ON THE GENETICS OF THE SPOTTED PATTERN OF THE GUINEA PIG

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Received May 20, 1936

INTRODUCTION

COAT patterns in which color is restricted to spots on a white ground are extremely common in guinea pigs, and have naturally attracted the attention of geneticists from the first. That the genetic situation is somewhat complicated is indicated by the fact that there are at present at least three decidedly different interpretations.

Variations in this type of pattern, ranging from mere traces of white in a few locations (toes, nose, between ears) to black eyed white, through intermediate grades showing much diversity in localization and often extreme asymmetry, were described by CASTLE (1905), who, however, attempted no genetic interpretation. GOODALE and MORGAN (1913) published data from which they concluded that "the spotted coat is a very complex affair depending presumably on a number of factors." They suggested the hypothesis that there may be one recessive factor (*ss*) necessary for any spotting with extension due to other factors (s_1s_1, s_2s_2, s_3s_3 etc.). IBSEN (1916) provisionally treated spotting as due to a recessive factor *s*, but noted "evidence is accumulating which seems to indicate that the relationship is not as simple as has hitherto been supposed."

In a list of guinea pig color factors (WRIGHT 1916) the symbol Σw was used for the "assemblage of unanalyzed factors which determine white spotting." In a review in the next year, however, (WRIGHT 1917), a pair of alleles *S*, *s* was listed as differentiating at least certain inbred stocks. The extreme variability of the pattern in both amount and localization of white even after 16 generations of brother-sister mating was cited as evidence for an extraordinary amount of non-genetic variability and as the reason for difficulty in genetic analysis. An effect of sex on spotting was noted. Females of each of 23 inbred strains were found to have slightly more white on the average than males. An effect of *s* as a modifier of the character of the tortoiseshell pattern of black and yellow was noted.

Early crosses between inbred strains

The data were not published in detail at the time. We shall present that bearing on the alleles *S*, *s* in condensed form. Twenty three strains were started in 1906 from single pairs by Mr. G. M. ROMMEL of the U. S.

TABLE 1

Distributions of 4 inbred strains of guinea pigs, 1916–22 in percentages. Grade O means solid color: X means a trace of white. The grades are at 5% intervals (1=2.5 to 7.5%). W (black eyed white) is distinguished from 20 (a trace of color). The median percentages of white are given separately for males and females in the last two columns.

STRAIN	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	O	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂	♀
34	100.0	0	0	0	0	0	0	0	0	333	0	0
39	0	35.2	32.9	14.3	7.7	6.7	2.3	0.9	0	659	13.9	24.4
35	0	0.8	4.0	10.1	19.7	29.4	27.1	8.6	0.3	1460	62.5	68.9
13	0	0	0	0.5	0.7	2.8	16.2	71.0	8.8	1278	95.0	96.4

Bureau of Animal Industry. These were maintained by exclusive brother-sister mating. The senior author took charge of the experiments in 1915 at which time 17 of the strains were still on hand. One of them (No. 34) had no white spotting (although much "silvering"). All of the others consisted wholly of tricolors but there were great differences among them in average amount of white. A drawing of the pattern of every animal was made at birth in a rubber stamp outline and the amount of white was estimated from an outline on tracing cloth divided into 20 squares. In table 1 is a condensed table of distributions in 4 strains. The distributions for 13, 35, 39, and two other strains have been published in full, separating males and females (WRIGHT 1926). Grade X means a trace of white and W, for black-eyed white, is distinguished from grade 20 with a trace of color. Strain 39 had least white of any of the spotted strains while 13 was at the opposite extreme.

During the years 1916 to 1919 many crosses were made between these and other strains (WRIGHT 1922). There were 1334 young from first crosses,

TABLE 2

The distributions of reciprocal crosses between strains 39 and 13, and of F_2. Note the similarity of F_2 to F_1 and the marked disagreement with expectation based on 25% strain 39, 50% F_1 and 25% strain 13 as indicated in the bottom line. In designating crosses in this and later tables, the female parent is given first.

CROSS	GRADE OF SPOTTING									NO.
	O	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
39×13	0	3.3	0	13.3	6.7	30.0	20.0	26.7	0	30
13×39	0	0.8	6.4	8.0	14.4	25.6	27.2	17.6	0	125
Total F_1	**0**	**1.3**	**5.2**	**9.0**	**12.9**	**26.5**	**25.8**	**19.3**	**0**	**155**
F_2	0	2.4	12.2	13.4	8.5	23.2	24.4	15.9	0	82
Expected in F_2 if 1 factor	*0*	*9.5*	*10.8*	*8.2*	*8.6*	*15.6*	*17.5*	*27.6*	*2.2*	

981 from crosses of F_1 to a third strain, 692 F_2's from two strains and 617 F_2's combining 4 grandparental strains. Many more were born in selection experiments from these crosses. In all of these cases, spotted by spotted produced only spotted. We shall cite data from the cross between spotted strains at opposite extremes, (13×39) in the same condensed form as above (table 2).

