TWO-WAY SELECTION FOR BODY SIZE IN RATS, WITH OBSERVATIONS ON SIMULTANEOUS CHANGES IN COAT COLOR PATTERN AND HOOD SIZE[1]

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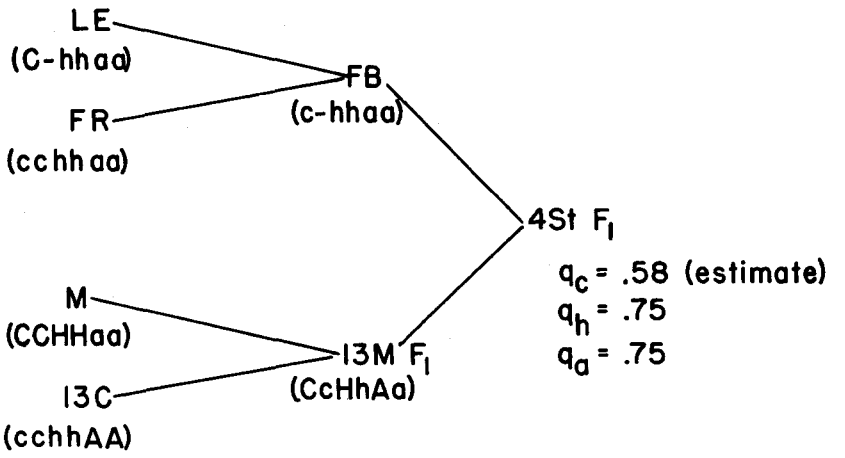
IN the course of a two-way selection experiment for body size in rats, we observed a number of differences, other than size, between the resulting lines (T. F. ZUCKER 1953, 1957). The question arose as to whether the observed correlations with body size were chance or obligate. To answer this question we are carrying on a second size selection experiment quite independent of the first. Results for the various characters possibly correlated with size and observed in both experiments will be reported elsewhere. Here we wish to describe the second size selection experiment now in its tenth generation of selection and will consider the effects of body size selection and restricted population size upon some characters not observed in the first experiment—coat color and pattern as determined at three major loci and the many loci affecting size of hood.

TABLE 1

*Constitution of foundation stock**



* Six or seven rats of each stock were used, about half of each sex, not sibs or half-sibs.

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MATERIAL AND METHODS

Foundation stock

The 4St stock was set up (see Table 1) from four established rat stocks each of which has been maintained for many years as a closed colony with avoidance of close inbreeding. LE (Long-Evans) was obtained from the branch colony established in the 1920's in the Anatomy Department of Columbia University by DR. P. E. SMITH. The Long-Evans stock originated somewhat earlier in California, from a crossing of laboratory albino females with local wild males. M (Merck) was developed at the Merck Institute for Therapeutic Research, Rahway, N. J., also from a cross between laboratory albino females (Sherman stock) and local wild males. M is a true-breeding black self extracted from this cross some years ago. Branches of this black stock are located at the National Institutes of Health (there called NIH black) and at Columbia University (formerly colony of DR. MAURICE SHILS, Public Health Institute, now of DR. W. B. STEWART, Department of Pathology). FR (Food Research) is originally of Wistar origin (Wistar noninbred albino colony brought to the Institute by DR. H. H. DONALDSON) and obtained from the branch colony of the Food Research Laboratories, Long Island City, N. Y., DR. B. L. OSER. 13C is of originally Sherman stock (colony of DR. H. C. SHERMAN, Department of Chemistry, Columbia University), which was in turn a branch of the Osborne-Mendel stock of the Connecticut Agricultural Experiment Station. This was originally a rather small rat. At Connecticut it has been bred up to a very large size, and in our hands it was selectively bred both up and down for body size. 13C was a moderately large line, but not the largest obtained from this first size selection experiment. After ten generations of selection, selection was relaxed for another eight generations. Of the other stocks employed, FR is definitely small, M and LE are middle-sized as rat stocks go.

The foundation stock 4St was heterozygous at three loci affecting coat color or pattern—albinism (*c,C*), hood-self (*h,H*) and agouti (*a,A*). *C* (nonalbino) and *A* (agouti) are fully dominant; *H* (self) is not. The initial allelic frequencies for these three genes, shown in Table 1, were calculated from the constitutions of the parent stocks which were either apparent from their phenotypes or established by the results of the crosses. Thus FR is phenotypically albino (*cc*). LE is heterozygous at the three loci in question, but we chose to use only phenotypically black (i.e., nonagouti) hooded individuals, which are *hhaa*, with one *C* and the other allele at this locus either *C* or *c*. The offspring of the FR × LE cross were, by litters, either all phenotypically black hoods, or about half black hoods, half albinos. This confirms the *hhaa* constitution for the FR rats, which we had expected since it is the known constitution of the Wistar noninbred albino colony. It also fixes the condition at the albino locus for each LE rat used.

13C is albino (*cc*). M is a true breeding black self, therefore *CCHHaa*. The cross turned out all agouti, with variable white patches on the belly and at the wrists. Heterozygotes at the hooded self locus are known to show such white spotting as appeared here; this is the pseudo-Irish, occasionally confused in the past with a true-breeding Irish which is the homozygote of another allele h^I at the

hooded locus (MUDGE 1908; CASTLE 1940, Figures 62 and 74 for *hH* Irish, Table 40 for h^1; see also CASTLE 1951). The 13C is, therefore, indicated to be *AA* and *hh*. Further results of this cross are summarized in Table 2 covering F_2 and backcrosses. No other known coat color variants have appeared in the two lines selected for large and small body size, called 4StL and 4StS, nor in the 13M stock resulting from the 13C × M cross, which has been carried on for nine generations. The original M stock occasionally shows a white spot in the groin indicating some spotting factor, but none of the individuals used showed this, and it has not turned up since.

