STUDIES ON SPOTTING PATTERNS

I. ANALYSIS OF QUANTITATIVE VARIATIONS IN THE PIED SPOTTING OF THE HOUSE MOUSE

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INTRODUCTION

The processes which lead to the formation of patterns during ontogeny in animals are at present very imperfectly understood. Concerning the mechanisms underlying even such apparently superficial patterns as the markings of the skin and hair in mammals we have almost no knowledge; yet they provide a rich and varied material from which it should be possible to discover some of the steps by which one part or area of the body comes to differ from another. Many of these patterns are hereditary and by selection, both in nature and under domestication, have come to be characteristic of species, varieties and family lines. They have been much used as material in the study of heredity and several of the variations have been shown to depend upon relatively simple genic differences. Thus to our interest in them for the light they may throw on some of the problems of individual development is added at once a new problem, how the genes influence a specific pattern, and one of the means for studying it, that is, the ability to bring certain pattern variations under the control of experimental breeding.

Of major importance from both points of view are the variations in white spotting in the domesticated rodents which can be bred rapidly in the laboratory. The various forms of white spotting which occur in other mammals are all known in rodents, so the generalizations from this order may provide a basis for understanding similar patterns common to other groups. The basic variation moreover is an apparently simple one in all mammals, the failure of the hair follicles of certain regions of the skin to form the normal pigments (melanins) which appear in other areas.

In rats, mice, guinea-pigs and rabbits differences between the white spotted and the solid or self colored (the wild type) condition depend upon gene substitutions; and the differences between several of the different spotting patterns within some of the species have also been shown to mendelize although in none of them has a complete genetic analysis been made.

1 The investigations reported were supported in part by the Fund for Research of Columbia University. The assistance of Mr. Nathan Kaliss, Mr. G. A. Lebedeff, Miss Ilse Michaelis, Miss Jeanne Coyne, and Mr. Gottfried Spänmar, at various points in the study, is gratefully acknowledged. A preliminary report has been published in abstract form (Dunn and Charles 1933).
The genes which have been identified have been listed and the effects of
the spotting genes in the different species have been compared by CASTLE
(1930a). Detailed descriptions and bibliographies will be found in CASTLE
(1930b) and in KEELER (1930). In the rat two mutant genes with major
effects on spotting are known: "hooded" in which the head and a stripe
along the back are colored and the rest of the body is white, and "Irish"
in which a white spot appears only on the belly. These behave as alleles
of each other and of the wild type condition. Within the hooded type there
is considerable variation in the extent of the white areas and this has been
shown by CASTLE and PINCUS (1928) to be due to mutant genes (probably
many with small effects) which influence the quantity of white spotting.
In addition there is variation in the hooded pattern in long inbred and
presumably homozygous lines, due probably to random developmental
variations not under genetic control. In the guinea pig one mutant gene s
is known which differentiates white spotted from self colored forms. How-
ever, ss animals isogenic for all other loci (in so far as long-continued
inbreeding can produce the latter condition) may vary from nearly
self colored to nearly all white, due largely, according to WRIGHT
(1920) to random developmental variations not directly under the control
of genes. Other mutant genes modifying the pattern effects of s may occur
(PICTE 1925; ILJIN 1928), but their existence and behavior apart from s
are not well attested. That multiple modifying genes with small cumula-
tive effects on the total extent of white spotting exist in the guinea pig
as in the rat is shown by the differentiation between long inbred pied fami-
lies in average extent of white spotting (WRIGHT 1920 and 1922), and by
the results of crossing inbred lines (WRIGHT and CHASE 1936).
In the rabbit two mutant genes with major effects on spotting are
known. One (English) when homozygous restricts pigment to eyes and
ears; the other (Dutch) when homozygous restricts pigment to the head
and posterior part of the body and produces a blue or wall eye. At least
three other genes, one of which may be an allele of Dutch, are concerned
in producing variations in the latter type of pattern from almost self-
colored to nearly all white, and there are probably in addition genes with
minor quantitative effects (PUNNETT and PEASE, 1925). No estimates of
the degree of non-genetic variation have been made, but its importance
is probably much less in rabbits than in guinea pigs.
The spotting genes of the house mouse will be discussed in detail below.
Here it may be noted that two genes with major effects have heretofore
been identified, one being found in the pied or blotched forms, the other
in the more irregularly spotted "variegated" forms and both together in
black-eyed whites. There is ample evidence of multiple genes with quanti-
tative effects modifying the extent of white spotting and indications of one
gene which restricts pied spotting to the head and belly only. In both the
house mouse and the deer mouse, other mutant genes with minor effects
on white spotting (blaze, tail or ventral spotting only) have been reported
several times. There is a considerable degree of non-genetic variation in
pied spotting of the house mouse, since inbred families may show individual variations over a wide range.

In each of the rodents studied one or more genes with major effects on
white spotting have been found and in each there is evidence of a number
of other genes with minor effects. The preservation of these many mutant
forms is probably due to conscious selection of variant types during the
long history of domestication of these species. Only a few mutant genes,
and these all with small effects (blaze, tail or belly spots) have been obtained
directly from the wild type. The more extreme departures from the wild
type, in which most of the body is white, appear in each case to be due to accumulations of several genes with quantitative effects. In none of these forms is all of the variation in extent of white spotting due to the action of mutant genes, but the degree of determination by genetic and other agencies varies from species to species. The highest degree of genetic determination appears in the rat in which the one pattern type (hooded) breeds true within fairly narrow limits. At the other extreme is the guinea pig in which much of the variation is non-genetic. The rabbit and the mouse occupy intermediate positions in this respect. This is of some importance in judging the suitability of the different species as material for an analysis of the action of the spotting genes; for the advantages of experimental control over the material can be obtained only where spotting is largely controlled by genes. Other requisites are the existence of a number of utilizable genes with individual effects considerable enough to be measured, expressed in a variety of patterns in all parts of the body and relatively independent of environmental variations. These conditions seem to be reasonably well met by the house mouse, as discussed below, and in addition this species has the largest number of other located genes and breeds most rapidly.

Many unsolved problems stand in the way of immediate use of any of these variations for the eventual questions proposed. The first is the lack of a sufficient genetic analysis of the spotting variations which in general form a continuous series of which only the grosser steps can now be attributed to specific genes. Such an analysis is needed in order to describe in quantitative terms the effects of the several genes of a complex, their interactions with each other and with other mutant genes, particularly with respect to the relative dependence of the effects of one spotting gene upon the effects of another (modifying relationships), their relative degrees of dominance in various combinations, and the extents to which their effects cumulate; and whether the effects of some are primarily on the pattern
(localization of white and colored areas) or are more purely quantitative or general. Questions concerning the allelism and location of the genes wait upon such knowledge of their individual effects. Much of this information can be obtained by the recognized technique of experimental breeding aided by quantitative observational methods. Many problems of interpretation are raised by the data already in hand, whether, for example, the same genetic constitution is present in both white and colored areas or whether these differences are due to somatic segregation or somatic mutation; whether the potentialities for formation of pigment and pattern are equal or diverse in the various surface regions; and in either case whether these conditions arise by embryonic localization in the usual sense or through some other mechanism. Finally what inferences do the data permit whereby the genes, whether or not the same in all points of the pattern, may be connected with more primary effects than the differences in the completed pattern?

The following papers are concerned chiefly with the presentation of new data from a genetical analysis of quantitative variations in (1) pied and (2) variegated spotting of the house mouse and (3) interactions between genes influencing spotting and those influencing background color; and secondarily with (4) the determination of specific localized patterns and (5) possible interpretations of the action of the genes involved with special reference to conditions in the mouse.

