VARIATION IN THE HOODED PATTERN OF RATS, AND A NEW ALLELE OF HOODED *

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THE earliest recorded studies of color variation in rats were made by CRAMPE in the pre-Mendelian period, 1877–1885, and were published in an important European agricultural journal.

CRAMPE's work was reviewed from a Mendelian point of view by BATESON (1903) and by BATESON's pupil DONCASTER (1906), who also carried out a considerable number of breeding experiments involving the color variations, wild gray, black, hooded, and albino.

Their findings we should now describe as showing the existence of three independent pairs of genes, (1) C, colored *vs.* c, albino, (2) A, agouti (gray) *vs.* a, non-agouti (black), and (3) H, self *vs.* h, hooded.

Contrary to the opinion of DONCASTER, it has since been shown that self (H) and Irish (h^i) are distinct dominant alleles of hooded (h). Irish has a white spot on the belly between and behind the front legs. This spot is lacking in self, which is pigmented all over. Hooded (known also as piebald) is a pattern in which the entire ventral surface back of the head is white. Dorsally pigment is limited to the head and shoulders (the " hood ") and a mid-dorsal stripe extending back to the tip of the tail. The pattern varies chiefly in a quantitative way, as seen in the schematic diagrams shown in figure 1. If the quantitative variation is in a plus direction, the hood grows longer and the back stripe wider. If variation is in a minus direction, the hood grows shorter and the back stripe narrower. The back stripe in extreme cases may become interrupted or disappear completely leaving the only pigmentation located on the dorsal side of the head.

MACCURDY and CASTLE (1907) confirmed the findings of BATESON and DONCASTER as regards the three gene pairs already named. They also undertook a study of variations of the hooded pattern, both when hooded rats are bred without outcrossing and when they are outcrossed to wild gray rats, and hooded individuals are recovered in F_2.

They found that a cross with wild rats tended to increase the pigmented areas, lengthening the hood and widening the back stripe. They estimated the variations in the hooded pattern in percentages of the dorsal surface posterior to the hood which were pigmented, observing a range from 0 percent to 75 percent, equivalent in grades of CASTLE and PHILLIPS to a range from -2 to $+3$. See figure 1 of this paper. They instituted experiments to see if selection of extreme variates, plus or minus, would be effective in changing the racial

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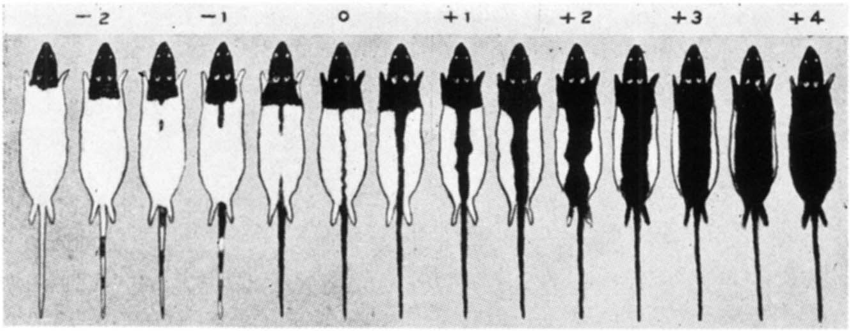

FIGURE 1.—Grading scale for hooded rats. After CASTLE and PHILLIPS (1914).

mean. For this purpose they employed a hooded stock which in three successive generations, including 183 individuals, had a range of variation from 0 to 35 percent (equivalent to grades -2 and $+1\frac{1}{2}$, figure 1). Eleven individuals from this stock having a mean grade of 11 percent produced 83 young with a mean grade of 9.6 percent. From these young were selected individuals having a mean grade of 7.8 percent, and from their young were selected parents of mean grade 1.7 percent to produce a third generation. The results of this selection experiment toward less pigmentation posterior to the hood may be summarized thus:

	Mean of parents	Mean of young	Number of young
Gen. 1	11.0	9.6	83
Gen. 2	7.8	5.6	61
Gen. 3	1.7	2.0	24

Selection clearly had been effective in decreasing the pigmentation.

In plus selections parents were chosen of mixed Irish and hooded ancestry. The respective means of parents and young in three different selections were as follows:

	Mean of parents	Mean of young	Number of young
Lot 1	12.0	15.8	111
Lot 2	23.0	22.5	70
Lot 3	40.7	36.2	64

None of these lots was continued into a second generation, but an increase in the grade of the parents is seen to be attended by a like increase in the grade of the offspring, supporting the idea that selection would be effective in changing the grade of the hooded pattern. This idea was admittedly contrary to the view of DE VRIES, then and subsequently widely held, that quantitatively varying characters can not be changed permanently by selection.