Breeding plan

The F_1 generation of 4St was subjected to some size selection but not according to the same formal scheme as all the later generations. The F_1 (parents 13M and FB) comprised 14 litters, and one of each sex was chosen from each litter at random. They were later mated according to size, and the six largest pairs were called 4StL; the six smallest were called 4StS. This was then a mass selection procedure of low selectivity (nearly 50 percent retained). Beginning with the second 4St generation, selection was handled according to the scheme used by FALCONER (1953a) in his mouse selection studies with selection purely within litters. In each generation there are six litters, each of four females and four males, totalling 48 rats. The best (i.e., largest or smallest as the case may be) male and best female of each litter are the breeders for the next generation, so that 25 percent of the population is selected, and there are six breeding pairs per generation. The most distantly related animals are mated, and if the female of litter A is mated with the male of litter B, the reverse pairing is avoided. We have

TABLE 2

Cross between 13C (albino) and M (black self)

		Albino‡ *cc*	Colored *C-*	Hooded black *C-hhaa*	Irish black *C-hHaa*	Self black *C-HHaa*	Hooded grey *C-hhA-*	Irish grey *C-hHA-*	Self grey *C-HHA-*
F_1									
	Found	0	88	0	0	0	0	55	0
	Expected†	0	88	0	0	0	0	55	0
F_2*									
	Found	66	264	11	29	21	28	78	32
	Expected	82.5	247.5	12.4	24.9	12.4	37.3	74.6	37.3
$F_1 \times M$									
	Found	0	74	0	15	12	0	17	19
	Expected	0	74	0	15.8	15.8	0	15.8	15.8
$F_1 \times 13C$									
	Found	23	32	0	0	0	13	17	0
	Expected	27.5	27.5	0	0	0	15	15	0

* The deficit of albinos in F_2 and $F_1 \times 13C$ is possibly real (exceeds 5 percent level of significance).

† Expectations are based on the assumptions that the albino stock 13C is *cchhAA*, the black self stock M is *CCHHaa*, and there is no linkage.

‡ The albino count was made at birth; classification of colored rats was made at weaning and thus involves a smaller number because of litter reduction.

generally mated the two best females of each litter to the best male of the chosen litter for mating for ten days, and then after a two-day interval mated them to the second best male, so that if the chosen best is sterile or slow breeding, the second best can be used without loss of time. Until recently the litter size was large enough (10 to 20) to allow the scheme to be carried through on the basis of one litter per pair, litters being reduced to eight shortly after birth in random fashion. In this way we have gotten nearly three generations per year, with both strains coming to breeding age at the same time. Recently, however, litter size in the small line has fallen below eight so that second litters are needed, causing some delay. The unselected 4St stock was not carried along to serve as control, and this is a flaw in the experiment. We do, however, have observations in the 13M strain that is not under size selection pressure and carried on with a comparable effective population size.

RESULTS

Body size selection

Animals were weighed at weekly or semiweekly intervals throughout their lives, and the resulting growth data will be discussed elsewhere. For present purposes of describing the progress of size selection we will use simply the weight at nine weeks, which was the principal determinant in selection. There is a large sex difference in rat growth; unlike the case with mice, the male grows *relatively* (as well as absolutely) much faster than the female, so that the ratio of male to female weight increases rapidly with age. This presents a problem in combining male and female size data. We have had a considerable degree of success in fitting *individual* as well as average postweaning growth curves with the equations

$$\log W = \log W\infty - 3.65/\text{age (weeks)} \text{ for males}$$

$$\log W = \log W\infty - 2.73/\text{age (weeks)} \text{ for females}$$

with $W\infty$ (weight at $1/\text{age} = 0$, or age $= \infty$), being the sole adjustable quantity for any individual curve (L. M. ZUCKER 1942, 1953). The equations illustrate the fact that weight ratios within the same sex remain constant with age, while the ratio of male to female weight increases continuously with age, being made up of the limiting weight ratio and an exponential factor containing the age and the difference between the characteristic male and female slopes (3.65–2.73). In groups of rats of very different average sizes, we find a constant ratio of male to female weight at nine weeks, with the value very close to 1.45. We will then assume that any observed female weight at nine weeks of age, when multiplied by 1.45, supplies the weight which the rat would have shown had it been a male. Female weights, so corrected, are averaged with male weights to give the average nine-week weight for any group.

Results are presented in Figure 1 in three different forms. Section A shows the ratio of mean nine-week weights in the two selected lines, plotted against generation number. This is a satisfactorily smooth curve, representing steady progress in selection. Section B shows separate plots for the two lines against calendar time together with a control strain (13M) not subjected to size selection. Section

