STUDIES OF A SIZE CROSS IN MICE

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IT WILL be conceded generally by students of genetics that body size, though clearly subject to heredity, is not inherited in a simple Mendelian way.

When races of unlike body size are crossed, the offspring are, in general, intermediate in body size, both in the first and in later generations of offspring. Most investigators in this field, being convinced that Mendel's law is universally valid, have accepted what is known as the multiple factor interpretation of this and other similar cases of intermediate or blending inheritance. On this hypothesis the genetic basis of differences in body size consists exclusively of mutated genes borne in the chromosomes. It is certain that such genes exist. Specific evidence for their existence will be described in this paper. But we are not convinced that no cell structures other than chromosomes are concerned in the determination of body size for the following reasons.

Some years since, a study was made of the inheritance of body size in a cross between races of rabbits, one of which was about four times as large as the other. The blending character of the inheritance indicated that, on a multiple factor interpretation, differential genes influencing body size must be so numerous and widely distributed as to be found in practically all chromosome pairs. An attempt was accordingly made, by crossing a large race in which four chromosomes carried dominant color genes with a small race carrying the corresponding recessive genes, to ascertain whether these four chromosome pairs bore any of the hypothetical genes influencing body size. The result was wholly negative. No difference in size could be detected between segregates which bore one or all of the dominant genes received from the large race parent and those which bore recessive genes derived from the small race parent. Nevertheless by embryological studies, CASTLE and GREGORY were able to show that the eggs of large-race and small-race rabbits develop at a different rate. Though the eggs are of the same size, that of the large-race rabbit undergoes cleavage more rapidly and thus produces a larger embryo. The more rapid rate of growth persists after birth and even until adult size is attained. Moreover, sperm as well as egg is influential in determining the developmental rate of the zygote. It seems, accordingly, that developmental rate is itself either gene controlled or controlled by some other feature of the organization of both sperm and egg.

About the time that this conclusion had been reached in our rabbit studies, GREEN made a cross between two species (or subspecies) of mice (*Mus bactrianus* and *Mus musculus*), the former of which is only about half as large as the latter. He applied the same criterion which CASTLE had used in the rabbit size cross for possible linkage between color genes and size genes, but with better success. He reported association in F_2 and backcross populations of larger size with the recessive color genes brown, dilution, and non-agouti, which came from the larger parent in the original cross. He inferred correctly that this implied the location in the three chromosome pairs under consideration of genes influencing body size. Later, when larger numbers had been studied, he concluded that the differences reported were of significant magnitude in one only of the three cases—that of the chromosome carrying the gene for brown. On this alone he based the argument for a multiple factor interpretation of body-size inheritance, supporting it later by evidence thought to indicate crossing over between the brown gene and one or more size genes located in the same chromosome.

We shall show that GREEN was undoubtedly right in assigning an influence making for greater body-size to both the brown chromosome and the dilute chromosome. The case for the non-agouti chromosome is doubtful. But we shall show, also, that the plus influence on body-size exerted by the brown chromosome and the dilute chromosome is due not, probably, to some hypothetical size gene linked with the color gene, but to the physiological action of that gene itself.

Our original plan was to verify, if possible, GREEN's findings by repeating his experiment with certain minor modifications. In this we had the cordial coöperation of Dr. GREEN, who supplied us with animals of the same inbred stocks which he had used in his experiment—namely, *M. bactrianus* and LITTLE's well known and long inbred dilute brown strain, both from the JACKSON MEMORIAL LABORATORY. We owe hearty thanks, in this connection, to Director C. C. LITTLE, and Drs. GREEN and MURRAY.

