also brown has a greater influence than dilution in both sexes in increasing the average weight and body length; but brown has in both sexes *less* influence than dilution in increasing length of the tail, exactly as in the reciprocal backcross. We may accordingly, for qualitative effects, combine the data from both backcrosses, weighting each in proportion to the number of individuals which it contains. This procedure gives us the weighted means printed in italics in table 3.

TABLE 3

Percentage change in body size effected by the gene mutations brown (b) and dilution (d) in reciprocal backcrosses between F_1 hybrids and the d br race

		FEMALES			MALES		
GENE	MOTHER	WEIGHT	BODY LENGTH	TAIL LENGTH	WEIGHT	BODY LENGTH	TAIL LENGTH
b	♀ F_1	3.18	1.45	1.56	4.30	2.04	1.20
b	♀ d br	2.37	1.28	.58	4.77	1.38	1.09
Weighted mean		*3.07*	*1.40*	*1.43*	*4.37*	*1.90*	*1.18*
d	♀ F_1	1.86	.82	2.36	2.30	.98	2.60
d	♀ d br	6.54	2.84	3.36	2.31	1.06	3.94
Weighted mean		*2.47*	*1.08*	*2.48*	*2.30*	*.99*	*2.80*

The second question, one of considerable theoretical interest, as it involves a possible differential maternal influence on the size of offspring, finds its answer in table 2. The d br (large race) mothers produce larger-bodied offspring in both sexes, if we rely on the criterion of body length alone, undoubtedly the most reliable criterion. The differences between the means of the two groups are statistically significant. If we judge by body weight, female offspring of the large race mothers are also significantly larger, but there is no significant difference in the case of males. As regards tail length, no significant difference is found in either sex.

RECIPROCAL BACKCROSSES OF F_1 ANIMALS FROM CROSS 2 TO THE D BR RACE

The F_1 animals from this cross showed (but were heterozygous for) the three dominant characters, agouti, black, and intensity. On backcrossing them with the d br race which is homozygous for the corresponding recessive alleles, non-agouti, brown, and dilution, eight classes of colored offspring were obtained. In the left half of table 4 and of table 5 will be found a summary of the data on the body size of each of these eight classes

TABLE 4

Comparison of the several classes of females produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

FEMALES	FROM ♀F_1×♂*d br*				FROM ♀*d br*×F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	22.99	92.12	73.55	20	23.37	93.40	72.65	.38	1.28	−.90
Blue Agouti	62	23.48	92.67	74.47	13	24.01	94.30	75.40	.53	1.63	.93
Brown Agouti	60	23.97	93.76	75.42	16	25.55	95.06	75.33	1.58	1.24	−.09
Dil. Br. Agouti	45	24.42	94.72	77.17	17	24.82	94.94	75.79	.40	.22	−1.38
Black	56	23.30	92.75	74.41	16	24.05	94.50	76.35	.75	1.75	1.94
Blue	62	23.74	93.66	76.65	15	25.33	94.33	76.25	1.59	.67	−.40
Brown	54	24.75	93.37	74.44	15	26.28	95.00	74.59	1.53	1.63	.15
Dil. Br.	47	24.52	94.51	77.25	12	25.42	94.09	77.12	.90	−.42	−.13
Totals and means	**442**	**23.91±.09**	**93.38±.10**	**75.42±.13**	**124**	**24.85**	**94.45**	**75.30±.26**	**.96**	**1.01**	**.01**

TABLE 5

Comparison of the several classes of males produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

MALES	FROM ♀F_1×♂*d br*				FROM ♀*d br*×♂F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	28.79	96.24	75.69	19	29.06	97.10	74.87	.27	.86	−.82
Blue Agouti	52	29.80	98.18	78.25	14	31.92	99.71	78.82	2.12	1.53	.57
Brown Agouti	63	31.27	99.45	76.59	22	32.48	99.05	78.11	1.21	−.40	1.52
Dil. Br. Agouti	61	30.76	98.52	79.19	16	32.86	98.68	79.85	2.10	.16	.66
Black	54	29.31	96.00	75.37	16	29.78	97.18	75.83	.37	1.18	.46
Blue	51	30.48	97.74	76.98	15	31.12	99.06	78.57	.64	1.32	1.59
Brown	51	30.87	98.34	77.63	12	32.54	98.41	77.41	1.67	.07	−.22
Dil. Br.	55	30.47	97.93	78.63	19	32.50	100.03	79.72	2.03	2.10	1.09
Totals and means	**443**	**30.40±.11**	**97.83±.11**	**77.27±.15**	**133**	**31.53**	**98.65**	**77.78±.30**	**1.31**	**.85**	**.51±.33**

as indicated by weight, body length, and tail length, when an F_1 female was the mother. In the right half of these same tables will be found corresponding data for the reciprocal backcross in which a d br female was the mother.