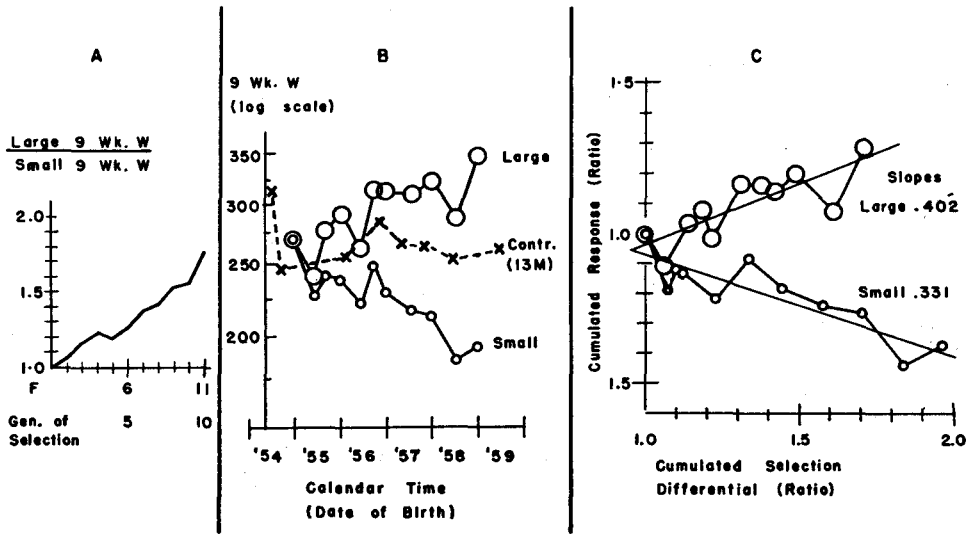

FIGURE 1.— Progress of body size selection. A. Ratio of nine-week weights of large and small strains against generation of selection. B. Separate plots of nine-week weights for large and small lines, and control line not selected for body size. Abscissa is calendar time. The first points for both 4St and 13M may possibly be high due to heterosis. The weight scale used is logarithmic. We have used ratios, or logs, or CV's throughout rather than differences or absolute weights or variance of absolute weights. The standard deviation tends to be proportional to mean weight, to be more constant on *log W* scale (cf. similar spreads of weight histograms in Figure 2B where successive class means are in geometric progression.) The ratio, not difference, of weights of two well-nourished and healthy individuals or groups of the same sex but different size remain constant during postweaning life. At any age the ratio, not difference, of male and female weights is constant. Consideration of ratios rather than absolute differences make much more biological sense.

C. Cumulated selection differential (calculated as weight *ratio*, not difference) against cumulated response (as weight ratio). This corrects the plot of section B for effects of more favorable selection differential in one line than in the other (as suggested by FALCONER 1953b). The slopes are realized heritability, and were calculated from the regression of response on selection differential.

C shows the same data in the form suggested by FALCONER (1953b)—cumulated response against cumulated selection differential. Sections B and C show marked irregularities not found in A, which must be due to environmental variation affecting both strains alike. The most prominent effect is that of going through the most rapid growth period during the hot summer weather (New York City, no air cooling). The summer of 1957 was relatively cool and free of long hot spells, and the graph shows it. Under these conditions it is not surprising that the coefficient of variation of the nine-week weight is highly variable and rather uninterpretable (see Table 3).

The slope of the plot made in section C of Figure 1 represents realized heritability. Slopes for the two lines are about the same. This is unlike the experience with mice, where selection down is more effective than selection up. In our first size selection experiment in rats, with the initial stock definitely smaller in size

TABLE 3

Coefficient of variation for nine-week body weight in control line and selected lines

	13M					4StS				4StL			
	Female		Male			Female		Male		Female		Male	
	CV*	N	CV	N		CV	N	CV	N	CV	N	CV	N
F_{3-4}	8.0	91	9.5	82	F_{3-5}	9.9	101	11.2	120	6.9	97	6.6	98
F_{5-6}	6.4	86	6.8	73	F_{6-8}	7.1	117	9.2	121	7.4	78	7.3	87
F_{7-8}	7.5	67	10.0	64	F_{9-11}	9.2	90	9.5	85	9.7	105	8.9	102

* CV's as calculated for each generation are extremely variable. We have included all available rats, some of them not in the main line of selection (i.e., litters of which one parent was second choice), in order to get as large numbers as possible. For further smoothing, we have averaged CV's for two or three successive generations, comprising in each case approximately one calendar year. All data on one line are approximately contemporary.

(nine-week weight about 230) than 4St, selection up was very much more successful than selection down (unpublished data). Two important factors contributing to asymmetry in two-way selection are dominance and the position of the initial phenotype in relation to the possible extreme phenotypic values (FALCONER 1953b). Dominance always favors selection towards the recessive. The effect of the initial phenotypic position is to favor selection towards the further phenotypic extreme and inhibit selection towards the nearer extreme. This effect, unlike dominance, can therefore cause reversal in relative rates of up and down selection as the initial phenotypic value in different selection experiments varies. Since, in mice and rats subjected to size selection, symmetry and opposite asymmetries have all been observed, it seems likely that the position of the initial phenotype in relation to the extremes has been an important variable, whatever the dominance situation may be.

Size of hood

CASTLE and co-workers (see reviews in CASTLE 1940, 1951) have shown that the size of the hood in *hh* rats responds readily to two-way selection, leading to two lines with very large and very small hoods, with ranges outside the range of the original stock. The genetic variability was not completely exhausted even by 20 generations of intensive selection. It was first concluded that "the genetic modification secured by selection did not involve the hooded gene itself but only the residual heredity (consisting probably of an association of genes which modified the somatic effect of the hooded gene itself)." There was later some evidence that eventually the selection also profited from a change at the hooded locus itself, with the appearance of a new allele h^I (true Irish) in the plus line, and possibly of the new allele h^n (notch) in the minus line.

Presumably in selection for body size one also makes progress by selecting for favorable configurations at many loci which modify by contributing plus or minus factors to the basic character, the rat physique. No doubt, body size selection will also concentrate the most favorable alleles of any possible allelic series affecting size, whether originally present in the population gene pool, or arising

by mutation during the course of selection. At any rate the two characters, body size and hood size, are alike in their continued response for many generations to two-way selection and in the conceptual structure which can be built up around the observed facts. It is of interest to see whether selection for the one character has any influence on the other.