THE PIED PATTERNS OF THE HOUSE MOUSE

In the house mouse is found the whole range of variation from unspotted or self-colored animals to those in which the whole coat is white, the only color appearing in the eyes. Two chief mutant genes influencing white spotting have been identified through the work of CUENOT, DURHAM, LITTLE, SO and IMAI and others. One of these (W) is found in the so-called black-eyed-white mice and in the “roan” or “variegated” forms, in which the black and white spots are generally small and scattered irregularly. These types will be discussed later (Part II).

The second major mutant gene (s) is found in the piebald (pied) or blotched forms shown in figure 1. The amount of white and the location of the white spots are extremely variable, but in pied forms generally the white and colored areas are discrete and clearly marked off.

In spite of the great variability, there is evidence of a general pattern which is common to most of these types. In pied forms the belly is usually more spotted than the back, that is, a greater proportion of its area is white. The areas of special predilection for white spotting, the so-called “points of depigmentation” of ILJIN (1928) are also common to most spotted mice. These consist of the feet and tail, which are white in nearly
all spotted forms; the navel or belly spot; the nose-tip and the blaze between the eyes, which when confluent form the “white-face”; paired shoulder spots on right and left of mid-line which are often joined to form a “collar”; paired lumbar or sacral spots often joined to form a belt. In the lighter forms more of these areas are white, and the individual white spots

Figure 1.—Variation in amount of white spotting in pied mice (genotype ss—). Successive skins differ by about five percent in amount of dorsal white.
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are greater in extent. The rump region and the areas about the eyes, ears and cheeks are nearly always colored. When pigment is confined to these areas in a regular pattern, the type is known to the fanciers as "Dutch-marked" (fig. 1, m–p). This seems to be a general background pattern which is common in some degree to most spotted mice, and it appears very commonly in other mammals. The fancier recognizes also a type known as "broken-marked" (fig. 1, i, j) in which black spots are irregularly disposed within the dorsal central white areas of the Dutch pattern. In the "even-marked" type of the fanciers these dark spots are symmetrically placed on either side of the mid-dorsal line.

Most of these forms may be obtained from mixed stocks of piebald mice. By inbreeding and selection, however, lines breeding true to greater or lesser degrees of white spotting have been produced by CUENOT, LITTLE, DURHAM and especially by fanciers, indicating that some of the quantitative variations within the pied type are hereditary.

Evidence of a similar nature was provided by DUNN (1920) who showed also that some quantitative modifiers of white spotting had similar effects on pied (s) and on variegated white spotting (W), indicating that some, at least, of the multiple genes involved were not specific modifiers of the different main spotting genes but had rather general non-specific effects. Pied strains with practically no white spotting and others with small amounts have also been isolated by selection (DUNN 1920 and DUNN and DURHAM 1925). One strain derived by selection from pied stock (ss) bred true for ten generations to a small amount of white on toes, tail or belly only. Finally, we have isolated by selection strains which are entirely white (fig. 1, t) except for the eyes (described as "all-white" later in this report) and others with intermediate amounts. There is no doubt that there are several genes with quantitative effects on pied spotting and that these may be fixed or rendered relatively homozygous by inbreeding.

Certain of the specific patterns appearing in the pied types are also under genetic control as discussed in a later paper.

Two preliminary problems are raised by the occurrence of these hereditary variations within the pied forms. (1) Do all the variations in pied spotting have a genic basis? (2) If so, what is the phenotypic relation of the subsidiary genes to the pied gene (s)? Do they produce their effects only when s is present or do they also produce effects sui generis?

To study these questions it was necessary to obtain pure strains of pied mice showing constant differences in spotting, to determine how these strains differed genetically, to describe if possible the quantitative effects of the genes involved, to isolate specific genes influencing spotting and to study the effects of these genes in animals in which the pied spotting gene was not present.
A grading scale similar to that previously described (Dunn 1920) was used in which the proportion (percent) of white on both dorsal and ventral surfaces of each animal was estimated. By reference to a standard diagram in which each surface was divided into twenty parts, it was found possible to estimate approximately the sizes of the white areas and to assign each animal a numerical grade of from 0–100 for each surface. This estimation was made for each animal at from 3–6 days of age and recorded in the form of a fraction, the numerator being the estimated proportion of white on the dorsal surface, and the denominator the proportion of white on the ventral surface. Thus an animal with no white spotting was 0/0; one entirely white 100/100, and so forth. The estimates were rechecked at about one month of age and occasionally later in life. Repeated estimates usually agreed and variations of more than 5 percent in the center of the range (50 percent white) were extremely rare. The accuracy of the estimations was also tested by planimeter measurements of the areas of the spots on prepared and tanned skins. It was found that the estimates were accurate within about ±5 percent in the center of the range and were somewhat better at the extremes. In practice, animals near the extremes (0–10 and 90–100) were graded to the nearest percent; others to the nearest 5 percent (compare footnote table 1). Since the dorsal grade is a reliable guide to the total amount of spotting, only this grade is used in the discussion and in the tabulations which follow.

In addition to this quantitative description the general pattern type and the location of specific white or black areas on the back were recorded for each animal.

THE PRODUCTION OF PURE STRAINS

At the beginning of the experiment an attempt was made to collect as many forms of pied mice as were available. Our own stocks yielded only the belted and white-faced types which had been fixed by inbreeding and selection (Dunn and Durham 1925).

These pattern types were extracted from a common parent after being crossed together in 1927. The white-face line was later crossed with self and re-extracted in its original form after four generations of selection. The new inbred lines of belted and white-face have now been carried through 20–25 generations of brother-sister matings. Each has bred true to its own pattern, with only minor variations. Within the belted type several families differing slightly in average width of the belt diverged during the early generations of inbreeding, indicating the segregation of genes with minor effects on amount of white spotting. Within each family, even after twenty generations of inbreeding, a considerable range of variation
in amount of spotting persists (table 1). The darkest family of Line 190 varies from 5 to 45 percent white dorsally, with modal grade at 15–20; the lightest family varies from 10 to 50 percent white, with mode at 35 percent. The whole range may be encountered within a single litter. Selection for greater or less amounts of spotting has failed to modify the range appreciably, no animals with more than 50 percent or less than 5 percent of white having been found. These results indicate that animals homozygous for $ss$ and for other genes affecting spotting exhibit a considerable degree of non-genetic variation. The genes affecting spotting seem merely to set limits within which other developmental factors, not under genetic control, cause fluctuations. The "white-face" type shows less variability, from about 2–12 percent white within a single family, with the mode at 5–10 percent of white.

In 1927, the various pied types maintained by English fanciers were collected; from them new inbred and selected lines were begun. The named varieties included "Dutch-marked," "broken-marked" and "even-marked"; typical exhibition specimens of each were purchased. In addition a random assortment of pied mice was obtained from the same fanciers. These included nearly the whole range of pied variations in both amount and pattern of spotting.

Attempts were first made to fix the spotting patterns, Dutch, even and broken, which are defined by location of black areas. This was unsuccessful in all three cases. These experiments are to be discussed in a later paper. Evidently the location of black areas in these patterns is not under genetic control but is the result of an occasional fortuitous combination of developmental influences acting in animals with $ss$ and other spotting genes.