Accordingly it was decided to carry the selection experiment farther. This was done by CASTLE and PHILLIPS (1914). They devised a more convenient and practical grading scale shown in figure 1. They took as foundation stock for plus selection the darkest animals left by MACCURDY on completion of his experiments, and for minus selection the lightest animals left by MacCurdy. The parents employed in starting the plus series ranged in grades from + 1½ to + 3; in the minus series all parents were close to grade − 1½.

The mean grade and the range in variation of the offspring in 16 successive generations of the plus series, and in 17 generations of the minus series are shown in figure 2.

The two series overlapped in range in generation 2, but thereafter their means drew steadily apart and their ranges did not overlap. In generation 16, in the plus series, a population of 1690 rats had a mean grade of + 4.13 and a range of from + 3¼ to + 5¾; in the minus series a population of 1980 rats had a mean grade of − 2.63 and a range from − 1 to − 4.

The difference between the mean grades in the two series amounted to 6¾ grade units. The modal phenotype in the plus series had the back completely black and a small amount of the ventral surface also black. The modal

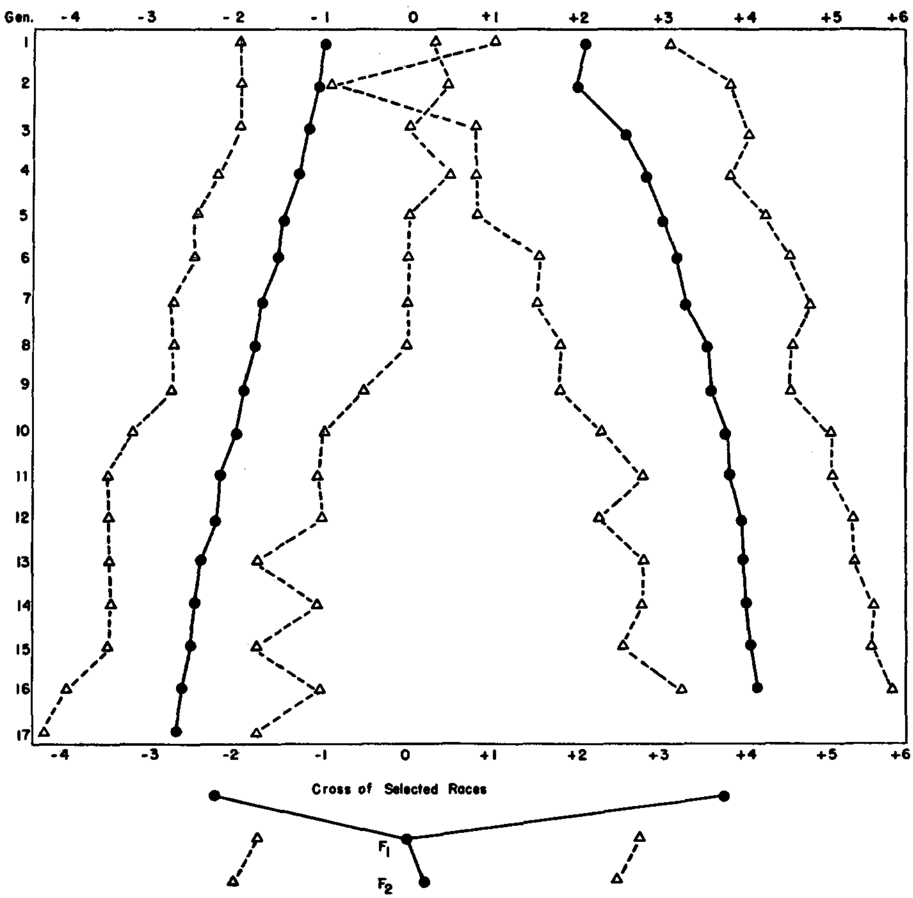

Figure 2.—Results of selecting hooded rats toward decreased and increased amounts of pigmentation through 16 successive generations. Heavy lines connect mean grades for each generation, broken lines connect grades of extreme variates in each generation, thus showing range of variation in each series.

type in the minus series had the body completely white, with pigment confined to the dorsal surface of the head.

With such evidence in hand, is it surprising that CASTLE should have thought, as late as 1916, that modification had been effected in the gene for the hooded character?

However, discoveries made subsequent to 1911 had shown that modification of the phenotype of Mendelizing characters may be effected by *other genes* associated with the essential gene for a Mendelian character. Might not this be true also for the gene for hooded in the rat?