Observations have been of two kinds. (1) Hood size was classified in four degrees for the first five and for the tenth generations. There has been no change of the average in either line. (2) In the tenth generation (ninth of body size selection), careful measurements were made of hood size in the two lines. As described by WRIGHT and CHASE (1936), a drawing was made within a rubber stamp outline, one for the dorsal and one for the ventral aspect. This was done by one person, and when the rats were four- to six- weeks old. Areas were measured by a planimeter. Two different areas were measured—the area of the colored stripe down the back starting at the edge of the band of solid color covering head and shoulders, and the total area of color over the whole body. The second measure includes a large nearly constant colored area, and the total is principally modified by variation in the first measurement—the stripe itself—although there is also some variation in the amount of white on the forelegs and in occasional small colored spots which show on the belly. Results with the two areas were very similar in sense. Figure 2 shows the distribution of measurements of the stripe area, compared with the distribution of nine-week body weights for the two lines. It is clear that selection for body size is having little if any effect on hood size; the only effect is the greater dispersion of hood size in the large line, with two new classes added to the histogram. At the present stage of selection there is no real difference between the two lines in respect to hood size.

One view of quantitative characters is that they are determined by a network of gene loci, very large in number and probably spread over all or many chromosomes. A common or even largely common set of such loci for our two characters is clearly ruled out. Two independent sets of genes, if each is numerous and widely distributed, would certainly show some linkage. Here the expected behavior under size selection is a little uncertain. The foundation stock cannot have been in full linkage equilibrium, but it is impossible to say how far out of linkage equilibrium it was. This depends on the extent to which pairs of linked loci were oppositely fixed or had very different allelic frequencies (i.e., were nearly oppositely fixed) in the different parent stocks. If the foundation stock was out of linkage equilibrium for many relevant loci, one would expect a gradual approach to equilibrium during the course of generations with some establishment of differences in hood size in the two lines, due to such linkage in the early stages of size selection, followed by no further change after some later stage. But our failure to find such an early difference in hood size does not rule out the possibility that both characters are controlled by large overlapping networks of genes; it only rules out the idea of a single set of pleiotropic genes principally determining both characters.

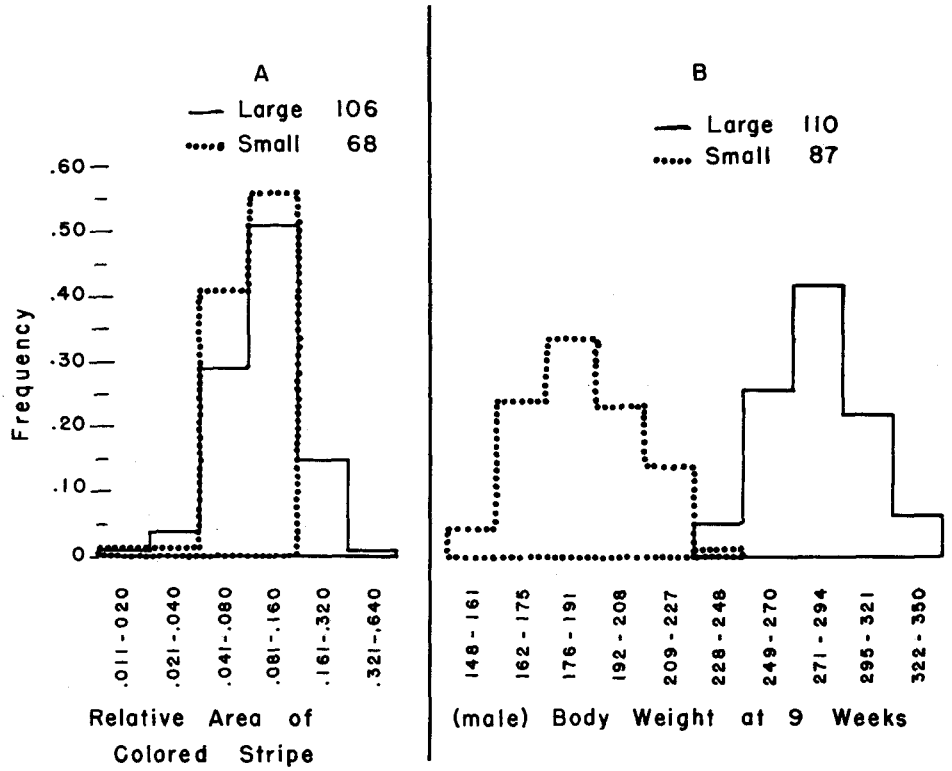

FIGURE 2.—Effect of nine generations of two-way selection for body size on size of colored hood. Our six classes of stripe area can be compared to the scale used by CASTLE and PHILIPS (see CASTLE 1940) as follows:

Our class	1	2	3	4	5	6
CASTLE and PHILIPS	—0.5, 0	0.5	1	1.5	2	2.5, 3, 3.5

Three major coat color genes

In contrast to the relative stability of the hood size in hooded rats during selection for body size, we find large changes in coat color pattern. The large line is now essentially restricted to albinos and black hooded rats; the small line has practically no albinos, has appreciable numbers of agoutis, has also self rats, and has large numbers of pseudo-Irish. Altogether the small line has much more color than the large one. MACARTHUR (1949) observed large net changes in color during his size selection experiment and suggested as possible explanations either chance (random drift), or a possible true association, the latter especially likely in view of other evidence from crosses that two of the genes concerned—brown and dilute—are associated with body size (GREEN 1931; CASTLE 1941; for objections BUTLER 1954). However, CASTLE had concluded from similar experiments that albino and agouti are not associated with size, and when our data are analyzed in terms of changes at the three relevant gene loci, the changes appear to be probably of chance origin.