TABLE 6

Percentage change in the body size of females effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
	♀ F_1						
a	Bact.	.46±.14	1.94	.21±.20	.33	.68±.28	.84
a	♀ d br	.83	3.40	.05	.05	1.38±.52	1.84
Weighted mean		*.54*	*2.26*	*.17*	*.26*	*.83*	*1.06*
	♀ F_1						
b	Bact.	1.40±.18	1.94	1.21±.20	1.30	.68±.28	.84
b	♀ d br	1.33	5.48	.64	.68	.71±.52	.94
Weighted mean		*1.38*	*2.71*	*1.08*	*1.16*	*.68*	*.85*
d		.13±.17	.54	.77±.20	.82	1.77±.25	2.37
d	♀ d br	.08	.33	−.07	−.07	1.52±.48	2.04
Weighted mean		*.12*	*.49*	*.58*	*.62*	*1.71*	*2.29*

TABLE 7

Percentage change in the body size of males effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 inidividuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
	♀ F_1						
a	Bact.	−.29±.21	−1.40	−.81±.22	−.83	−.23±.29	−.30
a	♀ d br	−.14	−.44	.03	.03	.59±.56	.76
Weighted mean		*−.25*	*−1.18*	*−.60*	*−.61*	*−.04*	*−.05*
	♀ F_1						
b	Bact.	1.30±.21	4.40	1.56±.20	1.60	1.50±.30	1.96
b	♀ d br	2.12	6.97	.78	.79	2.14±.58	2.79
Weighted mean		*1.49*	*4.99*	*1.37*	*1.41*	*1.64*	*2.13*
	♀ F_1						
d	Bact.	.32±.22	1.00	.74±.22	.75	2.10±.30	2.75
d	♀ d br	1.13	3.66	1.43	1.46	3.03±.57	3.97
Weighted mean		*.51*	*1.61*	*.89*	*.91*	*2.31*	*3.03*

The question as to the relative influence of F_1 mothers and d br mothers on the size of their offspring will be clear from an examination of these tables. In each of the eight color classes, in both sexes, the d br mothers produce the heavier offspring. The same superiority of the progeny of d br mothers is found as regards body length in seven of the eight color classes of each sex, and of course emphatically when all color classes are combined.

As regards tail length, there is no uniform superiority of the progeny of large race mothers, though in general, particularly among the males, d br mothers produce the longer-tailed progeny.

The relative influence of each of the recessive genes on the body size of the offspring in these backcrosses is shown in tables 6 and 7. Non-agouti females are larger than agouti females by all three criteria, weight, body length, and tail length (table 6), though as regards body length (the best criterion) the difference is negligible. But among the males a contrary relation is found, since agouti individuals are larger-bodied than non-agoutis by all three criteria. It is accordingly not shown that the non-agouti mutation, though derived from the larger parent race, has any consistent influence either to increase or to decrease body size.

The brown mutation (*b*) is shown in this cross, as in all others, to increase body size, as judged by all three criteria. Brown females are more than 2.5 percent heavier and brown males are 5 percent heavier than black individuals of the same sex. Brown females are also 1.1 percent longer bodied and brown males are 1.4 percent longer-bodied than black ones. Brown females have tails less than 1 percent longer than blacks, but brown males have tails more than 2 percent longer than blacks. By all criteria males show a greater increase in size than females, as in the backcrosses previously discussed.

Dilution, as in the other crosses, increases size in both sexes in lesser amount than brown as regards weight and body length, but in greater amount as regards tail length. Tail length is increased 2.29 percent in dilute females and 3.03 percent in dilute males, the corresponding increases for brown individuals being .85 percent and 2.13 percent respectively. A special influence of the dilute gene (or the dilute chromosome) on tail length is thus shown to occur in four backcross populations, namely, in the reciprocal backcrosses both from Cross 1 and from Cross 2.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO MALES OF THE MATERNAL (SE) RACE

This backcross, like those already described, was made reciprocally, but owing to an insufficient supply of se females being available, only the backcross in which F_1 mothers were used has as yet produced a population large enough for useful discussion. We shall accordingly, for the present, confine our attention to this alone.