In the course of these experiments it was noticed that while specific patterns involving regular locations of black areas did not become fixed under inbreeding, certain limits in the total amount of white spotting did, in certain lines, tend to become relatively fixed (table 1), and attention was then turned to producing inbred lines breeding true to a given degree of white spotting. Of first importance in this respect was the production of a stock breeding true to the maximum degree of white spotting, since this might be expected to contain the maximum number of genes tending to increase the extent of spotting. In one line (Dutch) such a selection made rapid progress, as shown in figure 2. The original range of the animals of this line was from about 65–85 percent of dorsal white. After three generations of selection several animals with no black spots at all appeared. This new form was called "all-white" to distinguish it from the genetically

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2 We are grateful to Professor F. A. E. Crew, Dr. J. N. Pickard, and Mr. Stewart Russell of the Department of Animal Genetics, University of Edinburgh for their help in obtaining material and for their kindness to one of us (L. C. D.) while a guest in their laboratory.
### Table 1

*Distribution of white-spotting in inbred strains used in the experiments.*

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>GENERATION*</th>
<th>SELF</th>
<th>PERCENT OF DORSAL WHITE†</th>
<th>n</th>
<th>m</th>
</tr>
</thead>
<tbody>
<tr>
<td>All white (L-19)</td>
<td>F&lt;sub&gt;10&lt;/sub&gt;-20</td>
<td></td>
<td>2 14 155 576</td>
<td>747</td>
<td>99.3</td>
</tr>
<tr>
<td>Broken (light)</td>
<td>(L-1a)</td>
<td>F&lt;sub&gt;8&lt;/sub&gt;-18</td>
<td>1 6 1 7 5 13 14 21 37 20 25 16 8</td>
<td>174</td>
<td>55.6</td>
</tr>
<tr>
<td>Broken (dark)</td>
<td>(L-1b)</td>
<td>F&lt;sub&gt;8&lt;/sub&gt;-18</td>
<td>3 6 9 20 24 18 24 25 23 10 3 3</td>
<td>168</td>
<td>32.5</td>
</tr>
<tr>
<td>Belted L-190</td>
<td>(light)</td>
<td>F&lt;sub&gt;12&lt;/sub&gt;-22</td>
<td>1 7 22 49 101 86 74 47 10</td>
<td>397</td>
<td>33.7</td>
</tr>
<tr>
<td>(medium)</td>
<td>F&lt;sub&gt;12&lt;/sub&gt;-22</td>
<td>9 33 58 49 66 35 22 7 2</td>
<td>281</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td>(dark)</td>
<td>F&lt;sub&gt;12&lt;/sub&gt;-22</td>
<td>10 65 76 54 58 26 19 3 1</td>
<td>312</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td>Belted L-228</td>
<td>F&lt;sub&gt;4&lt;/sub&gt;-7</td>
<td>18 33 47 32 8 7 3</td>
<td>150</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>Belted L-66</td>
<td>F&lt;sub&gt;4&lt;/sub&gt;-18</td>
<td>59 46 29 16 13 5 1 1</td>
<td>170</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>White-face</td>
<td>L-174</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;-12</td>
<td>20 37 2</td>
<td>59</td>
<td>6.5</td>
</tr>
<tr>
<td>L-118</td>
<td>F&lt;sub&gt;2&lt;/sub&gt;-14</td>
<td>7 89 35 3</td>
<td>134</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Dilute brown self</td>
<td>F&lt;sub&gt;8&lt;/sub&gt;-26</td>
<td>all</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black &amp; tan self</td>
<td>F&lt;sub&gt;19&lt;/sub&gt;-20</td>
<td>all</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The inbred generations included are, in general, those contemporary with the crossing experiments.

† In this and subsequent tables "o/" includes animals with white spotting on belly only; class 5 includes those with 1-5 percent; class 10, those with 6-12 percent; class 90 from 90-94 percent; and class 95 from 95-99 percent. In all other classes the midpoint of the class is used as the grade.
different "black-eyed-white" type. After the third generation only such all-white males were used for breeding and after the fifth only all-white of both sexes. From this time the strain bred true to an average of about 99 percent white. The majority were all-white, but a few with small black spots on rump, ear, or cheek have continued to appear. The eyes in all cases retain full dark pigmentation. The disappearance of dorsal black spots in response to selection appeared to follow a regular course described below (Part IV).

Two all-white families derived from a single mating in the fifth generation have been bred through the 23rd generation by brother-sister matings and have remained constant. The distribution of the spotting phenotypes in these lines is shown in table 1. The variation remaining in the stock is probably non-genetic since animals with a small black spot give about the same proportion of all-white young as those with no black at all. Reverse selections toward increased amount of pigment have been ineffective. Inbreeding and selection experiments were carried on with other pattern types which appeared in the early generations of the all-white selection experiment. Thus a type with a black spot on the rump only was inbred with selection for this type for ten generations. It fluctuated in grade of spotting from 70–100 percent white with mode at 85 percent but continually produced all-white, Dutch and half-Dutch spotted. Either the type selected was heterozygous or else the black rump condition was non-genetic. No pure line of this type was obtained.
Pure lines of other spotted and self-colored animals were produced by inbreeding. The spotting ranges of those used in subsequent experiments are shown in Table 1. This table illustrates two facts of importance in interpreting the experiments which follow: (1) there is a clear differentiation in range and average amounts of spotting among the inbred strains shown; (2) there is much variation, which persists from generation to generation within each strain and this is especially great wherever the average is near the middle of the range. Light-spotted lines, such as all-white, and dark spotted lines, such as 66 and 118, show less variability. The factors responsible for this variability, whether genetic or non-genetic, appear not to have equal measurable effects on all parts of the range, but to have lesser values in terms of area at the extremes than at the center of the range (cf. Wright 1920 and discussion p. 40). It is thus probable that the unit of measurement (percent of white) does not have the same significance throughout the spotting range.

When the all-white type had become fixed (7th–10th generation) it was crossed with dark-spotted and medium-spotted lines and with several self-colored strains.

### Table 2

**Inheritance of quantititative differences in pied spotting.**

<table>
<thead>
<tr>
<th>Percent of Dorsal White</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10 20 30 40 50 60 70 80 90 100</td>
</tr>
<tr>
<td><strong>P&lt;sub&gt;1&lt;/sub&gt; Line 118 (F&lt;sub&gt;1&lt;/sub&gt;)</strong></td>
</tr>
<tr>
<td>7 8 9 3 5 1 2 1</td>
</tr>
<tr>
<td><strong>P&lt;sub&gt;1&lt;/sub&gt; Line 9 all white (F&lt;sub&gt;1&lt;/sub&gt;)</strong></td>
</tr>
<tr>
<td>1 9 29 134</td>
</tr>
<tr>
<td><strong>F&lt;sub&gt;1&lt;/sub&gt; Line 9×Line 118</strong></td>
</tr>
<tr>
<td>1 1 4 3 10 7 9 9 7 1 2</td>
</tr>
<tr>
<td><strong>F&lt;sub&gt;1&lt;/sub&gt; Line 19×Line 118</strong></td>
</tr>
<tr>
<td>1 2 6 15 10 12 14 19 27 29 28 19 34 21 14 2 3 2</td>
</tr>
<tr>
<td><strong>BC F&lt;sub&gt;1&lt;/sub&gt;×Line 19</strong></td>
</tr>
<tr>
<td>5 16 33 24 15 10 9 12</td>
</tr>
<tr>
<td><strong>P&lt;sub&gt;1&lt;/sub&gt; Line 190a Belt (F&lt;sub&gt;1&lt;/sub&gt;)</strong></td>
</tr>
<tr>
<td>3 10 22 23 31 27 28 15 5</td>
</tr>
<tr>
<td><strong>P&lt;sub&gt;1&lt;/sub&gt; Line 19 all white (F&lt;sub&gt;1&lt;/sub&gt;)</strong></td>
</tr>
<tr>
<td>1 9 29 134</td>
</tr>
<tr>
<td><strong>F&lt;sub&gt;1&lt;/sub&gt; Line 190a×Line 19</strong></td>
</tr>
<tr>
<td>1 5 4 1</td>
</tr>
<tr>
<td><strong>F&lt;sub&gt;1&lt;/sub&gt; Line 190a×Line 19</strong></td>
</tr>
<tr>
<td>2 5 1 7 16 29 45 10 3 2 3 2</td>
</tr>
</tbody>
</table>

The crosses with white-face, belted and broken pied lines were to test the inheritance of differences in amount of spotting; and specifically to discover whether the genic differences involved were substitutions in several loci or multiple allelic substitutions at one locus.