Calculation of allelic frequencies: Initial values of all three allelic frequencies q_c, q_h, and q_a are known from the constitutions of the four stocks crossed. Subsequently, exact q's can be calculated only if the heterozygote is distinguishable from both homozygotes, and this is true only for the hooded locus. Table 4 shows successive values in the two lines for q_h calculated as the frequency of hooded rats plus half the frequency of pseudo-Irish. Table 4 also includes approximate values for q_h, from the square root of the frequency of hooded individuals. This approximate calculation assumes Hardy Weinberg equilibrium—i.e., random breeding. It is apparent that this is a very good approximation, although there is a slight tendency for the approximate calculation to be too low; we shall come back to this small discrepancy later. The close approach to Hardy Weinberg equilibrium in the population for one locus means that it should hold for the other two loci; this gives us a method for calculating q_c and q_a.

Genetic drift and gene fixation: Figure 3 shows the course of the three allelic frequencies during size selection. A character associated with body size would be

TABLE 4

Frequency of hooded allele h

F no.	2	3	4	5	6	7	8	9	10	11	12	
Selection no.	1	2	3	4	5	6	7	8	9	10	11	
4StS												
	.78	.74	.72	.53	.59	.64	.69	.75	.76	.72	.78	exact
	.74	.71	.73	.50	.56	.59	.66	.71	.79	.71	.78	approx.
4StL												
	.76	.80	.79	.81	.83	.93	.92	.95	1.00	1.00	1.00	exact
	.77	.77	.78	.78	.85	.93	.91	.94	1.00	1.00	1.00	approx.

Exact calculation: $q=[hh]+\frac{1}{2}[hH]$.
Approximate calculation, assuming Hardy Weinberg equilibrium: $q=\sqrt{[hh]}$.

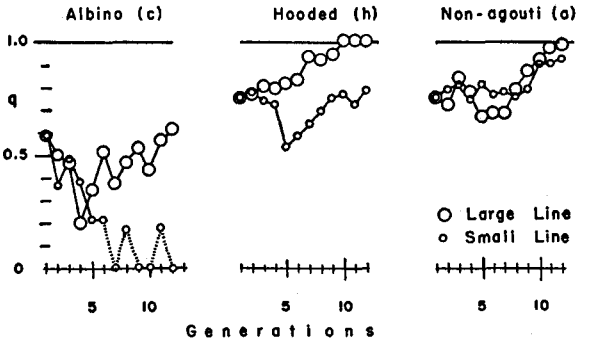

FIGURE 3.—Changes in three coat color genes during body size selection with 12 breeders per generation. For alleles c and a, q is calculated by an approximation which deteriorates rapidly in the neighborhood of $q = 0$; hence, the broken lines and repeated apparent but not real fixation of c in the small line.

expected to move in opposite directions in the two lines, and it appears that there are no continuing opposite trends. The possibilities for large cumulative genotypic changes due to chance—genetic drift—are considerable in small populations (CROW 1954; LI 1955, p. 316 *et seq.*). In the present case we are drawing a sample of 12 out of the possible infinite offspring population, and each individual is very poorly able to represent the average q of that infinite population, since q's for individuals can be only 0, 0.5 or 1. Therefore, a group of 12 is quite unlikely to give the same q as the previous group of 12. If the sampling error chances to go in the same direction for several generations there is drift, and long continued drift can lead to gene fixation. In the size selection experiment one of the six genes is probably fixed (h in 4StL, in doubt because of the possibility of albinos carrying H), and others are very close to being fixed. But if h were fixed in 4StL because of obligate association with size, one would certainly expect rather different behavior in 4StS than that shown.

As will be seen below (Table 5), the probability due to genetic drift of fixation

TABLE 5

Results of model breeding experiment replicated 120 times

Generation number*			F	$\bar{F}_t$	q	σ_q^2	$(F-\bar{F}_t)q(1-q)$	Number of homozygous lines	
								gg	*GG*
Inf. pop.	*gg*=.56; *gG*=.38; *GG*=.06		—.013†	. . .	.75	. . .	. . .	.	.
Parent	6F, 6M drawn from above, mated at random		—.002	—.039	.745	.0063	.007	0	0
1	6F, 6M offspring of above, mated to minimize inbreed		—.009	—.062	.747	.0113	.010	0	0
2	"	"	—.042	—.113	.741	.0145	.014	3	0
3	"	"	—.056	—.136	.748	.0171	.015	3	0
4	"	"	—.018	—.116	.747	.0215	.019	4	0
5	"	"	.067	—.092	.749	.0261	.030	7	0
6	"	"	.081	—.065	.749	.0296	.028	9	0
7	"	"	.085	—.106	.744	.0351	.036	13	0
8	"	"	.106	—.104	.741	.0393	.040	14	0
9	"	"	.172	—.030	.745	.0420	.038	16	0
10	"	"	.159	—.086	.743	.0453	.046	16	0
11	"	"	.183	—.093	.743	.0484	.052	18	0
12	"	"	.187	—.100	.737	.0560	.055	21	1
13	"	"	.223	—.124	.738	.0610	.071	24	1
∞			1.000			.189		90	30

* Generation number comparable to the number of generations of selection for 4St, and one less than the F number for 4St.

† Not zero, as it should be for an infinite population; the frequencies of the three genotypes were rounded off to two digits for convenience.