Reciprocal F_1's are intermediate and F_2 is also intermediate and only slightly more variable than F_1. It is impossible to find in F_2 25 per cent segregants with the distribution of strain 39 or 25 per cent like strain 13. The *minimum* number of segregating factors may be estimated at 4 or 5. No upper limit to the possible number can be set. It may be added that while males and females showed the usual slight sex difference there were no indications of sex linkage.

Let us now turn to the crosses between the single self-colored strain (No. 34) and spotted strains, all with much white. The latter will be treated collectively (table 3).

TABLE 3

The distributions of reciprocal crosses between strain 34 and miscellaneous spotted strains, of F_2, and of backcrosses of self or near self segregants to the spotted strains.

CROSS	GRADE OF SPOTTING									NO.		
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		0–5	6–W
34×spot	12.2	75.5	12.2	0	0	0	0	0	0	49	100	0
spot×34	11.1	77.8	11.1	0	0	0	0	0	0	18	100	0
Total F_1	**11.9**	**76.1**	**11.9**	**0**	**0**	**0**	**0**	**0**	**0**	**67**	**100**	**0**
F_2	9.4	53.1	9.4	12.5	0	6.2	9.4	0	0	32	71.9	28.1
1st Backcross	6.3	36.6	7.1	7.5	13.0	5.5	9.1	13.4	1.6	254	50.0	50.0
2–4 Backcross	8.6	31.9	6.9	6.9	7.7	11.2	9.5	16.4	0.9	116	47.4	52.6
Total Backcross	**7.0**	**35.1**	**7.0**	**7.3**	**11.4**	**7.3**	**9.2**	**14.3**	**1.4**	**370**	**49.1**	**50.9**

Complete dominance of self color is shown in only 12 per cent of the F_1 individuals but the average amount of white in F_1 is low, being always less than 27 per cent. In F_2, 28 per cent of the young exceed F_1, several being of high grade. There is a strong suggestion here of segregation of an incompletely recessive factor for high grade spotting. This is confirmed by the backcross of F_1 to inbred spotted. Exactly 50 per cent are above the limits of F_1. A second backcross of near self from the above matings to inbred spotted practically repeated the result from the first backcross. The same was true of small 3rd and 4th backcross generations. The young from these were derived only $\frac{1}{8}$ to 1/32 from the self strain but show no tendency to dilution of the heredity transmitted from the latter.

Non-genetic variability

The existence of the alleles *S*, *s* was first confidently asserted in a later paper (WRIGHT 1920) on the basis of a new experiment which had then reached the 7th backcross generation of self to inbred spotted. This experiment, ultimately carried to the 11th backcross generation, will be discussed later. The 1920 paper dealt primarily with an analysis of the variability within closely inbred strains. It was shown that in a typical strain (No. 35) descended at that time from a single mating in the 7th generation of brother-sister mating, there was variation from a trace of white to self-white but that this enormous variability (standard deviation about 22 per cent of the area of the coat) was almost wholly non-genetic. There was a correlation of only +.014 between parent and offspring (using a transformed scale designed to counteract the damping of variation in the neighborhoods of 0 and 100 per cent white). It was further shown that most of this non-genetic variability was of such a sort that environmental factors common to littermates played very little role. The correlation between littermates was only +.069.

While the intrastrain differences were thus proved to be non-genetic, the differences between strains are of course genetic. Both should be present in a random bred stock and this proved to be the case. The stock from which the inbred strains were derived had been maintained without even second cousin mating. In this stock the correlation between parent and offspring at this time was +.211 interpreted as indicating that 42 per cent (=2×.211) of the variance is genetic (leaving 58 per cent as non-genetic) on the assumption of no dominance or epistasis. Confirming this was the observation that the actual variance in the inbred stock was 57 per cent of that in the random bred stock on the transformed scale.

More extensive data on this question have been reported briefly in a later paper (WRIGHT 1926) together with data which indicate that the most important of the non-genetic factors common to littermates is the age of the mother. We shall discuss these and other data on the roles of heredity and environment in a later section.