The results of these experiments are shown in Table 2. In the upper part of the table are shown the frequency distributions of mice of a dark pied stock, of all-white stock and of hybrid generations from this cross. The dark line (118) had been derived from the white-face pied line described by Dunn and Durham (1925). The figures given are for the seven generations...
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of brother-sister matings immediately preceding the cross. The \( \text{F}_1 \) from this cross consisted of broken, Dutch, and belted mice, all with some white on the face. In amount of spotting the average was intermediate between the two parent types, but there was a great deal of variation, from 20 to nearly 80 percent of white. Other considerations indicate that much of this variation is not hereditary. \( \text{F}_1 \) animals from the center of the distribution were bred together to produce an \( \text{F}_2 \). The range and variability in amount of white spotting was greater in this generation than in the \( \text{F}_1 \), but the extreme parental types were not recovered. A few animals as dark as the white-face form were obtained but none had the white-face form of spotting; they were belted or had both belt and blaze spotting. A few very light animals were also obtained but no all-whites. A backcross of \( \text{F}_1 \) by all-white produced broken, Dutch and all-white types varying from 65 to 100 percent white.

The interpretation appears to be clear. White-face and all-white both contain one spotting gene (s) in common and differ by several spotting genes which act cumulatively and recombine in crosses. It appears certain that white-face and all-white do not differ only by alleles of one spotting locus since if they did, the parental and hybrid forms should have appeared in \( \text{F}_2 \) in a tri-modal distribution. Because of a considerable degree of non-genetic variation, especially in the center of the range, it is impossible to determine exactly the number of additional spotting genes concerned. The best estimate is from the backcross generation in which about 16 out of the 124 animals reproduced the range of the all-white type.\(^3\) This gives a ratio of about \( \frac{1}{3} \) all-white, corresponding to a difference of at least three major genes between the lines crossed. Comparison of the variance of \( \text{F}_2 \) and \( \text{F}_1 \) would lead to an estimate of about ten gene differences.

The essential point at present, however, is not the number of factors involved, which the coarseness of the grading scale and the non-hereditary variation make it impossible to determine, but the fact of recombination of a limited number of spotting genes, as shown by the frequent recovery of the all-white parent type.

In the lower part of table 2 are shown the results of crossing all-white with an inbred belted line (190a). The figures given for line 190a (light) are for the eight inbred generations (F16-17) preceding the cross. The small \( \text{F}_1 \) consisted of broken spotted mice intermediate in amount of spotting between the parent types. The \( \text{F}_2 \) was also intermediate but more variable and again the extreme parental types were not recovered. Seven out of 125 or 5.6 percent of the \( \text{F}_2 \) animals fell within the range of the all-white

\(^3\) Assuming that all-white normally consists of about 78 percent all-white, 17 percent which are 95-99 percent white and 5 percent below 95 percent white (the distribution of the \( \text{F}_{1-8} \) all-white parents).
type as compared with seven out of 258 or 2.7 percent of the F2's from the white-face cross. There may be fewer gene differences between belted and all-white than between white-face and all-white. There was no evidence of the clustering of frequencies about the grades of the parent types which should occur if belt and all-white represent allelic conditions of one spotting locus. It is concluded that belt and all-white contain one spotting gene in common (ss) and differ by several independent genes with quantitative effects on spotting.

All-white crossed with a darker belted line (L228) produced results similar to those above, but they add nothing of analytical interest. From the latter cross the dark belt was re-extracted by selection and inbreeding and after five generations of selection bred true to belt within the range of variation of the original belted line (L66 table 1). From the last two crosses to belt, all-white lines were also re-extracted by selections from F3. These have bred true to the same range of variation as the all-white parent stock.

The results indicated that these differences among the all-white, belt and white-face lines in amount of pied spotting are due to substitutions at several loci other than that of the known gene s. They do not support the interpretation of Cuénot who first recognized and studied variations in spotting and assumed them to be due to different conditions of the pied gene itself, either alleles or varying from it in "strength." His latest statement (1928, p. 207) is that "la panachure est conditionée par des facteurs de l'extension, multiples et allelomorphiques, dont l'effet chez l'individu s'additionne algébriquement." The context shows however that he assumes more than two such "alleles" present in the same individual, and his formulation can only be applied if one assumes that his spotting genes s1, s2, s3, and so forth are independent multiple genes, in which case their relationships to the pied gene are not clear. The results do agree with the interpretation of Little (1917b) who ascribed the variations in spotting to multiple modifying genes, and with the eventual interpretation of Castle's experiments on the hooded spotting of rats (Castle and Pincus, 1928). Whether the genes causing these variations are "modifiers" in the usual sense will be discussed in the next section.

ANALYSIS OF THE "MODIFYING" GENES

From the above experiments it was reasonable to assume that we had collected in the all-white stock several such "modifiers" which, when combined with ss, caused the whole coat to be white. We now proceeded to an analysis of these "modifiers." The experiments were designed to separate the modifiers from s and to accumulate them in pure stocks from which s had been excluded and to answer the question, "Do the pied 'modifiers' produce spotting in the absence of s?" It was thus necessary to cross all-
white by a given self-colored race; to backcross the F₁ animals (which should be heterozygous for s and probably for all "modifiers" as well) to all-white; to select the Ss offspring with the most white spotting and to backcross these to all-white; and to repeat this method of backcrossing to all-white until no further increase in the amount of spotting of the Ss animals was observable. The maximum number of effective "modifiers" should then have been obtained in homozygous condition in the Ss animals and these, when bred together, should give SS animals exhibiting the effect of the "modifiers" only. Ss animals from the earlier backcross generations should be heterozygous for some of the "modifiers" and homozygous for others, and by inbreeding these we hoped to obtain SS stocks with different "modifiers."

Three experiments of this type were carried out. The self races used were a long inbred stock of dilute browns (Little); a stock of blacks obtained from an English fancier and inbred in our laboratory for seven generations; and a stock of black-and-tans inbred for ten generations. None of these races had shown any white spotting. Only such tested races can be regarded as self-colored, since most stocks of mice show traces of spotting on toes or tail tips and thus may contain minor spotting genes.

