PIEBALD RATS AND THE THEORY OF GENES

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The study of heredity as an exact science dates from the rediscovery of Mendel's law in 1900. After the validity of the law had been established by abundant and conclusive evidence, the question arose, are the gametes pure. Is a character which disappears in crosses, and then reappears a generation later in 25% of the offspring, subject to contamination or modification during the process? The idea of gametic purity was at first looked on with favor. Bateson[1] although he never gave unqualified adhesion to this view, formulated it very clearly, thus. "The pure [homozygous] dominant and the pure recessive members of each generation are not merely like, but identical with the pure parents, and their descendants obtained by self-fertilization are similarly pure. If they are pure, surely the male and female elements of which they were composed must also be pure."

My own experimental studies of heredity, begun in 1902, early led me to observe characters which were unmistakably *changed* by crosses and so I have for many years advocated the view that the gametes are not pure in the sense expressed by Bateson. Moreover it was observed that characters which mendelize in crosses may, even when uncrossed, show fluctuating or graded variation in consequence of which systematic selection is able to produce very diverse races as regards a single mendelizing character, the ordinary allelomorph of which is wholly excluded from the experiment. This observation shows that characters may vary otherwise than by contamination and I was in consequence led to adopt the hypothesis that unit-characters are "inconstant" in varying degrees, but probably never perfectly constant.

This view has been repeatedly challenged, either by those who questioned the evidence cited in support of it, or by those who first substituted a different concept, 'gene,' for that of 'unit-character' and then denied that a 'gene' can vary. Dissent to the evidence for character variability has gradually disappeared as others have independently undertaken to study the visible characters of organisms as affected by crossing or systematic selection. The findings are commonly such as I have described in the case of the hooded pattern of piebald rats, which I have been studying for several years. This pattern is a simple recessive in crosses with the self pattern of wild rats, but it usually emerges from such crosses in a modified form, the amount of white in the pattern being either increased or diminished according to what stock is selected for experimental study. Even when uncrossed and bred as pure as possible, I have always found a certain amount of genetic variability to persist in a hooded race, so that selection, plus or minus is effective in changing it. The facts as I have described them are now pretty generally accepted as correct, but two different views as to their interpretation have been suggested, both of which can not be true. These two views were outlined by Castle and Phillips[2] when the first part of the experimental data was published. We considered the evidence then in hand inconclusive as between the two interpretations and planned experiments to yield, if possible, decisive evidence for one or the other. This evidence is now complete, but before I undertake to summarize it, I wish to outline the alternative interpretations to be tested. They center about the concept of the 'gene,' to which reference has already been made. The term gene or gen was introduced by Johannsen in an attempt to simplify the ideas involved in the previously current term, unit-character. By unit-character was understood (1) any visible character of an organism which behaves as an indivisible unit in Mendelian inheritance and (2) by implication, that thing in the germ-cell which produces the visible character. Johannsen[3] pointed out that these two things were logically distinct, suggested the term gene for the hypothetical germ-cell determiner, and made it clear that it is not possible to say how many germinal determiners (genes) are involved in the production of a single visible character, but only how many are present in alternative forms (as allelomorphs). He therefore advised the entire discontinuance of the use of the term unit-character and proposed to discuss the subject of heredity exclusively in terms of genes. This is the so-called genotype theory.

Before this theory could be accepted unreservedly, it has seemed desirable to know whether all observed inheritance phenomena can be expressed satisfactorily in terms of genes, which are supposed to be to heredity what atoms are to chemistry, the ultimate, indivisible units, which constitute gametes much as atoms in combination constitute compounds. It also seemed desirable to know whether a single gene is indeed invariable like an atom (or a simple chemical compound).

Much study has in recent years been given to these questions with the result that (1) to express all heredity in terms of unvarying genes, it is necessary to suppose that besides the single gene indispensable to the production of a visible character, its gene proper, there occur also other genes whose action is subsidiary. Their action may not be indispensable to the production of a character, yet they certainly modify its visible form. These are called modifying genes. In some cases they are known to have other functions also. Thus the gene proper of one character may function also as a modifying gene for another character. But in the majority of cases the only ground for hypothecating the existence of modifying genes is the fact that characters are visibly modified.

As an alternative to the theory of modifying genes, the theory has been considered that genes may themselves be variable and if so, genes purely modifying in function might be dispensed with.

These are the alternative views that we had in mind in our experiments with the hooded character of rats. It had been established that the hooded character varied but that it gave unifactorial inheritance ratios. The question to be determined was whether the single gene plainly in evidence was or was not variable. To test the point it was necessary to make the 'residual heredity' as nearly constant as possible, whether this consisted of modifying genes or not. For the purpose of determining whether the gene proper for the hooded character had or had not varied in the course of our selection experiments, we proposed to utilize two very diverse races of hooded rats produced by many generations of selection in opposite directions. They were (1) *a plus selected race* in which the pigmented areas had been increased as much as possible by selection, and (2) *a minus selected race* in which the pigmented areas had been reduced as much as possible by selection, both having been derived at the outset from a common stock. In the course of the selection they had become very different in appearance, the plus race being practically black all over as seen from above, the minus race white all over except for a black hood on head and shoulders. In classifying the young of each generation of rats an arbitrary set of grades was found useful. (See fig. 1.)