There is a remarkable contrast between the situation in the guinea pig and that in the rat. In CASTLE'S (1916) selection experiments with hooded rats, the standard deviation among the offspring of the later generations averaged only about 3 per cent of the range from solid color to solid white. Yet progress by selection was possible up to the end, demonstrating the presence of considerable genetic variability. The correlation between mid parent and offspring (10th to 16th generations) averaged about +.25 and +.26 in the minus and plus series respectively. Thus a standard devia-

tion of 3 per cent in an unfixed strain of rats is to be compared with one of about 20 per cent in a stock of guinea pigs in which all animals have been shown to have almost identical genetic constitutions. The same contrast is also doubtless indicated by the difference in regularity and symmetry. The patterns of hooded rats form a practically linear series of grades with a high degree of symmetry. In guinea pigs nearly every individual has its own characteristic pattern and there is usually asymmetry. Why the same character should behave so differently in different rodents is not known. The fact of an enormous amount of non-genetic variability in the guinea pig must be taken into account in any discussion of the genetics of white spotting in this animal. The phenotypes of single individuals are practically worthless here as indicators of genotypes.

Eleven generations of back crossing

The 11-generation backcross experiment, referred to above, was reported at the 1923 meeting of the American Society of Zoologists, but only

TABLE 4

Distribution of grades of spotting in F_1, F_2 and backcrosses to spotted strains, following a cross of a self-colored male to spotted females. The backcrosses are classified according to the amount of ancestry of strains 35 (median about 65% white) and strain 13 (median at 98% white).

	GRADES OF SPOTTING									NO.
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
spot×self	26.7	70.0	0	3.3						30
F_2	10.0	45.0	10.0	10.0	10.0	5.0	10.0			20
Backcrosses to spot										
3/4 blood or more 35	7.7	44.3	7.7	17.3	7.7	1.9	9.6	3.8		52
Intermediate	1.8	33.9	8.8	5.8	10.8	11.0	14.3	12.8	0.8	398
3/4 blood or more 13	0	21.2	11.3	12.5	6.2	1.3	11.2	33.8	2.5	80

a brief abstract was published. The first cross was between a self-colored male (unrelated to strain 34 or to the other inbred strains referred to above) and tricolor females from various inbred strains. Most of the young showed a little white spotting, the most extreme being a female with 30 per cent. None showed any yellow spotting. A small F_2 generation showed 25 per cent with spotting beyond the limits of F_1. F_1 was backcrossed with tricolors of various strains and produced about 50 per cent with no more white than F_1 and about 50 per cent with larger amounts. Each of these classes was about equally divided between animals with and without yellow spotting.

Selfs or near selfs of this generation were backcrossed again to inbred tricolors producing young which were 7/8 blood tricolor by ancestry but the distribution of types was substantially as in the first backcross genera-

tion. This process was repeated through 11 backcross generations, producing at length animals which were 99.98 per cent of tricolor ancestry but the results remained the same.

The tricolor strains used varied from ones which averaged about 65 per cent white (No. 35) to ones with over 95 per cent (No. 13). In table 4 all backcross generations are combined, but a distinction is made between those which were $\frac{3}{4}$ blood or more of strain 35, those which were $\frac{3}{4}$ blood or more of strain 13 and those which came of intermediate tricolor ancestry.

Obviously the line between *Ss* and *ss* must be drawn at different points, depending on the tricolor strain used. In table 5, the line was drawn above

TABLE 5

Persistence of genes S and E through 11 generations of backcrossing of self (or near self) segregants ($SsEe^p$) to inbred tricolor stocks (sse^pe^p). Criterion for separating Ss and ss as described in text.

NO. OF BACK-CROSSES	AMOUNT OF SELF ANCESTRY	LITTLE OR NO WHITE		STRONGLY SPOTTED		TOTAL	
		NO YELLOW $SsEe^p$	TORTOISE Sse^pe^p	NO YELLOW $ssEe^p$	TRICOLOR sse^pe^p	*Ss*	*ss*
1	1/4	25	26	29	18	51	47
2	1/8	19	22	14	16	41	30
3	1/16	12	6	5	6	18	11
4	1/32	12	10	4	6	22	10
5	1/64	6	8	20	14	14	34
6	1/128	6	7	6	7	13	13
7	1/256	7	7	9	7	14	16
8	1/512	13	10	17	18	23	35
9	1/1024	15	14	18	9	29	27
10	1/2048	19	14	21	13	33	34
11	1/4096	5	4	4	2	9	6
Total		139	128	147	116	267	263

grades 3 and 4 for males and females respectively of the group with $\frac{3}{4}$ blood of strain 35, above 7 and 8 for the males and females respectively of the intermediate group, and above 11 and 12 for the males and females of those with $\frac{3}{4}$ blood of strain 13. These were the points which most nearly divided these groups on a 1:1 basis. It is of course recognized that this is somewhat arbitrary and that there was probably real overlapping in each case.