This backcross is of interest not only because it gives additional evidence on the effect of brown and dilution on body size but also because it yields data on the effects of two additional gene mutations, pink-eye and short-ear. Since short-ear is closely linked with dilution, the number of phenotypes produced by the backcross is eight, except when a crossover occurs (about once in a thousand). The number of individuals produced was larger in this than in any of our other backcrosses. We present data on a population of 930 females and 917 males, a total of 1,847 individuals. It would be a needless use of space to report the detailed study which has been made of the variation of each color class in weight, body length, and tail length.

TABLE 8

Comparative variability of females of the several color classes produced by a backcross of F_1 females to the se race

		WEIGHT		BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Black	126	23.12±.18	2.95±.12	91.00±.20	3.25±.14	79.20±.26	4.13±.18
Blue se	133	22.82±.15	2.66±.11	90.27±.16	2.73±.11	78.77±.23	3.96±.16
Brown	124	22.71±.16	2.65±.11	90.59±.20	3.30±.13	79.32±.28	4.62±.19
D br se	124	22.49±.17	2.77±.12	90.92±.19	3.20±.14	78.29±.27	4.55±.20
PE Black	118	23.21±.17	2.81±.12	90.32±.20	3.21±.14	78.12±.25	3.90±.17
PE Blue se	98	22.02±.16	2.44±.12	89.93±.25	3.71±.18	78.13±.29	4.22±.20
PE Brown	119	24.09±.20	3.31±.14	91.42±.20	3.34±.15	78.91±.23	3.84±.16
PE d br se	88	22.50±.19	2.65±.13	90.76±.19	2.99±.15	78.03±.30	4.18±.21
Totals and means	**930**	**22.83±.06**	**2.78±.04**	**90.65±.07**	**3.21±.05**	**78.75±.09**	**4.14±.06**

We shall content ourselves, therefore, with a summary of the data. Tables 8 and 9 show, for each sex separately, the mean values and variability as regards the size characters studied in the case of each color class. Table 10 shows the influence of each mutant gene on the size characters studied. In the case of short-ear and dilution, it is possible to estimate only their

TABLE 9

Comparative variability of males of the several color classes produced by a backcross of F_1 females to the se race

		WEIGHT		BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Black	126	28.11±.19	3.20±.13	94.32±.20	3.24±.14	80.72±.28	4.50±.20
Blue se	117	27.81±.19	3.04±.13	94.36±.17	2.64±.12	80.75±.29	4.40±.20
Brown	101	30.31±.25	3.81±.18	96.41±.24	3.61±.17	82.74±.35	5.11±.24
D br se	117	28.46±.21	3.47±.15	95.33±.22	3.60±.16	81.06±.29	4.52±.20
PE Black	100	28.42±.22	3.24±.15	94.51±.23	3.40±.16	80.49±.33	4.70±.23
PE Blue se	121	26.69±.18	2.98±.13	93.12±.19	3.09±.13	79.85±.29	4.65±.21
PE Brown	136	29.46±.18	3.20±.13	95.83±.19	3.39±.13	81.53±.29	4.84±.20
PE d br se	99	27.02±.25	3.68±.18	94.42±.19	2.79±.13	81.16±.25	3.53±.18
Totals and means	**917**	**28.38±.08**	**3.60±.06**	**94.80±.07**	**3.37±.05**	**81.01±.10**	**4.60±.07**

combined effect. In other backcrosses we have found that dilution regularly increases body size, but it is clear from this cross that short-ear decreases size more than dilution increases it, so that their combined action is a decrease even greater than the increase made by brown, which, up to this time, had been found more influential than any other single gene mutation on body size. The decrease in weight amounts to 3.67 percent for females, 5.74 percent for males. How great the decreasing effect of short-ear on body size would be, apart from dilution, it is impossible at present to state. Among other gene mutations which decrease body size must undoubtedly be included dwarf and waltzing, but as yet we have no data on their quantitative influence.