In the first cross which we made, on which we shall report in this paper, we used as mothers, not LITTLE's dilute brown race but a derivative of it established by GATES, in which pink-eye and short-ear had been added to the assemblage of recessive characters. This race accordingly was homozygous for the five recessive characters, pink-eye, dilution, short-ear, brown, and non-agouti. It had been closely inbred in brother-sister matings for several generations. Two of the five recessive genes, namely, dilution and short-ear, are borne in the same chromosome and presumably very close together, since the occurrence of crossovers is only about one in 1000. Accordingly, four chromosomes are in this race tagged with recessives, namely, (1) brown, (2) dilution and short-ear, (3) pink-eye, and (4) non-agouti. For brevity, we shall refer to this race as the s.e. race. As the other parent race we used not wild *M. bactrianus*, as GREEN had done, but a supposedly domestic derivative of it, the long inbred strain of black-and-white Japanese waltzing mouse used by Gates (1926). This carries three independent recessive characters, piebald, waltzing, and non-agouti.

In weight 17 adult males of the s.e. race range from 22 to 33 grams, average 26.2. Gates (1926) reports the average weight of adult Japanese waltzing males to be 17.6 grams. The weight of five adult F_1 males ranges from 21.0 to 29.8 grams, average 25.4. These fragmentary records indicate (and the general impression which one gets when handling the animals coincides with it) that the F_1 animals are nearly, though not quite, as large as the larger parental race. In vigor, productiveness, and longevity, however, the hybrids are far superior to either parent race.

The F_1 animals were crossed reciprocally with LITTLE'S dilute brown race in order that segregation in size might be observed in relation to brown and dilution only without complication from pink-eye and short-ear which would continue to be recessive in whatever backcross animals received them from the F_1 parent. For the "d br" race, having been deliberately inbred, chiefly in brother-sister matings, since 1909, according to GREEN, should be completely homozygous for all genetic factors (except for possible new mutation), and it is free from pink-eye and short-ear, though homozygous for dilution and brown.

The backcross animals accordingly fall, as regards color, into four classes, (1) black, (2) dilute black (blue), (3) brown, and (4) dilute brown.

GATES (1926) thought that there was a tendency in a cross similar to this for the chromosomes to emerge from the cross in the same associations in which they entered it, so that in F_2 or in backcrosses a higher percentage was obtained of character combinations identical with those found in the parents than one would expect from chance alone. But GREEN did not observe any such tendency toward an association between chromosomes in the cross which he made between *M. bactrianus* (the supposed ancestor of the waltzing mouse) and the "d br" race of LITTLE. Further, a reëxamination of the data recorded by GATES raises a question as to the significance of the statistical deviations from expectation which he reported. It has seemed desirable, therefore, to reopen the question in connection with this investigation.

If GATES' association principle were valid for this cross, we should expect the parental combinations *intense black* and *dilute brown* to exceed in frequency the recombinations *dilute black* and *intense brown*. In the cross between F_1 females and "d br" males a population of 1001 animals recorded at weaning time (age 3 to 4 weeks) has been tabulated from the records without selection. They consist of (1) black intense, 235; (2) black dilute, 268; (3) brown intense, 249; and (4) brown dilute, 249. The sum of the parental combinations (black intense and brown dilute) is 484, which is less than the sum of the recombinations (black dilute and brown intense), which equals 517. The probable error (expected chance deviation from equality in a population of this size) is 10.6. The observed deviation is 16.5, which is less than twice the P. E. and so not significant. It is also contrary in character (being minus rather than plus) to a deviation which might be due to persistent association of the chromosomes. The conclusion is warranted that in this cross, as in so many others which have been studied, the segregation of each chromosome pair occurs quite independently of other chromosome pairs, and continued association or dissociation of genes borne in different chromosomes is quite a matter of chance. We may therefore dismiss as groundless a criticism by CASTLE of GREEN'S interpretation of his results based on the association principle outlined by GATES.

From the cross, F_1 ♀ ×"d br" ♂, 1236 mice have been reared, weighed monthly or oftener between the ages of 4 and 6 months, and then chloroformed and measured as to body length and tail length by SUMNER'S method, keeping the body under a uniform tension of 20 grams. In tables 1 and 2 the animals are classified as to sex, color, and maximum weight at or prior to six months of age. In tables 3 and 4 they are similarly classified as regards body length at six months of age.