The results of reciprocal crosses to the several self types and of backcrossing the hybrids to all-white for five to six generations were essentially similar and are combined in table 3. The first generation was self or nearly so. Actually 15 animals had no white whatever; 31 had a small white spot on the belly, covering not over 15 percent of its surface; two had somewhat larger belly spots, while three had, in addition, a small white blaze between the eyes. The self alleles of s and the "modifiers" are apparently nearly completely dominant when all are heterozygous. The backcross of F₁ to all-white produced two groups of progeny in about equal numbers. One group was self or dark spotted, varying from 0 to 40 percent dorsal white and included only three animals with no white spotting at all (self). The other group had more white spotting, varying from 55 percent to all-white. The two groups were clearly separated by two zero classes. The dark group proved to be Ss and the light ss. It was thus immediately evident that the "modifiers" from all-white when homozygous produce a considerable amount of white spotting in the presence of one self or wild type allele of s, and thus are not specific modifiers in the sense of Bridges (1919). The chief difference between the Ss animals of the first backcross and the Ss animals of the F₁ is that the former contain some "modifiers" in homozygous condition while in the latter all are heterozygous and produce little or no spotting. Ss animals of the BC₁ generation, from above the mean of the distribution, were again backcrossed to all-white. The BC₂ so produced again consisted of two groups of spotted mice, but the extent of
Table 3

Results of crossing “all-white” by self lines and of backcrossing $S_1$ animals repeatedly to “all-white.”

<table>
<thead>
<tr>
<th>PARENTS (LIGHT SELECTION)</th>
<th>PERCENT OF DORSAL WHITE</th>
<th>$S_1$</th>
<th>$s_1$</th>
<th>$m$</th>
<th>$n$</th>
<th>$s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁ “all” × self &amp; self × “all”</td>
<td>0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 n</td>
<td>51 0+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₁ F₁(S₁) × all</td>
<td>30 61 33 27 30 21 2 4 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₂ BC₁(S₁) × all</td>
<td>2 7 16 20 36 38 36 19 4</td>
<td>1 2 7 19 33 22 31 28 29 18</td>
<td>209 10.61</td>
<td>190</td>
<td>32.84</td>
<td>169</td>
</tr>
<tr>
<td>BC₃ BC₂(S₂) × all</td>
<td>1 7 15 25 30 28 23 10 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₄ BC₃(S₂) × all</td>
<td>2 5 10 14 15 10 — 3 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₅ BC₄(S₂) × all</td>
<td>1 5 2 6 6 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DARK SELECTION)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁ Self × all</td>
<td>2 1 2</td>
<td></td>
<td></td>
<td></td>
<td>23 0+</td>
<td></td>
</tr>
<tr>
<td>BC₁</td>
<td>4 22 11 4 8 5 2 3</td>
<td>2 4 8 4 10 11 13 7 5 8</td>
<td>10.93</td>
<td></td>
<td>59</td>
<td>86.53</td>
</tr>
<tr>
<td>BC₂</td>
<td>2 11 8 8 3 4 2 3 1 2</td>
<td></td>
<td>1 5 5 12 12 7 49</td>
<td>15.75</td>
<td>42</td>
<td>90.95</td>
</tr>
<tr>
<td>BC₃</td>
<td>1 1 3 1 9 9 11 8 2</td>
<td></td>
<td>6 15 28 45 25.27</td>
<td>49</td>
<td>97.24</td>
<td></td>
</tr>
<tr>
<td>BC₄</td>
<td>1 2 2 2 1</td>
<td></td>
<td>1 3 5</td>
<td>30.00</td>
<td>9</td>
<td>97.22</td>
</tr>
</tbody>
</table>
white spotting in each group was much increased over BC₁. The darker (Ss) group contained no self-colored animals at all, and some were nearly half white. Of the lighter (ss) group nearly half were 100 percent white and only one was as dark as 70 percent white. The lighter Ss animals were again backcrossed to all-white and in BC₃ the extent of white spotting again increased although by a lesser amount than between BC₁ and BC₂. In BC₄ there was a slight increase in amount of spotting but no increase in BC₅. After five backcrosses the fertility and fecundity of the backcross animals had fallen so low, probably as a result of introduction of most of the genes of the poorly fertile all-white line, that it was impossible to carry the experiment further. The results show, however, that the chief object had been attained by the fourth backcross, that is, the backcross animals showed no further increase in the amount of white spotting, indicating that all or most of the effective genes had been introduced from the all-white race.

As a further test, the lightest Ss animals (45–60 percent white) from BC₃, BC₄, and BC₅ were inbred and their lightest Ss progeny again selected. Two generations of such selection produced no increase in the average grade of spotting over that obtained in BC₄. Similarly nine of the lightest animals (45–60) from these generations were again backcrossed to all-white but no increase in amount of white spotting over that of BC₄ was obtained.

The interpretation of these results is apparent when one compares the results obtained with those expected from the random assortment of genes with quantitative effects on white spotting in the system of matings used. To facilitate this comparison the results of the backcrossing experiment are shown graphically in figure 3, in which the average percents of white spotting for the individuals of the F₁-BC₅ generations are plotted. The Ss and ss groups are shown separately. The average amount of spotting in the Ss animals rises from zero in F₁ and reaches a maximum of just under 40 percent in BC₄. It appears to approach 40 as an asymptote. There is a similar rise in the grades of extreme individuals in each generation, but very little alteration of the range. The persistence of a constant degree of variation comes about presumably through a fortuitous balance between the diminishing effects of segregation and the maintenance of non-genetic variation which is greater when spotting phenotypes are nearer the middle of the range. The averages of the ss group rise similarly from BC₁ (when this class first appears through segregation) and approach 100 percent white, the all-white type.

These curves show a general resemblance to those describing the approach toward homozygosis, after an outcross, brought about by repeated backcrossing without selection, to a homozygous parent type. Under such
a system the proportion of individuals heterozygous in any one particular locus is halved in each generation and thus the proportion of individuals homozygous in all of the loci which differentiate the strains approaches 100 as an asymptote, at a rate which depends on the number of such loci. In the present case over 80 percent of the individuals are expected to be homozygous after the fifth backcross if the number of loci is seven or less, or over 90 percent if the number of loci is three or less.

The general similarity of the approach toward limiting values of white spotting and of the theoretical results expected from random backcrossing is sufficient to indicate the general mechanism responsible for the results. The theoretical expectancies, however, are based on random matings

![Graph showing changes in average percent of white spotting in continued backcrossing of Ss animals.](image)
while in our experiment the lighter individuals in each litter were selected for backcrossing. There is evidence that the selection practised has not had any considerable effect. In a special experiment the darkest (least spotted) animals were selected for backcrossing. Only animals less than 7 percent white were bred in any generation. The results are shown in table 3 and the averages are plotted in figure 3. Stringent selection against increase of white spotting did not alter materially the tendency shown in the light selection experiment for the percent of white spotting to increase in each backcross generation up to the fourth, although the spotting averages in the dark series are somewhat below those of the light series. This indicates that the increase in amount of spotting in these experiments is due chiefly to the increasing homozygosis in the backcross animals of the genes of the all-white race.

The relative ineffectiveness of selection is probably due to the presence of a good deal of non-genetic variation in white spotting and to the presence of several modifying genes. The $Ss$ animals of the BC$_2$ generation for example vary from 20 to 60 percent white, although over 90 percent of them should be homozygous for the mutant genes of the all-white type (exclusive of the $Ss$ pair) if there are as few as three and over 80 percent if there are as many as seven. The persistence of the wide range of variation throughout the five generations of backcrossing to a homozygous type (figure 3) finds here its explanation. The lightest animals of the last two backcross generations produced progeny with this same wide range of variation. Finally the absence of any sensible parent-offspring correlations between generations after BC$_1$ shows that selection cannot have played a very important part. The results to be expected from random matings would therefore resemble fairly closely the actual results shown in table 3 and figure 3, although the averages in each generation might be slightly below those of the light selection experiment and above those of the dark selection experiment.