CASTLE and PHILLIPS had crossed with each other modal representatives of plus and minus series after 5 or 6 generations of selection separately. The young obtained both in F_1 and F_2 were of intermediate grade, though somewhat more variable in F_2 than in F_1. See bottom of figure 2. They had also from time to time made crosses with wild rats to test the permanency of the modifications made by selection in the hooded pattern, from generation 2 to generation 10. The hooded young extracted from such crosses in F_2 were in the minus series changed from a grade of –2 in the hooded grandparent to a grade of about + ¼ in the grandchildren, a total loss of the effects of minus selection amounting to 2¼ grades. In the plus series the selected hooded parents were of grade about + 3½, the extracted hooded grandchildren being of practically the same grade, unchanged by a cross with wild.

The difference between the two selected races had accordingly been reduced by a cross with a wild race, principally by a change in the minus race, but the difference still persisted to the extent of about 3¼ grades. Would further crossing with wild rats reduce it further, or perhaps abolish it? To test this question CASTLE (1919) made three successive crosses with

TABLE 1

Results of crossing the plus selected race with a wild race.

	Mean grade	Standard deviation	Number of young
Control, uncrossed plus race generation 10	+3.73	0.36	776
Once extracted hooded F_2 young	+3.17	0.73	73
Twice extracted hooded F_2 young	+3.34	0.50	256
Thrice extracted hooded F_2 young	+3.04	0.64	19

TABLE 2

Results of crossing the minus selected race with a wild race.

	Mean grade	Standard deviation	Number of young
Control, uncrossed minus race, generation 16	−2.63	0.27	1,980
Once extracted hooded F_2 young	−0.38	1.25	121
Twice extracted hooded F_2 young	+1.01	0.92	49
Thrice extracted hooded F_2 young	+2.55	0.66	104

wild rats, using an uncrossed plus stock of mean grade +3.73, and an uncrossed minus stock of mean grade −2.63. The results are recorded in tables 1 and 2 and shown graphically in figure 3.

The plus selected stock by three successive outcrosses to wild, was made lighter by only about half a grade. But the minus selected stock was made darker by all of five grades. The two stocks were after the third cross with wild just about half a grade apart. Presumably additional crosses would have completely extinguished this small difference.

The logical conclusion to be drawn from this experiment was and is that the progressive changes made in the hooded pattern by 16 successive generations of selection were due wholly to multiple modifying factors which became associated with the gene for hooded, that gene itself having remained meanwhile unchanged. In accumulation of modifiers the minus series had departed more from the wild race than had the plus series.

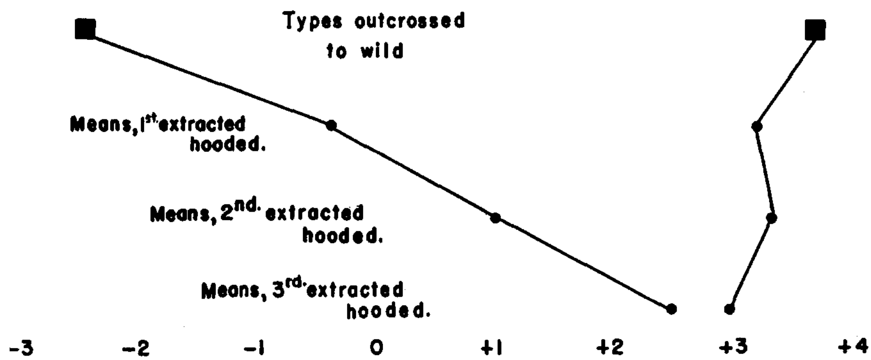

FIGURE 3.—Results of three successive crosses of selected minus and plus rats to wild (self gray) stock, showing the mean grade of the hooded young recovered after each outcross. After CASTLE (1919).

Change of phenotype in a one-gene Mendelizing character was, by the experiment just described, shown to be a matter of accumulation of modifying factors rather than a change in the gene itself. Nevertheless evidence had been obtained incidentally that major changes may take place in the gene itself, altering the phenotype abruptly and permanently. One such mutation observed by CASTLE and PHILLIPS was a mutation from the hooded pattern (h) to the Irish pattern (h^i), in which the entire coat is pigmented except for a white spot of varying size between the fore legs and extending thence backward with increase in its size. This mutation occurred in the tenth generation of the plus selected series, in two individuals, sired by the same male, whose grade was +4. Both mutant individuals were of much higher grade than their parents, *i.e.*, more nearly black all over. They were graded +5½ and +5¾ respectively, +6 being the grade assigned to an all black rat.

Extensive breeding experiments made with the mutants proved that both were heterozygous (hh^i) and bore associated modifiers of the plus race. For when the mutant male was mated with females of the plus race, half the young were mutants similar to their sire in grade, the other half being not different from ordinary individuals of the plus series in grade. But when the mutant male was mated with females of the minus race, half the young were mutants of lower average grade than their sire, and the others were much lower in grade than the average of the plus series. This result showed that modifiers introduced from the minus parent lessened the pigmented areas in the offspring, both in the mutant hybrid group (hh^i) and in the homozygous hooded group (hh).