Brown, as in other backcrosses, has a tendency to increase all size characters studied. Pink-eye, on the other hand, though also derived from the larger parent race in the original cross, has a tendency to decrease size in the backcross. This tendency is manifested in all characters studied except weight in the case of females. Here some extremely fat individuals made the average weight for all pink-eyed females exceed that of the dark-eyed,

TABLE 10

Influence of particular genes on body size in the backcross to the se race, as indicated by percentage increase or decrease (−) of the average.

	FEMALES			MALES		
	WEIGHT	BODY	TAIL	WEIGHT	BODY	TAIL
Brown	.68	.60	.10	3.80	1.53	1.45
SE+Dilution	−3.67	−.40	−.74	−5.74	−1.01	−.82
Pink-eye	.74	−.09	−.76	−2.77	− .19	−.69

but as regards body-length and tail-length, pink-eyed females as well as males, fell below the dark-eyed. The effect of pink-eye in decreasing body size is small but consistent in both sexes (except for weight in females) and so may be accepted as genuine.

We are now in a position to consider the theoretical question, is it linkage with size genes borne in the same chromosomes, or is it the physiological action on growth of the mutant genes themselves which is responsible for the effects noted in these mouse crosses? The larger parent introduced mutant genes which mark four of the twenty chromosomes of the mouse, namely, (1) agouti, (2) brown, (3) dilution and short-ear, and (4) pink-eye. On the linkage hypothesis we should expect to find larger body size associated with each of these genes in the backcross segregates. From the results of our study it appears that there are no linked size genes in the agouti chromosome or the pink-eye chromosome, since agouti individuals do not consistently differ from non-agoutis, and pink-eyed segregates are actually smaller than dark-eyed, contrary to expectation based on the linkage hypothesis. Dilution is associated with larger size when short-ear is not present, and brown is regularly found in the larger-bodied segregates. For the linkage hypothesis we have then, thus far, two positive and two negative tests. But under further examination of the positive cases, the linkage hypothesis breaks down. The case of dilution and short-ear affords a crucial test. Dilution by itself increases size, but when short-ear is present, size is decreased. But dilution and short-ear are closely linked, crossovers occurring less than once in a thousand times. If linkage with a size gene were responsible for the larger size of dilute animals, this gene should become effective irrespective of the presence of short-ear, since it would become homozygous when *either* dilution or short-ear became homozygous; but we find that dilution increases body size even when short-ear is present as a heterozygote; yet when short-ear is homozygous, size is decreased even in the presence of dilution. We are thus forced to assume a physiological action of short-ear on growth. If we concede the existence of such an effect to short-ear, there is no reason why we should not concede also to dilution an influence of a contrary character. Indeed that seems to be the only logical explanation of the observed facts. But if short-ear and dilution affect growth by their physiological action and thus influence body size, there is every reason to suppose that similar action is exercised by brown. FELDMAN (1935) has presented evidence supporting this view.

Size inheritance has long been supposed to result from the joint action of many genes, but in mammals we have hitherto lacked evidence of what these genes were or how they acted. For the mouse we are now able definitely to identify as genetic modifiers of body size the mutant genes brown, dilution, and short-ear. They act through their influence on general body size as manifested in length of trunk and tail and total body weight. This means probably that their action begins early and continues throughout growth. For dilution we have the interesting observation that besides its general action on body size, it exercises a special influence toward increase in the length of the tail, not great enough, however, to counteract the contrary influence of short-ear when present. WRIGHT, DAVENPORT, SUMNER, and others have found evidence of the existence in mammals of special genetic influences local in their action. This demonstrated action of dilution supports that interpretation. It must mean, in physiological terms, that though the dilution gene is probably active throughout growth and thus influences size of the animal as a whole, it is especially active at that stage in development when the tail is being formed.

There is a genetic difference between *Mus bactrianus* and the Japanese waltzer in length of tail. The tail in *M. bactrianus* is relatively shorter. This difference is manifested in the backcross populations. For animals of the same body size, those descended from a Japanese waltzer have longer tails than those descended from *M. bactrianus*. The backcross populations produced by d br males, when mated with F_1 females from Cross 1 and from Cross 2, may be compared as to their size indices in table 11. The animals derived from Cross 2 are seen to be about 1 percent longer-bodied and 10 percent heavier, but their tails are 3 or 4 percent shorter. This is independent of the action of dilution in lengthening the tail, because its influence is present equally in both populations, and its action is positive in both cases, as already stated. But the genetic basis on which dilution may act is different in the two backcrosses. *M. bactrianus* has a genetic complex for shorter tail length than the Japanese waltzing mouse.