Symbols: Quantities calculated for a subpopulation are labelled with a subscript—q_t, $[gg]_t$, F_t. The average of these quantities for all the subpopulations is shown by a bar—$\overline{q_t}$, $\overline{[gg]_t}$, $\bar{F}_t$. Since the inbreeding coefficient is indeterminate for homozygous lines, $\bar{F}_t$ includes only unfixed lines whereas the other quantities are calculated for all the lines. Quantities calculated for the over-all large population obtained by pooling all the subpopulations are shown with plain symbols—q, $[gg]$, F. $q=\overline{q_t}$; $[gg]=\overline{[gg]_t}$; but $F \neq \bar{F}_t$.

of one gene by the 11th generation of this breeding scheme and an initial q of 0.75, is 0.154, so that in sets of four trials (corresponding to our four rat cases with initial q of 0.75), the expectation would be

none fixed	1 fixed	2 fixed	3 fixed	4 fixed
0.512	0.373	0.102	0.012	0.000

The rat results—one out of four probably fixed—are in good agreement with this expectation.

Expectation for fixation at the albino locus, with initial $q = 0.58$, would be less because the starting point is farther from either end. The near fixation in one out of two trials as has developed in the 4St breeding experiment is, therefore, not the most probable finding, although quite possible. However, in the data of the 13C × M cross (Table 2) there is a just significant deficit of albinos, suggesting that in one, at least, of the original albino stocks *c* may be linked to a gene unfavorable for early survival. If this were the case, it would also explain the fact that in the first four generations of selection albinism decreases rapidly in both lines. As the populations approach equilibrium with regard to this linkage, drift can send the albinism back up again, as in the 4StL. Or conceivably, albinism might even yet turn out to be associated with large body size, a relation obscured by an opposing linkage phenomenon; opposite trends have indeed been maintained since the fourth generation. It is of course also possible that for the other loci there is some partial association with size which is so far obscured by genetic drift operating to oppose the directed change.

Inbreeding and outbreeding effects: The fact that in Table 4 the approximate calculation for q_h is usually slightly below the exact one is a reflection of the fact that these populations usually deviate from Hardy Weinberg equilibrium by showing a slight *deficit* of homozygotes and an excess of heterozygotes. The apparent inbreeding coefficient calculated for the hooded locus is about − 0.1 (corresponding to a difference between the two calculations of Table 4 of 0.01, 0.02 or 0.025 for q of 0.1 or 0.9, 0.7 or 0.3, and 0.5 respectively). The inbreeding coefficient for this breeding plan should be positive, rising by about 0.02 per generation. The reason for this apparent discrepancy is twofold; the first element in the discrepancy is the fact that the expectation of a positive F applies to a statistically large population made up of many subpopulations each carried on by this breeding plan, and not to any subpopulation alone.

It has been shown (WAHLUND; see account in LI 1955, p. 297 *et seq.*) that small subpopulations each carried on with random breeding (subpopulation F_i averaging zero) combine into large populations in which the over-all F rises steadily with generation number; the value of F depends on the spread in q_i values of the subpopulations, which in turn depends on the effective subpopulation size. Then the average subpopulation inbreeding coefficient $\overline{F_i}$ (each one calculated in relation to the corresponding q_i) remains zero, but the over-all population inbreeding coefficient F (calculated in relation to the overall q, or $\overline{q_i}$)

rises steadily. There is, therefore, no inconsistency between an expected positive F and observed $\overline{F}_i$ of zero.

However, our F_i values average near -0.1. This deviation from Hardy Weinberg equilibrium cannot be explained by any hypothetical linkage to genes under selection. In the selected breeder population one can of course expect deviations from equilibrium with regard to genes under selection, and therefore with regard to any linked genes not yet in linkage equilibrium. But no matter how distorted the parent population, the offspring population must be in Hardy Weinberg equilibrium with regard to each gene, provided the selected rats are mated at random. It is the use of non random mating which produces the observed Hardy Weinberg disequilibrium in the offspring population, and this statement will now be verified in a model experiment involving the same breeding plan, but no selection and no linkage.

Model breeding experiment

The breeding scheme employed in the rat experiment involves two necessary consequences of small population size (CROW 1954): (1) the piling up of common ancestors and (2) large random sampling error, or (1) inbreeding and (2) genetic drift, together with a third feature, the mating of the most distantly related, which was introduced to minimize inbreeding effects. The coat color data illustrate the effects of sampling error and of mating the most distantly related, but are incapable of illustrating the effects of the piling up of common ancestors and the interplay of this feature with the others. To illustrate this interplay more completely, and to obtain readily some quantitative expectations for the behavior in our rat subpopulations, we have carried out a model breeding experiment. The model is a single gene locus with the random feature supplied by a table of random numbers and the breeding plan as in the rat experiment except that there is no selection. This is a proper model for our results on the coat color genes, assuming there was no (direct or indirect) selection for these. The model experiment was repeated 120 times. Then we can see empirically how inbreeding coefficient, gene equilibrium and genetic drift operate both in subpopulations and in the total population.

Procedure: Let us start with an allelic frequency $q_0 = 0.75$ (where g and G are the two alleles at a locus) and with an equilibrium population. Then the frequencies of the three genotypes gg, gG and GG are (in two-digit numbers) 0.56, 0.38 and 0.06. Let us assign the numbers one through 56 to genotype gg, 57 through 94 to gG, 95 through 00 to GG; draw 12 two-digit numbers from a random number table and write out the corresponding 12 genotypes. This gives us the first 12 breeders; we will call the first six females, the last six males, and mate one with seven, two with eight etc., to get a random mating. Matings $gg \times gg$, $GG \times gg$ and $GG \times GG$ have only one kind of offspring. In $gG \times gG$, assign the numbers one and two to gg offspring, three through six to gG, seven and eight to GG; draw two one-digit numbers from the table, rejecting nine and zero, and the two corresponding genotypes are two random offspring from this mating. For

matings $gg \times gG$ or $GG \times gG$, assign numbers one through four to one type of offspring, four through eight to the other, and proceed as before. In this way one can set up the six matings in each generation and draw at random the two offspring from each pair, which constitute the 12 breeders of the next generation, and so on as long as one likes. As in the rat experiment, the most distantly related individuals are mated. The allelic frequency for each generation is that of the 12 parents of that generation, the value which would also be found in a large population composed of equal numbers of offspring of those 12 parents; it differs by sampling error from the allelic frequency of the actual 12 individuals drawn from this infinite population.