Although the main object of these experiments was to obtain a given end result rather than to study the process by which it was obtained, it is worth while to draw attention to several inferences from the results. The distribution of the $Ss$ progeny in the first backcross has a single mode at 5 percent white. This makes it probable that no one gene in addition to $s$ has a major effect on spotting, since such a gene, segregating in this generation, should produce bimodality, of which there is no clear indication. This conclusion is confirmed by breeding tests of the darkest BC$_1$ animals which should carry the wild type allele of such a gene and again segregate for it. This is shown not to occur (compare BC$_2$ generation in dark selection experiments). Distributions describing the $Ss$ progeny in subsequent backcrosses are also monomodal and indicate variations of
the continuous type, probably due to minor effects of several genes combined with non-genetic variation. The distributions of the \( ss \) group gradually approach that characteristic of the all-white type and become identical with it in the BC\(_5\). From the proportion of the all-white type in the BC\(_1\) distribution it is possible to estimate that at least three genes with major effects in addition to \( s \) are required to produce the all-white phenotype. About 13 percent of the \( ss \) group in BC\(_1\) reproduce the phenotypic distribution of the isogenic animals in all-white strains. This corresponds closely to the 12.5 percent of individuals expected to have no wild type alleles in BC\(_1\) if three major genes are segregating independently of \( s \). The proportion of the all-white type in the \( ss \) group in BC\(_2\) is 55 percent, which is somewhat above the expectation for three genes, but even a slight effect of selection would influence these proportions to such an extent as to make comparisons unsafe beyond BC\(_1\) (when no selection could be practiced). In addition, other spotting genes with very small effects are probably present, but the number cannot be well estimated from the present data.

Considering now the end result of the backcrosses to the all-white type, it is apparent, from the failure of further backcrossing or of selection to increase the amount of spotting in the \( Ss \) type, that nearly all of the effective spotting "modifiers" from all-white had been collected in this type. The pied gene \( s \) was then eliminated by crossing together the \( Ss \) animals of the BC\(_2\)–BC\(_5\) generation and by inbreeding those of genotype \( SS \). The cross should produce a population consisting of \( \frac{1}{4} SS : \frac{1}{2} Ss : \frac{1}{4} ss \). The actual results are shown in table 4, line 4. Only two clearly distinguishable types of offspring were obtained: all-white \( ss \), and dark spotted animals similar in appearance to the \( Ss \) parent type. No solid colored young were found. It was thus apparent that the \( SS \) group was contained within the dark spotted phenotype, and since they were white spotted the "modifiers" were proved to produce spotting in the absence of the pied gene \( s \). They are thus not specific modifiers but spotting genes in their own right.

In order to distinguish \( SS \) and \( Ss \) animals, a large sample of the progeny of the \( Ss \times Ss \) cross (referred to in our records as BCF\(_1\) generation) was tested by individual matings with all-white (\( ss \)) animals. Those which gave only one type of spotted offspring and no all-whites were classified as \( SS \); those which gave two types (dark spotted \( Ss \) and all-whites \( ss \)) were classified as \( Ss \). One all-white offspring was sufficient to indicate the parent's genotype as \( Ss \). The production of ten or more \( Ss \) offspring only was assumed to indicate the parent's genotype as \( SS \), since such a result from an \( Ss \) parent has a probability of only \( 1/1024 \). This proved to be a safe working criterion.

The results of these tests are shown in table 4. \( Ss \) animals from BC\(_2\),
### Table 4
Results of interse matings of BC animals from table 3 and of progeny tests of BCF1 animals so produced; and of inbreeding Type “K,” that is, SS animals with other spotting genes from “all-white.”

<table>
<thead>
<tr>
<th>Line</th>
<th>Generation</th>
<th>Parents</th>
<th>Percent of Dorsal White</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/12 3 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100</td>
<td>SS</td>
</tr>
<tr>
<td>1</td>
<td>2BCF1</td>
<td>2BCS×2BCSs</td>
<td>2382125</td>
<td>126299</td>
</tr>
<tr>
<td>2</td>
<td>3BCF1</td>
<td>3BCS×3BCSs</td>
<td>27692220251483331</td>
<td>2192612338</td>
</tr>
<tr>
<td>3</td>
<td>5BCF1</td>
<td>5BCS×5BCSs</td>
<td>135344442</td>
<td>36269</td>
</tr>
<tr>
<td>4</td>
<td>Total BCF1</td>
<td>29101835253020175331</td>
<td>2143817856</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BCF1’s; SS by test</td>
<td>44531</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BCF1’s; Ss by test</td>
<td>261469781</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Test BCF1; SS×all</td>
<td>2181115335629722</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Test BCF1; Ss×all</td>
<td>5111818422818852</td>
<td>143295155132</td>
<td></td>
</tr>
</tbody>
</table>

**Type K**

<table>
<thead>
<tr>
<th>Line</th>
<th>Generation</th>
<th>Parents</th>
<th>Percent of Dorsal White</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>F1</td>
<td>see line 5</td>
<td>12171915126</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F2</td>
<td>F2SS×F2SS</td>
<td>121715126</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F2</td>
<td>F2SS×F2SS</td>
<td>121715126</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F4</td>
<td>F4SS×F4SS</td>
<td>815138542</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F4</td>
<td>F4SS×F4SS</td>
<td>1016911186</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Total F1×4</td>
<td>3255534440214</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
BC₃ and BC₅ gave similar results when bred together. They produced progenies of which about ⅔ were of the all-white type while about ⅓ were dark spotted (line 4). Of the dark spotted type 72 were tested by matings to all-white. Eighteen proved to be SS and 54 proved to be Ss. The former type (table 4, line 5) included animals with from 10 to 35 percent of white; the latter type (line 6) varied from 15 to 60 percent white. Although the average grade of SS (18.9) is clearly darker than that of Ss (32.3) the distributions overlap. The average grades differ by about 13 percent which might be taken as an estimate of the effect of a second S gene when it is substituted for s in this Ss type. The samples of SS and Ss animals on which this is based however are probably not random since the number of SS tested is small, and since the average of Ss is somewhat below the 37 percent average characteristic of this type as established in BC₅. It is clear nevertheless that with approximately the same genetic backgrounds, that is, with several other spotting genes in homozygous condition, ss produces about 100 percent white; Ss about 35 percent white and SS about 20 percent. The spotting of the last phenotype is due entirely to the action of other spotting genes obtained from the all-white type. For convenience we have called this type “K,” although it will be shown that it contains a complex of several spotting genes. From the comparison of the genotypes above it can be seen that ss with the “K” complex gives all-white; substitution of one S gene reduces the amount of spotting by 65 percent; substitution of a second S gene entails an additional reduction of spotting of only about 15, from 35 percent to 20 percent white. From this relationship can be obtained a measure of the relative dominance of S when combined with the “K” complex. In the absence of the “k” genes, S is practically completely dominant to s, that is both SS and Ss are solid-colored; in the presence of the “k” genes S is incompletely dominant, the phenotype of Ss being shifted to about a fifth (actually 15/80) of the distance between SS and ss. If complete dominance of S be called 1, then the relative dominance of S in the presence of the “k” genes is about .80.

**ANALYSIS OF THE NEW SPOTTING GENES (“K” COMPLEX)**

The experiments up to this point have demonstrated that the pied mice with which our experiments began contained several spotting genes in addition to the heretofore known gene s; that combination of a number of these other genes with s produces the all-white condition in which pigment appears in the eyes or in very small amounts on the head or rump; that these genes are not alleles of s but are inherited independently of s; that they are not specific modifiers of s since they produce their typical effect (white-spotting) when s is eliminated from the genotype, and finally that they materially change the dominance relations of S and s. It remains
now to study the inheritance and expression of these newly isolated spotting genes.