TABLE 11

Mean size of backcross animals from Cross 1 and Cross 2 compared

	WEIGHT	BODY LENGTH	TAIL LENGTH
Females, from Cross 1	22.25	92.17	77.20
Females, from Cross 2	24.85	93.38	75.30
Ratio	111.6	101.3	97.5
Males from Cross 1	28.99	96.71	80.84
Males from Cross 2	31.53	97.83	77.78
Ratio	108.7	101.1	96.2

Correlation is fairly high between body length and weight; $.684 \pm .012$ in the case of males, $.642 \pm .013$ in the case of females in the backcross to the *se* race, our largest population. Between body length and tail length the correlation is less close, owing to the greater variability of tail length probably through environmental influences. The coefficients obtained for the same backcross populations as those already mentioned were for males $.466 \pm .018$, and for females $.406 \pm .019$. It is again noteworthy that higher coefficients are obtained for males than for females, indicating that growth processes are more advanced in the case of males than of females, and to a greater extent genetically determined, accidents of development and errors of observation being relatively smaller.

SUMMARY

1. Crosses were made between females of an inbred race of *Mus musculus* having pink eyes, short ears, and dilute brown non-agouti coat, mated (1) with black-and-white Japanese waltzing mice or (2) with *Mus bactrianus*.

2. Cross 1 produced dark-eyed, long-eared, intense black F_1 individuals. Cross 2 produced dark-eyed, long-eared, intense agouti F_1 individuals. In size the F_1 animals from both crosses were nearly as large as the larger parent race, and of remarkable vigor, fecundity, and longevity.

3. F_1 individuals from Cross 1 were reciprocally backcrossed to LITTLE's dilute brown race of *Mus musculus*. In both backcrosses brown individuals were of larger body size than black ones, and dilute individuals were larger than intense ones by three different criteria, namely, (a) mature body weight, (b) body length, and (c) tail length.

4. The influence of brown was found to be greater than that of dilution as judged by body weight and body length, but as regards tail length dilution was found to be more influential than brown.

5. Of the reciprocal backcrosses, that in which the pure *musculus* (d br)

race was the mother produced consistently larger offspring as regards weight, body length, and usually as regards tail length also.

6. F_1 individuals from Cross 2 were also reciprocally backcrossed to LITTLE's dilute brown race. In these backcrosses, as in those from Cross 1, brown individuals were larger than black ones, and dilute individuals were larger than intense ones by all three criteria, weight, body length, and tail length.

7. As in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, brown was more influential than dilution in increasing weight and body length, but dilution was more influential than brown in increasing tail length. This leads us to the tentative conclusion that dilution has a special effect on tail length over and above its effect on general body size.

8. Again as in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, the pure *musculus* race as mother produces larger-bodied offspring than the reciprocal backcross, regularly in weight and body length, and usually in tail length also. The larger racial or individual size of the mother is thus shown to be a factor in producing large body size in her offspring. Such influence, if genetic, must be extra-chromosomal, as has been shown by LITTLE as regards the inheritance of susceptibility to spontaneous cancer in reciprocal crosses and backcrosses in mice.

9. The F_1 animals from Cross 1 were also backcrossed to the maternal parent race, the pink-eyed short-eared dilute brown race of GATES. There resulted 8 color classes, segregation occurring simultaneously for three independent characters, (1) pink-eye, (2) brown, and (3) the closely linked characters, short-ear and dilution.

10. In this backcross, as in those already described, brown individuals were larger-bodied than black ones. Pink-eyed individuals were slightly smaller than dark-eyed ones, and agouti individuals showed no consistent difference, being larger-bodied in the case of females but smaller in the case of males. Dilute individuals we should have expected to be larger-bodied than intense in this as in other backcrosses, but because of its regular association with short-ear, which strongly reduces body size, the short-eared dilute classes were actually smaller-bodied than the intense long-eared classes by all three criteria employed.

11. Linkage with size genes is an inadequate explanation of the size differences found in backcross populations. Direct physiological action on growth of the mutant genes studied is the interpretation preferred by us. As regards their influence on size, brown and dilution have accelerating effects, pink-eye has a slightly retarding, and short-ear a strongly retarding effect. Agouti probably has no effect.

12. Fairly high correlation coefficients indicate that general growth

processes rather than local ones are chiefly affected by these mutant genes, but a special local action of dilution on tail length is indicated.

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