Model breeding experiments to illustrate effects of close inbreeding, using a random number table in this way, have been published by BONNIER (1955). We were not aware of this until after we had done a good deal of work with our procedure.

Results: Table 5 summarizes the results in terms of inbreeding coefficients (for total population and average for the 120 subpopulations), allelic frequency and its variance, and gene fixation. It seemed advisable to check the procedure by comparing the results with theory where possible. Figure 4 compares the findings with expectation for the variance of allelic frequencies and for population inbreeding coefficient, or rather, the inverse function heterozygosis ($1\text{-}F$). The expected steady increase in variance of q (corresponding to drift in the individual q's) begins at once because the sampling error is present in each generation. The expected decrease in heterozygosis due to breeding from small population begins only after the fourth generation, when the 16 great great grandparents required for each rat must have been supplied by the 12 individuals of the first set, so that some were used twice. Up to this point there were no common ancestors; therefore, no loss of heterozygosis on this account; and, in fact, there is in the early generations an actual rise in heterozygosis (negative F) due to the avoidance of matings of close relatives. Thus we have for a few generations inbreeding and dispersion of q effects dissociated from each other in sense. CROW (1954) has discussed another case in which these two generally related effects of small population size can diverge from each other.

As in the rat experiment, the average subpopulation inbreeding coefficient $\overline{F_i}$ fluctuates around -0.1 (actual average -0.09), which presumably simply reflects the avoidance of mating of close relatives in the preceding generations and is not cumulative. Within each subpopulation there is a noncumulating relative excess of heterozygotes in relation to the q_i for that subpopulation, while in the total population there is a steadily growing deficit of heterozygotes in relation to the pooled q for the entire population.

Inbreeding coefficients, based on breeding history, apply to large populations, not to subpopulations; but they also apply to individuals with many gene loci. We can consider the 120 replicate breeding experiments as one experiment on a model in which each individual has 120 gene loci. Considering this pool of 120 genes, the individual with a history of inbreeding—a positive inbreeding co-

efficient due to common ancestors—has relatively more loci in a homozygous condition than would be expected by chance.

Unexpectedly it was found (cf. Table 5) that

$$\sigma_q^2 \cong (F-\overline{F_i})q(1-q)$$

This looks like an extension of the equation (WRIGHT, WAHLUND; see LI 1955, p. 298) for subpopulations of small size within which breeding is fully random,

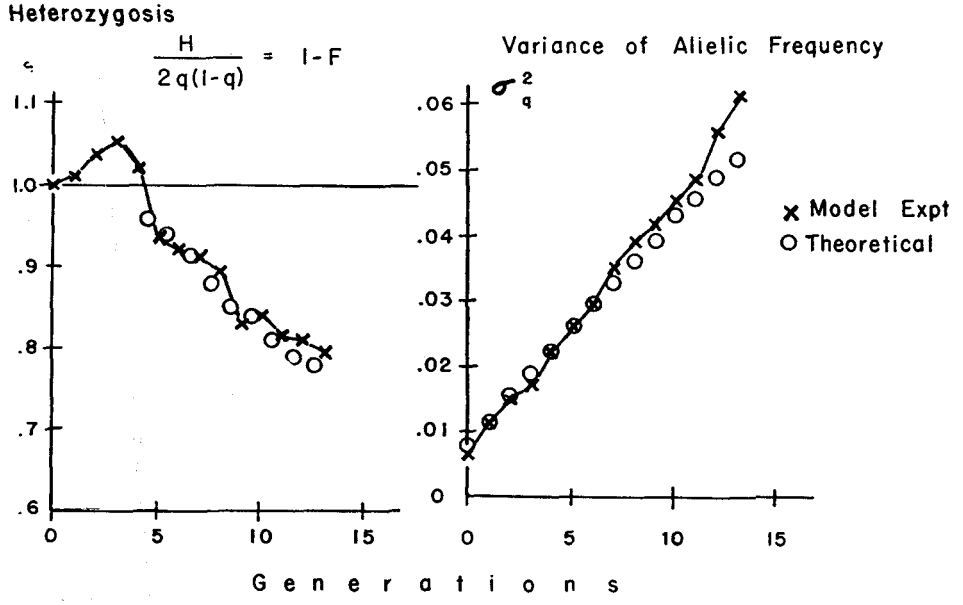

FIGURE 4.—Comparison of empirical model with theory.

Heterozygosis: theoretical values calculated as the average of the predictions for 16 and for eight breeders, when only the most distantly related are mated (WRIGHT; see LI 1955, p. 209, Table 59). The quantity plotted is $H_t/2q(1-q)$. Results are not plotted against generation number because the common ancestry which lowers heterozygosis makes a first partial appearance in the fourth generation (four of the 12 individuals of F_0 each provide two great great grandparents), and a more complete appearance in the fifth generation *et seq.* (where all 12 individuals of the (t-4)th generation appear more than once as an ancestor in each individual's ancestry); therefore, the calculated values are plotted midway between 4th and 5th, then 5th and 6th, etc. The values for the model were calculated as (1-F) from the data of Table 5.