The order of increasing size is the same for weight and for body length in both sexes. Blacks are smallest, then come blues, next browns, and last dilute browns. The only exception is found in body weight of dilute brown females, which are no heavier than intense browns, although as regards body length they are decidedly longer than intense browns. Body length is, as suggested by GREEN, a more reliable indicator of general body size than weight, for certain individuals in both the F_1 and the backcross populations have a tendency to become very fat after they have reached the age of 4 or 5 months, so that their weights displace the average upward. Body length, on the other hand, is apparently not affected by accumulation of fat, though it is clear that only individuals of large size become excessively fat. Body length is also a linear dimension, whereas increase in body weight depends upon increase in all correlated dimensions and thus produces greater variation. This is shown in the higher coefficients of variability for weight than for body length. For females the C. V. in weight is 10.8–12.0 and in body length 3.4–3.6; for males the C. V. in weight is 9.8–11.1, and in body length 2.9–3.8.

If we compare all black pigmented individuals (whether intense or dilute) with all brown pigmented ones of the same sex (whether intense

TABLE 1

Variation in weight of females of four different color classes from matings, ♀ $F_1 \times$ ♂ *d br (Little).*

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																		NO.	MEAN	S.D.	C.V.
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
Black	2	1	3	15	16	28	35	23	13	11	6	5	1	0	1				160	21.46±.12	2.33±.08	10.8
Blue		2	2	11	17	24	21	35	23	16	11	9	0	0	4	0	1	1	177	22.32±.13	2.69±.09	12.0
Brown			4	6	13	22	30	20	21	11	17	11	2	5	2				164	22.61±.13	2.62±.09	11.5
Dilute Brown			4	5	10	28	24	28	21	12	12	10	4	2	1	2	1		164	22.61±.14	2.67±.10	11.8
All Blacks	Black and Blue combined																		337	21.91±.08	2.55±.05	Dif. means,
All Browns	Brown and Dilute Brown combined																		328	22.61±.12	2.65±.07	.70±.14
All Intense	Black and Brown combined																		324	22.04±.09	2.52±.06	Dif. means,
All Dilute	Blue and Dilute Brown combined																		341	22.45±.10	2.67±.07	.41±.13

TABLE 2

Variation in weight of males of four different color classes from matings, ♀ $F_1 \times$ ♂ d br (Little).

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																								NO.	MEAN	S.D.	C.V.
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41				
Black	1	0	0	0	3	2	13	13	12	18	21	13	19	6	6	3	3	1	2						136	28.24 ± .16	2.77 ± .10	9.8
Blue		1	2	1	3	5	10	8	17	17	13	18	14	9	11	7	1	0	3	0	2	1	1		144	28.64 ± .17	3.04 ± .12	10.6
Brown					2	5	3	14	10	15	23	22	17	6	7	3	7	3	3	0	2				142	29.09 ± .18	3.24 ± .13	11.1
Dilute Brown						2	1	4	14	18	19	23	24	13	7	6	6	4	2	2	2	0	1	1	149	30.01 ± .17	3.18 ± .12	10.5
All Blacks	Black and Blue combined																								280	28.34 ± .13	3.40 ± .09	Dif. means,
All Browns	Brown and Dilute Brown combined																								291	29.56 ± .12	3.23 ± .09	1.22 ± .17
All Intense	Black and Brown Combined																								278	28.67 ± .12	3.16 ± .09	Dif. means,
All Dilute	Blue and Dilute Brown combined																								293	29.34 ± .13	3.52 ± .09	.67 ± .17

TABLE 3

*Variation in body length in mm. of females of four color classes from matings, ♀ $F_1 \times$ ♂ *d br* (*Little*).*