CURTIS and DUNNING (1937) made careful studies of variation in the extent of the hooded pattern in several different inbred hooded races used in cancer investigations. They found that in each race the pattern varied about a characteristic modal grade, differences between them being assumed to be entirely due to modifiers rather than to differences in the gene for hooded pattern. But they also observed two independent instances of mutation from h to h^i, comparable with the case of mutation reported by CASTLE and PHILLIPS.

How frequent in occurrence such mutational changes in the gene for hooded are, we can only conjecture. CASTLE and PHILLIPS observed one in a plus selected population of 8,941 hooded rats. But their minus selected series contained no observed mutation either plus or minus, though it consisted of 11,704 carefully graded hooded rats. So for their entire experiment, the score is one mutation in a population of 20,645. CASTLE and PINCUS (1926) observed one minus mutation (lowering the average grade about ½ grade) in 2062 young. Curtis and Dunning observed two mutations from h to h^i in populations totaling 5,640. Combining all the data, the demonstrated occurrences of mutation in the gene for hooded are four in 28,347, or approximately one in 7,000.

NOTCH, A NEW ALLELE OF HOODED

Quite recently there has been observed a major mutation of the gene for hooded (h) in a minus direction, which it is my purpose to describe as a new allele (h^n), to be called *notch*. See figure 4. Unfortunately I cannot state under what conditions or in what stock it first occurred. My attention was called to it when I observed some rats closely resembling the most lightly pigmented variants of the long extinct selection series, of generations 13–17, reported in 1916. They made their appearance in 1947 as recessives extracted from linkage crosses. They had no connection with the prolonged selection experiment terminated in 1919. Out of curiosity I put them aside and bred them together. Their young varied very closely about a mode of -3, from which they ranged not more than half a grade in either direction. See the graph at the top of figure 7.

Since they looked so much like the final product of a long continued minus selection, I was curious to know whether they too were a mere product of accumulated minus modifying factors, or were due to a mutational change in the gene for hooded itself. To test the matter, I made a cross of a typical -3 male of the new " notch " stock with a typical $+2$ female of a pink eyed,