From the "K" spotted type originating as described above, inbred lines have been established which have bred true to the new condition within certain limits of variation for five generations. The F₁ generation (table 4, line 5) consists of those animals from BCF₁ which were tested and found to be SS. The lighter of these (15 percent white and over) were bred to produce an F₂ and similar selection was practiced in subsequent generations. In spite of such selection, the limits of variation have remained about the same (from 3 to 35 percent of dorsal white) for five generations, indicating a relatively homozygous genotype. The persistence of variation in F₆ shows that it is probably largely non-genetic. The spotting of this type is always in the mid-dorsal region, usually as a typical white belt extending around the body. The belly is usually spotted to about the same extent as the back instead of being more spotted as in other pied types. In general appearance, however, this type is not to be distinguished from belted pied.

The hereditary basis of the new type has been tested by crossing it with the same homozygous self races (dilute brown and black-and-tan) which were used in the all-white crosses (table 5). The F₁ animals from these crosses (line 1) are self, or nearly so, never having more than a very small white spot (5 percent or less) on the belly, showing that the wild type alleles of the spotting genes are nearly completely dominant. In an F₂ of 193 animals, only 32, or about 16 percent, showed any dorsal spotting, and none of these reproduced the extreme spotting grade of the "K" grandparents (table 5, line 2). Most of the F₂ spotted animals had only a small white spot on the back, covering less than 5 percent of the surface. The average grade of the F₂ spotted was about 5 percent compared with an average grade of about 15 percent for the "K" parent type. Of the 161 animals that showed no dorsal spotting, 60 were noted as having ventral spotting and the spots were sometimes as large as those occurring on "K" type animals. The remainder were either solid colored or were killed or died before the ventral spotting was established. There appeared to be, however, continuous variation from solid colored to about 15 percent white, making an exact analysis difficult and uncertain. F₁ was backcrossed also to the "K" parent type (table 5, line 3). Out of 167 progeny 68 were like the F₁ type (self or small ventral spot) and 99 showed some dorsal spotting. The significant departure from a 1:1 ratio shows clearly that more than one spotting gene is concerned. If these BC animals showing a ventral spot are classified as "spotted" the total of these is 128 or 76.6 per cent of the total. Such a ratio should be obtained if two major genes, either one or both of which, when homozygous, lead to white spot-
### Table 5

Results of crosses involving the new spotting form, "K."

<table>
<thead>
<tr>
<th>PARENTS</th>
<th>SELF</th>
<th>PERCENT OF DORSAL WHITE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/</td>
<td>5</td>
</tr>
<tr>
<td>1. &quot;Self×&quot;K&quot; 25/</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>2. F1 &quot;K&quot; × F1 &quot;K&quot;</td>
<td>101</td>
<td>60</td>
</tr>
<tr>
<td>3. F1 &quot;K&quot; × &quot;K&quot;</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>4. &quot;K&quot; F4</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>5. Line 190 F19</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>6. Line 190×&quot;K&quot;</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>7. F1 190 &quot;K&quot; × &quot;K&quot;</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8. F1 190 &quot;K&quot; × L-190</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>9. Lightest from 8 inter se</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

* Reciprocal crosses of d br and black-tan self races by light "K" animals.
ting, differentiate “K” from self. Here again there was intergrading variation and while the general situation is clear, that is that a small number of spotting genes differentiate “K” from the wild type, the exact number and effect of each cannot be established with certainty from the present evidence.

The new type “K” has been shown to be indistinguishable somatically from the belted pied type. It is interesting to observe the results of crossing the two similar types. In table 5, line 4, the range of “K” for the fourth inbred generation is shown compared with the range of the 19th inbred generation of the belted line 190 (line 5) from which animals to be crossed with “K” were taken. “K” is somewhat less spotted on the average, but the distributions of the two lines coincide over most of the range. The F₁ (table 5, line 6) is clearly much darker than either parent. Reversion has occurred toward the wild type condition, indicating that each race contains at least some of the wild type alleles of the spotting genes of the other race and that the loci involved show partial dominance. F₁ animals were backcrossed to each parent type. The backcross to “K” (table 5, line 7) produced 100 offspring showing a continuous variation in spotting from self to 35 percent white; this represents an extension of the range of the “K” type at the self end, indicating segregation of wild type alleles from 190. The distribution shows evidence, more clearly marked in the offspring of a number of individual pairs, of modes at 5 and at 30 percent white. The median falls in the 6-10 percent class, but there is no low or zero frequency here and consequently no clear evidence of simple segregation of even one gene. It is likely, however, that the lower group is largely SS and the upper mainly Ss.

The F₁ animals were also backcrossed to line 190 (table 5, line 8). Here two groups of spotted animals are clearly evident; a dark group of 108 individuals varying from 0 to 15 percent white with one mode at 1-5 percent; and a lighter group of 113 individuals varying from 35-75 percent white, with no clear mode, but with highest frequencies between 50 and 65. This represents segregation of S and s, the darker group being Ss, the lighter ss. Members of the lighter group when bred inter se produced only light spotted animals showing them to be homozygous ss. It is clear that the darker group shows less spotting than the “K” parent type, proving that wild type (self) alleles of the “k” genes have been introduced from the pied (190) parent. This group is darker also than the F₁ type, although like F₁, it is Ss; it contains a few entirely unspotted animals and many with spotting only on the belly. Such animals do not occur in either “K” or 190 parent types and must be due to recombination of wild type alleles of the secondary spotting loci from 190, with S from the “K” line.

Similarly the light ss group has much more white spotting than the 190
parent type and over 60 per cent of this group exceeds the upper range of the 190 type. This is undoubtedly due to recombinations of ss from 190 with other spotting genes from the “K” parent. Some or all of these genes, when combined with ss, must influence spotting even when heterozygous, since the 190 parent must be assumed to be pure for the wild type alleles of at least some of these genes.

Accordingly, the lightest animals from BC \( F_1(K/190) \times 190 \) (table 5, line 8) should have in addition to ss the maximum number of other spotting genes (most of them in heterozygous condition) and when inbred should produce some animals homozygous for all spotting alleles involved in the parent race. Such BC animals from the spotting classes 60–75 have been bred together with the results shown in line 9. The 107 offspring ranged from 25 to 95 per cent white, with one mode at 60. They obviously had more white spotting than the population from which they were chosen. Two out of the 107 offspring fell within the range of all-white. This re-synthesis of the all-white type from the cross of two dark spotted lines shows that the latter contain different spotting genes, the effects of which cumulate when combined in the homozygous state. The number of gene differences involved is difficult to estimate, but it is probable that at least three with major effects are concerned as well as others with minor effects. The arrangement of these in the parent types must follow some such scheme as follows: Belt 190, \( ss(AABBCC \cdots nn) \); “K” race, \( SS(aabbcc \cdots nn) \); \( F_1, Ss(AaBbCc \cdots nn) \); all-white, \( ss(aabbcc \cdots nn) \).

The data from the backcross of \( F_1(190/K) \times “K” \) (table 5, line 7) are not well adapted for testing this hypothesis because of the continuous nature of the variation and the impossibility of separating genetic from non-genetic variations wherever such a condition obtains. However, the general character of the distribution and the limits of variation correspond to the predictions imposed by the above scheme. Thus about \( \frac{1}{8} \) of the progeny from this backcross should be as dark as “K” or darker (\( \frac{3}{4} SS, \frac{1}{4} SsAa \), etc.) and animals with more spotting than “K” should arise where \( Ss \) is combined with the other important spotting genes \( (a, b) \) in homozygous form, since in other combinations \( Ss \) gives self or very little spotting as in \( BC \ F_1(190/K) \times 190 \), and in \( F_1 \) all \( \times \) self. Such recombinations are limited to 1/8 of the progeny of this backcross (\( Ss aabbcc \)) and probably fall within the upper parts of the “K” range. Consequently the backcross to “K” should give only “K” types and a proportion of very dark spotted forms increased over the \( F_1 \) \((K \times 190)\) proportion by the segregation of wild type spotting alleles from 190 and this is the result obtained.