COLOR	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	104	NO.	MEAN	S.D.	C.V.
Black		2	1	0	5	4	8	10	19	28	22	17	10	7	8	12	2	1	1	1			158	90.88±.17	3.22±.12	3.54
Blue	1	0	0	0	3	4	5	10	12	15	24	23	23	15	18	4	9	6	2	1	1		176	92.13±.17	3.26±.12	3.53
Brown				1	1	2	5	6	13	14	11	21	16	25	23	5	12	3	2	1	1		162	92.68±.17	3.21±.12	3.46
Dilute Brown		1	0	1	1	3	1	8	10	12	11	21	14	19	21	15	10	4	4	2	0	1	159	93.00±.18	3.40±.12	3.65
All Blacks	Black and Blue combined																						334	91.51±.12	3.32±.08	Dif. means, 1.33±.17
All Browns	Brown and Dilute Brown combined																						321	92.84±.12	3.26±.08	
All Intense	Black and Brown combined																						320	91.79±.12	2.72±.08	Dif. means, .74±.17
All Dilute	Blue and Dilute Blue combined																						335	92.55±.12	3.36±.08	

TABLE 4

*Variation in body-length in mm. of males of four color classes from matings, ♀ $F_1 \times$ ♂ *d br* (Little).*

COLOR	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	NO.	MEAN	S.D.	C.V.
Black	1	0	0	1	0	1	1	3	2	7	6	14	9	20	13	15	15	14	6	4	2	1				135	95.52±.20	3.47±.14	3.63
Blue		2	0	0	1	1	2	2	2	5	7	9	10	12	18	20	22	12	7	4	1	2	0	2		141	95.91±.20	3.68±.14	3.83
Brown							3	1	1	1	7	4	8	15	14	23	19	21	13	4	2	1	2	1	1	141	96.96±.17	3.09±.12	3.18
Dilute Brown							1	0	1	1	0	3	5	9	13	16	26	23	14	12	11	6	2	1	2	146	98.46±.16	2.93±.11	2.97
All Blacks	Black and Blue combined																									276	95.77±.14	3.50±.10	Dif. means,
All Browns	Brown and Dilute Brown combined																									287	97.73±.10	3.11±.08	1.96±.17
All Intense	Black and Brown combined																									276	96.26±.14	3.38±.10	Dif. means,
All Dilute	Blue and Dilute Brown combined																									287	97.21±.14	3.57±.10	.95±.17

or dilute), we find the latter invariably longer-bodied and heavier in both sexes. For females, browns are 1.4 percent longer-bodied and 3.2 percent heavier; for males, browns are 2.04 percent longer-bodied and 4.3 percent heavier. See table 5.

If we compare the intense pigmented individuals (whether black or brown) with dilute pigmented (whether black or brown) of the same sex, we find the latter invariably larger and heavier. The difference is less in the

TABLE 5

Comparison of females with males as regards body length and weight in different color groups.

AV. BODY LENGTH	FEMALES	MALES	RATIO
All Blacks	91.55	95.77	100:104.6
All Browns	92.84	97.73	100:105.2
Ratio, Black:Brown	100:101.40	100:102.04	Mean=104.9
All Intense	91.79	96.26	100:104.8
All Dilute	92.55	97.21	100:105.0
Ratio, Intense:Dilute	100:100.82	100:100.98	Mean=104.9
Av. Weight			
All Blacks	21.91	28.34	100:129
All Browns	22.61	29.56	100:131
Ratio, Black:Brown	100:103.2	100:104.3	Mean=130
All Intense	22.04	28.67	100:130
All Dilute	22.45	29.34	100:130
Ratio, Intense:Dilute	100:101.9	100:102.3	Mean=130

case of dilution than it was in the case of brown, but still significant statistically in every case. See table 5. For females, the dilutes are 0.82 percent longer-bodied and 1.9 percent heavier; for males, the corresponding values are 0.98 percent and 2.3 percent.