Variance of q: Theoretical values were calculated from the equation given by CROW (1954)

$$\sigma^2_{q_t} = \frac{q(1-q)}{2N_e} + \left(1 - \frac{1}{2N_e}\right)\sigma^2_{q_{t-1}}$$

where N_e is the effective population number. For the first value, representing 12 individuals drawn from an infinite population, the second term vanishes, and N_e is 12—i.e., $2N_e$ is 24. For all subsequent values however the effective population number is twice the number of breeders, so that $2N_e$ is 48, because each breeding pair contributes two offspring to the population, rather than the offspring contributions per pair following a Poisson distribution. (CROW 1954).

so that $\overline{F_i}$ would be zero. This equation is: $\sigma_q^2 = Fq(1-q)$, and is readily derived:

$$Fq(1-q) = [gg] - q^2 = \frac{\sum_i [gg]i}{N} - q^2$$

For random breeding in the N subpopulations, $[gg]_i = q_i^2$, so $Fq(1-q) = \frac{\sum_i q_i^2}{N} - q^2$ and this is the same as variance of q_i if we ignore the difference between N and $N-1$. If one tries to carry out a similar derivation for the extended equation by writing out the values of F and of the F_i's in terms of q and the q_i's, this does not reduce to the variance of q without two quite impossible simplifications. The equation must, therefore, rest on some other derivation, possibly involving a relation between inbreeding coefficient of the offspring and the parent q or variance of q. (It should be noted that since F is indeterminate in value for a homozygous population, $\overline{F_i}$ is calculated for only those lines not yet fixed, whereas F, q and variance of q include all lines.) The relation is certainly very closely true in this experiment and applies equally well to a similar model breeding experiment with the same breeding scheme but with two-way selection (L. M. ZUCKER 1960), where in one line fixation finally reaches 100 percent with the observed relation continuing to apply up to the end.

Gene fixation amounts to 18 out of 120 cases by the eleventh generation (see Table 5). Obviously the immediate basis for this is the genetic drift, not the inbreeding (common ancestors) *per se*. Fixation has already started by the second generation when the heterozygosis in the general population is greater than one; fixation proceeds steadily during the next three generations at which point, for the first time, inbreeding—loss of heterozygosis makes its appearance.

For each gene there is random drift of the allelic frequency until it gets too close to one or zero. Although eventually, when all genes are fixed, a fourth of the loci will be fixed *GG* and three fourths *gg* (because initially $q_0 = \frac{3}{4}$), the early fixations are all necessarily *gg* because the starting point for the drift is so much closer to one than to zero. Actually drift towards lower q is faster than drift towards one; by the eleventh generation, when 18 have been fixed *gg* (i.e., have at some time reached $q = 1$), 27 loci have at some time reached 0.5, an equal distance in the other direction, but of course they are not caught at $q = 0.5$. The larger number moving to 0.5 than to one is probably a real difference. The possible rate of drift differs with the type of mating; thus $gG \times gG$ is the most favorable for drift, since in extreme cases the offspring can differ by 0.5 unit of q from the parents, while matings of heterozygote with homozygote allow a maximal change of only 0.25. Thus the rate of drift should be greatest when q is near 0.5, while asymmetry of gene fixation is greatest when q is near one end.

Evidence of obligate association between characters

The terms satellite or correlated characters have been used for characters which concentrate in a line being selected for some other character. If such a satellite character is due to a single gene, and the population size is small, the association

may be purely a chance one, due to genetic drift. If, however, one is dealing with a truly polygenic character, determined by equivalent contributions from many loci, with a plus contribution from one locus capable of neutralizing a minus contribution from another, such a character should be quite stable under the effects of genetic drift. Hood size is possibly such a character. Obligate association between two such characters should be readily obvious without confusion from drift. (If such a character starts near the extreme end of its range, however, a slow net drift away from the end is likely. This is because drift towards the near end cannot be as numerically large as drift towards the center. This effect is possibly illustrated by the slight downward trend of q in Table 5.) One can also readily envisage a type of character dependent on many loci where a favorable change at one locus cannot counterbalance an unfavorable change at another locus. Fertility is probably an example. Unfavorable drift at any one locus tends to spoil the character irrespective of favorable changes at other loci. Such characters should be practically as much subject to genetic drift in small populations as are unit characters. It is, therefore, usually necessary to get independent evidence that an apparent satellite character is obligately so, either by a repetition of the selection experiment *de novo*, or even better, by selecting for the satellite character to see whether the other character will then also be concentrated (SOKAL and HUNTER 1954; WEIR *et al.* 1953). We will report elsewhere on several characters associated with body size in two independent size selection experiments.

SUMMARY

A two-way size selection experiment is being carried out on a rat stock heterozygous for three major coat color genes and an unknown large number of genes modifying the size of the colored area in hooded rats. After ten generations of within-litter selection there is a nearly 80 percent difference in mean body size, with rate of progress in the two directions about the same. So far there is no evidence of correlation between any coat color character and body size.

The three single gene loci are subject to marked genetic drift owing to the small population size (12; effective size 24), with one locus probably fixed and others near to fixation.

An empirical model illustrating the genetic drift and closely matched to the actual rat breeding plan has been set up and replicated 120 times, allowing the calculation of inbreeding coefficient within subpopulations and for the over-all population, genetic drift as measured by the variance of the allelic frequencies, rate of gene fixation, etc., all of which agree well with relevant theory. From an initial allelic frequency of 0.75 the probability of fixation of a gene rises about 0.016 per generation, with the first 17 percent being fixed at the near end of the range of q. The results of the rat experiment are in good agreement.

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