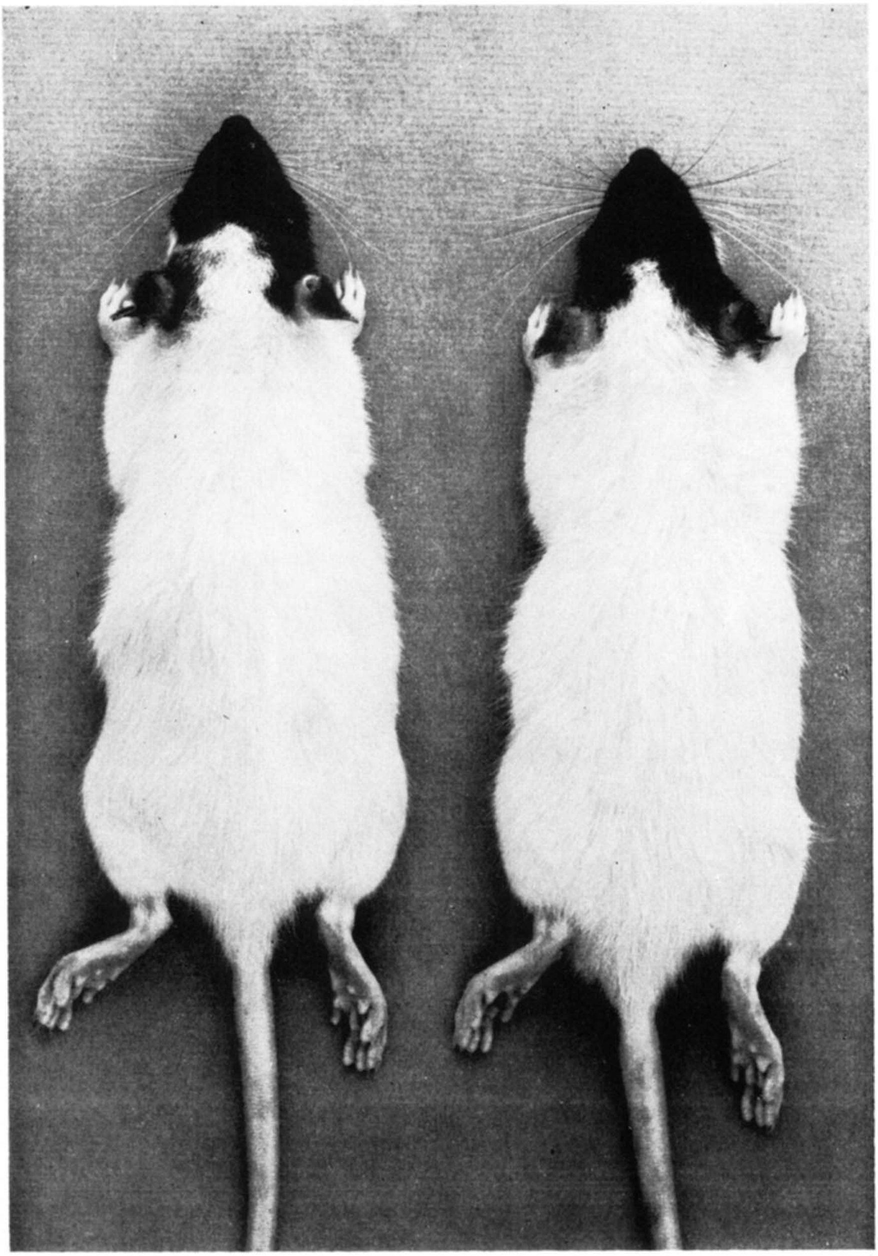

FIGURE 4.—Typical "notch" rats, grade —3.

hooded race, which had been bred brother with sister for four generations and which ranged from +1 to +2½ in grade. See figures 5 and 7.

The resulting litter of F_1 young were all much alike and were graded −1½, being considerably nearer to the notch parent than to the pink-eyed parent in grade. They were back-crossed to notch individuals of grade −3, producing

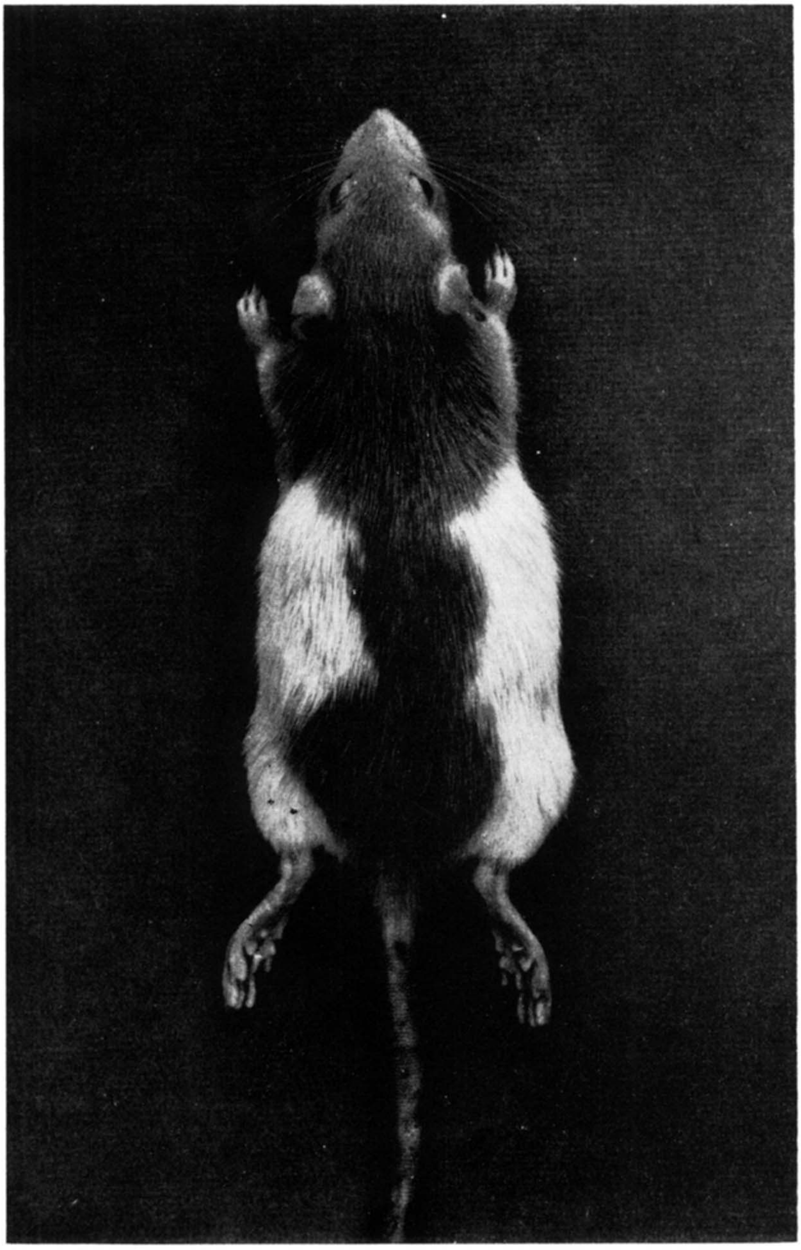

FIGURE 5.—A pink-eyed hooded rat of grade + 2.

a population of 398 individuals, the grade distribution of which is shown in figure 7.