The correlation between body-length and weight is high, being $0.65 \pm .01$ in the case of females and $0.66 \pm .01$ in the case of males. See tables 6 and 7. This supports the conclusion which one of us has based on a long series of investigations indicating that the genetic agencies which affect size are chiefly general rather than local in their action.

An interesting relation between the size characters of females and males is shown in table 5. Males are consistently about 5 percent longer-bodied than females of the same color group, but in weight they are about 30 percent heavier, the first difference being based on a linear measurement, the second on a mass or three-dimensional evaluation.

The differences between color groups are also greater relatively, as well as absolutely, in the case of males when compared with females. Brown makes an increase in body length (over black) of 1.40 percent in the case of females but of 2.04 percent in the case of males. Brown also increases weight by 3.2 percent in the case of females, but by 4.3 percent in the case of males.

Dilution consistently has a lesser effect on size than brown, but its effect is regularly more pronounced in the case of males than of females. Thus dilution increases body length by .82 percent in females, but by .98 percent in males. It increases weight by 1.9 percent in females, but by 2.3 percent in males. The male mouse is a larger animal than the female. It grows beyond the stage at which growth is normally arrested in females, and in the final stages of growth the differential effect of genes borne in the chromosomes carrying brown and dilution is more strongly in evidence than before.

It remains to consider the question whether the differential effect on growth exercised by the brown and dilution chromosomes is due to the genes for brown and dilution respectively or to some gene or genes linked with them. FELDMAN (1935) has recently published data which indicate that in races of mice, whether of large or of small body size, browns in mixed litters are regularly larger than their black sibs.

In the cross described in this paper, we introduced from the *musculus* parent into the hybrids two closely linked recessive characters borne in the same chromosome, namely, dilution and short-ear. We have shown that in the backcross to "d br" males the individuals which are homozygous for dilution are larger than those which are heterozygous (the blacks and browns). Dilution (or something linked with it) must be regarded as an influence increasing size when homozygous. Short-ear does not reappear in this backcross, though present as a recessive in substantially all dilute individuals, since it would be transmitted to all individuals to which the F_1 parent transmitted dilution, unless a crossover occurred between short-ear and dilution.

But in a different backcross, to be described more fully in a subsequent paper, short-ear does make its appearance, and we are thus able to estimate its influence on size. When our F_1 females are backcrossed to the parental *musculus* race (pink-eyed short-eared dilute brown), the same four classes of offspring result as in the backcross already described, namely, black, blue, brown, and dilute brown; but in this case blues and dilute browns are short-eared. They are also regularly inferior in size to the blacks and browns respectively, which are long-eared. In other words, the tendency of homozygous dilution to *increase* body size, whether associated with black or with brown, is more than offset by a tendency of short-ear, when homozygous, to *decrease* body size.

But dilution and short-ear are closely linked with each other, with crossovers occurring about once in a thousand times. Any hypothetical size gene closely linked with one should also be closely linked with the other. Yet homozygous dilution increases size, and homozygous short-ear

TABLE 6

Correlation between body length and weight in 656 backcross females. $r = .65 \pm .01$.

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	
15		1								1															2
16	1				1	1																			3
17		1	1	2	2			6																	12
18					5	6	3	7	6	4	3		1	1			1								37
19			1		1	3	9	4	12	5	6	8		3		1									53
20						1	3	9	14	25	10	16	7	5	7	2									99
21					1	1	3	3	9	15	18	23	13	9	8	3	2	1							109
22								2	8	9	20	16	15	16	10	6	3								105
23						1	1	1	2	5	3	10	11	12	16	9	5	3							79
24									2	3	4	4	6	6	13	3	4	3	1						49
25								1			3	3	4	5	7	8	7	4	2	2					46
26									1	1	1	1	5	7	6	3	5		2	2	1				35
27										1		1		1	1		1		2						7
28															1	1	2	2	1						7
29												2			1		2	1		1	1				8
30																	1							1	2
31														1				1	1						3
	1	2	2	2	10	13	19	33	54	69	68	84	62	66	70	36	33	15	9	5	2			1	656

TABLE 7

Correlation between body length and weight in 534 backcross males. $r = .66 \pm .01$.