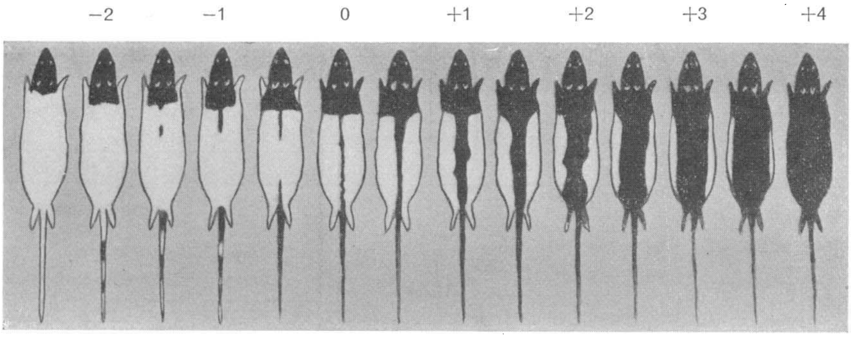

FIG. 1. A SET OF "GRADES" USED IN CLASSIFYING THE OBSERVED VARIATIONS OF THE HOODED CHARACTER OF RATS

Pictures at the extreme right and extreme left of the series show the modal conditions of the plus selected and of the minus selected races respectively.

In order to compare the genetic value of the gene proper for the hooded character in one of these races with its value in the other, it was necessary first to eliminate all modifying genes or else to make them similar in the two races. To do this the plan was adopted of making repeated crosses of each race with a third race, entirely free from the hooded character, thus combining with the residual heredity of the third race the hooded character from each of the selected races. A race of wild rats was chosen as the third race and tables 1 and 2 show how crosses with this race affected the grade of the hooded character as recovered in hooded individuals in the F_2 generation.

A first cross of the plus race (table 1) lowered the grade of the hooded character from about +3.73 to +3.17. A second cross brought a slight rise in the mean grade of the extracted hooded young to +3.34, and a small group of 19 hooded young extracted from a third cross had a mean grade of +3.04. It will be observed that the hooded character was lowered not over three-fourths of a grade by three successive crosses. This fact led me to conclude provisionally in 1916[4] that the hooded gene proper had really changed in the course of our selection experiments, since after the crosses it *remained different* from what it had been originally. This view is obviously erroneous in the light of the results obtained from the minus crosses subsequently studied.

TABLE 1

RESULTS OF CROSSING THE PLUS SELECTED RACE WITH A WILD RACE

	MEAN GRADE	STANDARD DEVIATION	NUMBER OF HOODED 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 ON HOODED 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

The crosses of the minus selected race were started some six generations later in the history of our selection experiments than were those with the plus selected race. They gave results much more striking than those of the plus crosses. (See table 2.) The mean grade of the minus race, when the crosses were started, was −2.63. The F_2 hooded young from a first cross, with the wild race were of mean grade −.38, a change of over two grades. A second cross produced hooded F_2 young chiefly *plus* in character, mean grade +1.01. A third cross produced F_2 hooded young exclusively plus in character, mean +2.55. One family in this lot of thrice extracted hooded young, consisted of 14 hooded individuals of mean grade +3.05, almost exactly identical in mean grade with the thrice extracted hooded young of the plus series (table 1).

This result indicates that three crosses with a third race had sufficed practically to eliminate whatever differences had been produced in the minus and plus races respectively by long continued selection in opposite directions. Those differences accordingly were based on residual heredity, not on changes in the hooded gene proper. For when the residual heredity was equalized, the hooded character appeared substantially the same in the two races. These findings harmonize with the idea that the residual heredity in question consists of several modifying genes independent of the hooded gene proper. Another point favoring that interpretation is the increased variability of the hooded character following the first cross, and its subsequent decrease following the second and third crosses. See the column, standard deviation, in tables 1 and 2.

These results favor the widely accepted view that the single gene is not subject to fluctuating variability, but is stable like a chemical compound of definite composition and changes only similarly, by definite steps (mutation in the sense of Morgan, not of DeVries). They offer no obstacles to the proposition of Johannsen (ably supported by East), that a gene terminology is adequate to express all known varieties of inheritance phenomena.

The full results of this investigation will be published by the Carnegie Institution of Washington.

[1] Bateson, W., *Report I to the Evolution Committee of the Royal Society*, 1902, p. 12.

[2] Castle, W. E., and Phillips, J. C., *Carnegie Inst. Washington, Pub.*, No. 195, 1914.

[3] Johannsen, W., *Elemente der exakten Erblichkeitslehre*, 1909.

[4] Castle, W. E., and Wright, S., *Carnegie Inst. Washington, Pub.* No. 241, 1916.

BUD VARIATION

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The common experience of horticulturists and plant breeders is that propagation by buds, cuttings, layering, etc. (asexual propagation) yields a comparatively uniform progeny, while propagation by seed (sexual reproduction) and especially that which involves mating of unlike parents whether of the same or of different species or races, is likely to give decided variation among progeny. On the other hand, common experience and practice recognizes the widespread occurrence of bud variations and the importance of utilizing them in developing new types of important commercial races, or in maintaining old races at a high standard, as is well illustrated by the recent studies (Shamel and others 1918) of bud variations in the citrus fruits.

In scientific and theoretical breeding, much attention has been given to the study of heredity in sexual reproduction. In many species this is the only method that can be utilized, and a knowledge of such heredity is of great practical as well as of theoretical interest. When, however, the question arises re-