If the notch and hooded parental phenotypes are due to different alleles of hooded, the formula of the respective parents would be h^nh^n and hh respectively, and the formula of the F_1 individuals would be h^nh, modifying factors

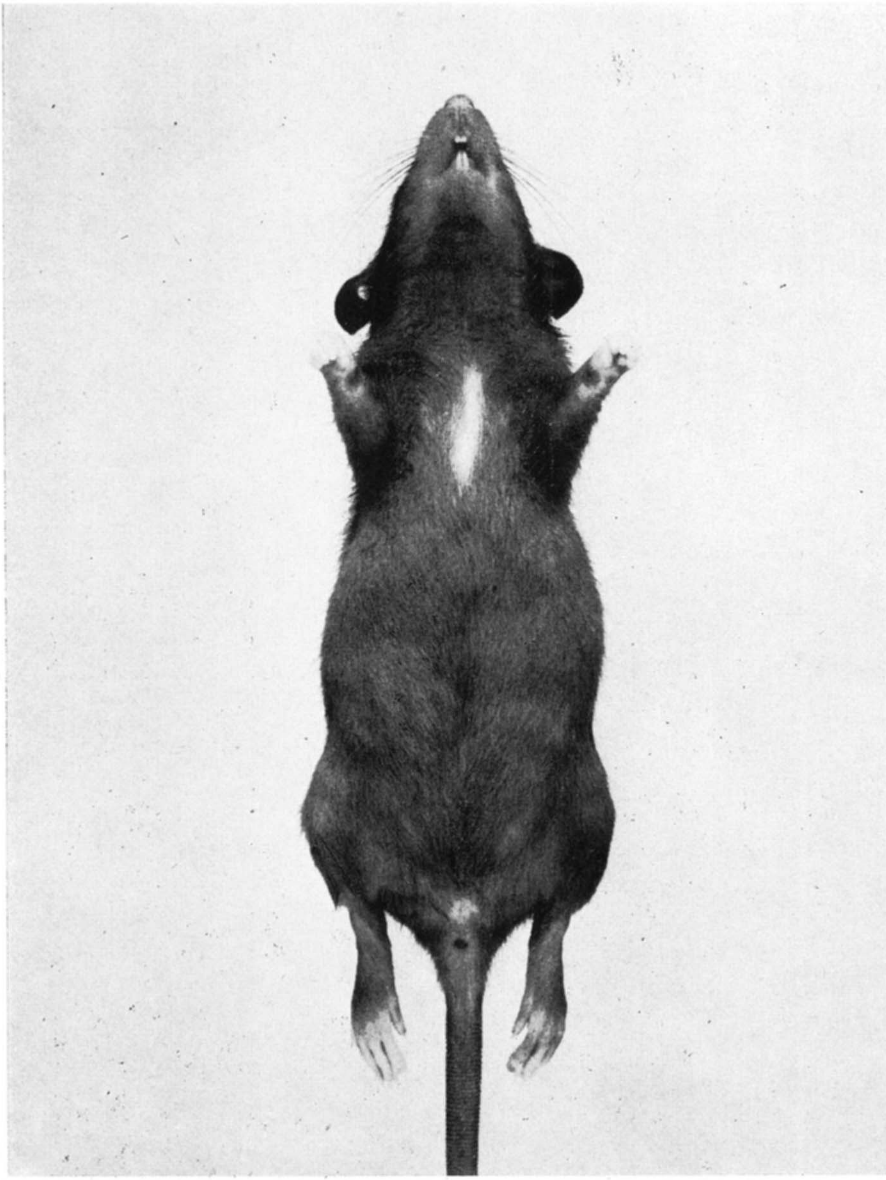

FIGURE 6.—A black race (Irish) rat of grade + 5 3/4.

being disregarded. The back-cross mating might be expressed as $h^n h \times h^n h^n$, and would result in equal numbers of $h^n h$ and $h^n h^n$ individuals. The former should resemble the F_1 parent in grade, the latter should resemble the notch parent in grade, except as a possible result of a regrouping of modifying factors.

Included in the undoubted members of the $h^n h$ (hybrid) group would be all individuals to the right of class − 2 which is the largest and most central of the grade groups. In the h^nh^n group would fall all individuals to the left of class -2. The former number 107, the latter 176.