	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	
19					1																				1
20	2																								2
21							1																		1
22				1		1	1		2				1	1											7
23						4	1	2	1	2	1	1	1		1										14
24					1	1	1		4	2	6	5	3	2		2									25
25						1			1	8	7	4	5	2	4	2	1	1							36
26							1	2	2	3	5		9	6	4	6	1			1					40
27							1		3	1	7	7	11	12	14	4	6	1							67
28											4	2	10	9	14	15	11	6	1		1				73
29												3	7	11	12	17	11	9	1	4					75
30								1		2		1	6	10	14	9	13	8	4	2		1	1		72
31								1				1			2	9	7	5	3	3					31
32													2	2	2	6	5	1	6		4		1	1	30
33														1	2	4	2	2	4	1		1			17
34															1	2	6	1		1	2	1		1	15
35																	3	2	1	1					7
36									1					1		2	2	2	1	1					10
37																						1			1
38															1	1				1			1	1	5
39															1										1
40																1					1	1			3
41																			1						1
	2			1	2	7	6	6	13	19	30	22	55	57	72	80	68	38	22	15	8	5	3	3	534

decreases size, even in the presence of dilution. It is difficult to avoid the conclusion that these genes are, by virtue of their own physiological action, genes influencing body size. For if the influence on size were due to a third gene linked with short-ear and dilution, it should become operative when *either* short-ear or dilution become homozygous; but we find that size increases when dilution becomes homozygous but decreases when short-ear becomes homozygous, their action being qualitatively contrary.

SUMMARY

1. A cross was made between a pink-eyed dilute brown short-eared race of house mouse and an inbred strain of black-and-white Japanese waltzing mouse. Adult males of the parent races weighed, on the average, 26.2 and 17.6 grams, respectively. The F_1 hybrids were uniform black in color, and males, when adult, weighed about 25.4 grams.

2. A backcross was made between F_1 hybrid females and males of LITTLE'S dilute brown race. The progeny fall into four color classes, black, blue, brown, and dilute brown, with no statistically significant differences between their respective frequencies. A backcross population of 665 females and 571 males was raised to the age of six months, each animal being weighed at monthly intervals and its body-length measured at six months of age.

3. In body-size, whether estimated by maximum weight or by body length, brown animals are larger than blacks, and dilute animals are larger than intense ones, the difference being greater in the case of brown than of dilution, but clearly significant in both.

4. Males are larger than females and show a greater influence on size of the brown and the dilute characters than females do.

5. Reasons are given for regarding the superior size of brown and dilute animals as due to the physiological action of the genes for those characters rather than of specific size genes borne in the same chromosomes.

6. The coefficient of correlation between adult weight and body length is, in the case of females, $.65 \pm .01$, and of males, $.66 \pm .01$.

LITERATURE CITED

CASTLE, W. E., 1929, 1931 Size inheritance in rabbits. J. Exp. Zool. **53**: **60**.
1932 GREEN'S studies of linkage in size inheritance. Amer. Nat. **66**.

FELDMAN, H. W., 1935 The brown variation and growth of the house mouse. Amer. Nat. **69**.

GATES, W. H., 1926 The Japanese waltzing mouse. Publ. No. 337, Carnegie Inst. Wash.

GREEN, C. V., 1931 Size inheritance and growth in a mouse species cross. J. Exp. Zool. **59**.
1933 Further evidence of linkage in size inheritance. Amer. Nat. **67**.
1935 Apparent changes with age in crossing-over between colour and size genes in mice. J. Genet. **30**.

GREGORY, P. W. and W. E. CASTLE, 1931 Further studies on the embryological basis of size inheritance in the rabbit. J. Exp. Zool. **59**.

SUMNER, F. B., 1927 Linear and colorometric measurements of small mammals. J. Mammalogy **8**.