The conclusion seems clearly established that the new spotted form “K” differs genetically from the belted pied form which it closely resembles
phenotypically in a number of genes which affect spotting. One of these, (s) has a considerable effect when in combination with the others; at least three others have lesser effects, and there may well be others with very minor effects. An estimate of the effect of S can be obtained by comparing the differences in spotting between the Ss and ss animals from the backcross of F1(190/K) × 190 (table 5, line 8). The other genes are probably distributed at random between these two groups and except for common genes such as c · · · n, at least one wild type allele of each is present in each individual. The Ss group has about 2.5 percent of dorsal white; the ss group has an average of about 56.5 percent white. The difference attributable to s is thus about 54 percent. This is to be compared with our estimate of 65 percent for the effect of S from the data on backcrosses of Ss (from self) to all-white (page 34). The two estimates are roughly similar; the difference may be due to the presence of wild type alleles of two of the genes in the backcross to line 190, which probably reduces the effectiveness of s. No good estimate of the effects of the other spotting genes can be obtained from the above data. It should be remarked, however, that the difference between the grade of ss (56.5 percent) from the backcross to line 190 and the grade of ss when the other spotting genes are homozygous as in all-white (grade 100) is about 43.5 percent, which is attributable to the combined effects of all the other spotting genes.

A few crosses of “K” spotted with a white-faced pied line having an average spotting of from 5–10 percent have produced only self-colored animals; this is a reversion to wild type similar in kind to that which occurred in crosses of “K” by belted pied, but more complete. Apparently white-face, although known to contain ss, has the dominant alleles of all other major spotting genes of the “K” type. The white-faced line may then be represented as ss(AABBCC · · · nn?). If this is so, then the white-faced and belted pied lines should differ by only a single major spotting gene. The evidence on this point is still incomplete but the indications point to the correctness of the above assumptions. Thus belted pied (line 190) by white-face has produced to date 18 animals varying only from 1–10 percent of dorsal spotting, that is all are within the range of the white-faced line and none within the range of the belted line. The F2 consists of 10 animals in the 1–10 percent group and 4 with 25–30 percent spotting, and no intermediates. A single gene difference is sufficient to explain the present evidence. Moreover, white-faced pied crossed with another dark pied line (line 66) has produced in F1 only dark pied (1–10 percent) white, and a similar range in F2 and in backcrosses to line 66. These two lines then appear to have the same genes influencing the amount of spotting and to differ from the wild type only by the pied gene s.
DISCUSSION

The set of mutant genes additional to the previously known $s$ now found to be concerned with the production of spotting in fanciers' races of pied house mice suggests a phenomenon which may well be more common at least in the inheritance of quantitative characters than has so far been realized. That is the fundamental identity of dominance modification and "gene interaction."

It has been seen that $s$ is not, as commonly thought, the "main" mutant gene for pied spotting, with other genes as modifiers merely altering the amount. Instead, the "K" complex of at least three mutant genes has been shown to have effects *sui generis* and these are found to be qualitatively identical with those of $s$ (that is, tending to suppress pigment development in some part of the skin). Among the alleles of these four or more loci many combinations are possible of which few have yet been clearly identified phenotypically. But even among these few a comparison may show the relations typical of "gene interaction" or of dominance modification, depending on which set of genotypes is compared phenotypically. The former is seen in the demonstration that $s$ with one or more genes of the "K" complex produces about 25 percent of spotting ($L_{190}$); the whole "K" group without $s$ produces slightly less spotting in the same general area of the body; but $s$ and the "K" genes in homozygous combination suppress pigment in all parts of the skin, though not the eye, producing the "all-white" phenotype with dark eyes. Dominance modification is shown by the previously discussed shift of the $Ss$ phenotype from practical identity with the $SS$ phenotype in the absence of the "K" complex to about a fifth of the interval from $SS$ to $ss$ in the presence of the "K" group of mutants.

This double aspect of the data finds a possible basis in the dependence of the quantitative phenotypic effect of any single gene upon the total reaction set by the remaining genes of the spotting group in the fashion assumed by Sinnott (1935) for squash shape and by Wright and Chase (1936) for guinea pig spotting. The latter presumably comes closer to the present case. Wright and Chase assume essentially (in their "inverse probability transformation" of percent of white spotting) that successive substitutions of equal spotting genes produce at first small alterations in amount of white, then larger changes and finally again decreasing change as the amount of white approaches 100 percent, alleles and non-alleles behaving indistinguishably. If then we compare the effects of substituting one and two recessives of the same locus, in the presence of different numbers of recessives at other loci, the wild type gene might be nearly dominant at one end of the range of spotting variation and nearly recessive at the other (light) end, with an approach to intermediacy where the other genes determine a level of white near 50 percent. Thus in Wright and
Chase's scheme gene interaction and dominance modification are only different aspects of a more general relation of phenotype to genotype.

That a relation of this sort is involved in the case of mouse spotting seems fairly plausible from its consistency with what has so far been found. A direct experimental test of the scheme is obviously desirable. Although not yet possible in the guinea pig, it may be so in the house mouse, where the number of loci involved is reasonably small and where the several mutants are not equal in effect, s probably exceeding the others.

The evidence shows also that the variations in spotting of the pied types are not due to reverse somatic mutations of s to S since they occur after s has been eliminated. By extension the same reasoning militates against the assumption of somatic mutation as a prime cause of spotting variations in this material.

The test of the complex relationships of these spotting genes in combination can be made only from more extensive evidence from crosses between strains differing only in single spotting loci. Such strains are being prepared by resolving the "K" complex into its components.

SUMMARY

Quantitative variation in the pied patterns of house mice (consisting of blotches of colored and white fur) extends over the whole range from a few white hairs to an entirely white coat.

Stocks breeding true to limited ranges of spotting variation (dark, medium and all-white races) and to certain localized patterns (white-face and belt) have been isolated by inbreeding.

By crossing these together and with self-colored (unspotted) races, it has been shown that all of the original spotted forms dealt with contain a spotting gene s. Forms with much white spotting (all-white) contain in addition at least three other spotting genes with quantitative effects which are inherited independently of s. Races showing different degrees of white spotting differ in the number of spotting genes which they contain.

These additional genes are not specific modifiers of s, since they produce spotting when s is absent. The effects of s and of the other spotting genes depend in part upon the combinations in which they appear. Thus, when other spotting genes are substituted for their type alleles, the relative dominance of S is shifted from 1 to .80. The effects of the spotting genes cumulate but not in a simple manner. They appear to cause greater quantitative changes in spotting when acting in phenotypes near the center of the spotting range than at either extreme.

In addition to variations due to gene mutations, a considerable degree of non-genetic variability is characteristic of all the homozygous spotted strains. The basis for this has not yet been analyzed.

Somatic mutation is probably not a prime cause of spotting variations.
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BIBLIOGRAPHY


CUÉNOT, L., 1904 L'hérité de la pigmentation chez les Souris. (3e note) Arch. Zool. exp. et gen. 4e ser. 2: XLV-LVI.