The undistributed class of -2 individuals represents overlapping of the two genotypes. It includes 115 individuals. If we distribute them so as to make the two theoretical genotype groups equal, each group will contain 199 individuals. Thus 23 individuals of the -2 group will be assigned to the h^nh^n

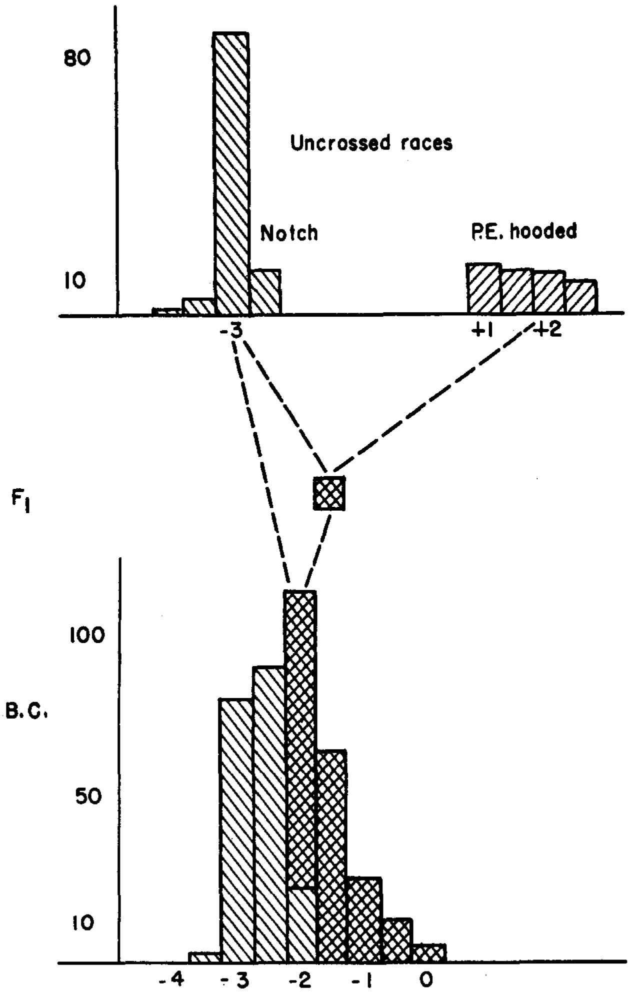

FIGURE 7.—Results of crossing notch with p.e. hooded, and of back crossing F_1 to notch.

genotype and the remaining 92 individuals to the hybrid (h^nh) genotype, as indicated in figure 7. The mean grade of the former group will then be -2.71, which is only a quarter grade less than the mean of the uncrossed notch parental group. The mean grade of the hybrid group (h^nh) will be -1.58, deviating only a trifle from the grade of the parental F_1 group (-1.50). These slight alterations in mean grade are possibly due to regrouping of modifying factors in which the two parents may have differed, or they may be without statistical significance.

If we assign the entire -2 class to the hybrid genotype, the averages will be changed only slightly, becoming -2.75 and -1.62 respectively.

We see accordingly that the results thus far obtained support the hypothesis that the difference of more than four grade units between the parental short-hooded and pink-eyed hooded groups was due to a difference in alleles of the hooded gene rather than to a difference in modifying factors.

As a further test of the hypothesis, it was proposed to attempt to equalize the distribution of modifying factors by crossing each race with a third non-hooded race. For this use a black race was employed which had been brother-sister inbred for four generations without the occurrence of any hooded individuals among the progeny. Its phenotype was about $+5\frac{3}{4}$, figure 6 (small ventral white spot) with an occasional $+6$ self black individual. Its genotype may be assumed to be h^ih^i, the Irish, small belly-spot allele of hooded.

The mating notch (grade -3) $\times$ black produced young with the belly entirely white, often with a sprinkling also of white hairs low on the sides. Their grade would be close to $+4$. The mating pink-eyed hooded $\times$ black produced young with white belly spots, grading about $+5\frac{1}{2}$.

An F_2 population was produced from each of the crosses, yielding as expected about 25 per cent of hooded individuals.

The hooded individuals obtained from the notch cross (129 in number) had a modal grade of $-2\frac{3}{4}$, average -2.65. See figure 8. They represent the recovered h^nh^n genotype with modifiers derived in part from the black race, in part from the uncrossed notch race.

The hooded young obtained from the pink-eyed hooded cross (numbering 101) represent the recovered *hh* genotype with modifiers derived in part from the black race, in part from the pink-eyed hooded race. Their modal grade is $+2$, average, $+1.41$.

This result indicates that notwithstanding a partial equalization of modifiers in the two groups of extracted hooded individuals, those groups involve strikingly different alleles of hooded, differing by about four units of our grading scale.

To make assurance doubly sure by further equalization of modifiers, a second outcross to the black race was made of hooded individuals extracted from the first outcross. See figure 8. The individuals selected for the outcross were an extracted notch, grade $-2\frac{3}{4}$, and an extracted hood of grade $+1\frac{1}{4}$.

The hooded young extracted in F_2 from this second outcross numbered 135 in the notch series. They had a modal grade of -2, average -1.98. In the series extracted from the pink-eyed hooded outcross, the young numbered 106, their modal grade was $+2$, average, $+1.95$. See figure 8.

In both series the modifiers should now theoretically be three-fourths derivatives from the black race, one-fourth derivatives from the uncrossed parent race. In both series the second outcross made the extracted hooded group *darker* than it had been after the first outcross. This indicates that modifiers in the black race were more plus in character than those of either uncrossed

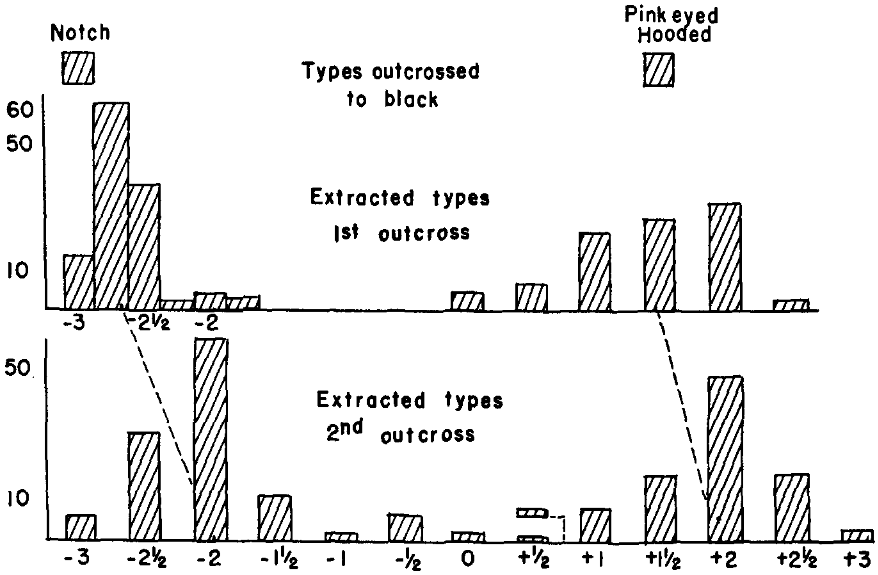

FIGURE 8.—Results of twice out-crossing notch and p.e. hooded to black Irish, and of extracting the hooded variates in F_2. The grade of the extracted hooded in each cross is shown in the figure.

race. This further equalization of modifiers left the two types, notch and hooded, still four grade units apart, very much as they had been in the uncrossed races, though the two had then probably differed somewhat in modifiers. Now they were substantially alike as to modifiers, but still quite distinct, evidently alleles, not modifications of a single genotype, such as were the extreme types secured in the selection experiments of CASTLE and PHILLIPS.

SUMMARY

The hooded pattern of rats is one of the earliest discovered Mendelian unit-characters of mammals and has been studied more extensively than that of any other mammal. It varies in the extent of its pigmented areas through the action of two different genetic agencies. One of these, always present, consists of multiple modifying factors, presumably not themselves capable of producing a coat pattern, but acting as modifiers of the action of the gene for hooded. By selection the total genetic action of these modifying factors may be changed, so as to result either in increased or in decreased areas of pigmentation. With the progress of selection in a definite direction, either plus or minus, variability in the pattern decreases somewhat but never disappears entirely. Presumably the number of modifying factors (genes other than the gene proper for hooded) is very large, or their interactions so complicated, that a condition of complete stability of genetic agencies for pattern production is unattainable.

The gene for hooded pattern (h), a simple recessive in heredity to uniformly pigmented coat of wild rats (H), is subject to rare spontaneous mutations resulting in changed amounts of pigmented areas. Such mutations have

led to the production of the allele h^i (Irish) resulting in a marked increase of pigmented areas, covering the entire body except a small belly spot. Another allele described in this paper for the first time is h^n (notch) effecting a reduction of the pigmented areas equaling or exceeding the most extensive decrease effected by long continued minus selection through the agency of modifying factors.

The incidence of mutation at the h locus is indicated to be something like one in 7,000. The alleles of hooded at present demonstrated are H self, h^i Irish, h hooded, and h^n notch, in an order of decreasing areas of pigmentation.

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