While no great stress can be put on the approximate equality of the groups with low and high grade spotting, the persistence of a bimodal distribution through 11 generations of backcrossing of selected low grades to pure tricolors can only be interpreted as evidence of transmission of an undilutable unit (*S*). The persistence of a group with no yellow spotting through the 11 backcrosses of course indicates transmission of a second

unit (E) and the ratios indicate random assortment relative to S,s. The deficiency of tricolors is undoubtedly due to the failure of yellow spotting to appear in some of constitution sse^pe^p which were nearly or wholly white. The fact that the white spotting factor s behaves as a modifier of the tortoiseshell pattern was obvious in these data. In Sse^pe^p with little white, the yellow was usually restricted to scattered hairs or an irregular brindle. In sse^pe^p there was a more or less complete segregation of black and yellow into distinct spots on the white ground. These effects have also been noted by ILJIN (1928). The yellow spotting factor on the other hand had no apparent modifying effect on the white pattern.

Other interpretations of spotting

PICTET has also reported on extensive experiments with white spotting in guinea pigs. From his descriptions and illustrations, it seems clear that the patterns in his stocks are similar to those with which we have worked. His conclusions, however, are very different. He finds four major pairs of alleles, two affecting head only and two the trunk only, no one of which can be identified directly with our single major pair S, s.

In a paper (1925) dealing with white on the head he distinguishes self color, frontal white (self except for a median streak) and lateral white (white on cheeks as well as nose). Pure selfs by albinos gave 78 self to 82 frontal (approximately 1:1). Crosses between these F_1 types gave 54 self: 54 frontal: 36 lateral (exact 3:3:2) apart from albinos. He assumes two pairs of alleles with interactions as below.

	FF, *Ff*	*ff*
UU, *Up*	Frontal	Self
pp	Lateral	Lateral

It is assumed that the selfs were of type *UUff* and that all of the albinos happened to be *ppFf* (the albino factor being correctly interpreted as independent of spotting). Results from later generations are interpreted as in harmony with this hypothesis.

Later papers (1930, 1931) analyze spotting of the trunk. While it is stated that in most cases the extent of white is similar on head and trunk, it is held that these regions are subject to independent systems of genes because the correlation is not complete. In particular it is noted that animals with "generalized" white on the trunk may have a self colored head while one with a self colored trunk may have any amount of white on the head. Thus while the symbols U and p are used for genes affecting the trunk in these papers, they are apparently not considered to be related to the head factors assigned these same symbols in the earlier paper.

From the 1931 account, 5 original matings of self by spotted gave 42 spotted in F_1, and 52 spotted to 19 self in F_2, suggesting a dominant gene

for spotting. On the other hand, 5 other matings of self by spotted gave 49 self in F_1, and 50 self to 19 spotted in F_2, suggestive of recessive spotting. The spotting in these two cases differed in character. The white in the recessive spotting was limited to the feet and small spots on the nape of the neck and in the sternal and perianal regions (apart from the head). This is called localized spotting. In the dominant type, the white was more or less extended over the body, ranging from narrow streaks across the back or along the belly to the completely white type (with black eyes). This is called generalized spotting.

The descendants of the above crosses were interbred. The total ratios agree with expectation from formulae assigned to parents according to the following scheme of combination effects.

	Pp, *Pp*	*pp*
UU, *Uu*	Generalized	Self
uu	Localized	Localized

($\chi^2 = 1.2$, n = 12 probability more than .999 of worse fit from random sampling.)

IBSEN (1932) makes the following statement in regard to white spotting. "White spotting (*s*) is quite variable in its expression in the guinea pig. There are probably a number of modifiers concerned and in addition there seems to be some variation that is entirely somatic. The dominant modifier *Fa* (face) causes the white spotting to be restricted entirely to the face, while its allelomorph, *fa*, permits white to appear in other parts of the body as well. Evidence has been accumulating which seems to indicate that there is a modifier of *Fa*, thus being a modifier of a modifier. This factor, *Na*, (narrow) causes the white face to be narrow while its allelomorph, *na*, permits it to widen. *Na* seems to be completely dominant to *na*. There are other types of white spotting in guinea pigs, such as white belt and white rump and it is even possible by selection to produce animals that are entirely white. None of these, however, can be readily fixed by selection thus lending support to the supposition that there is more than one pair of modifiers concerned in the production of each type. It would probably require much research to make a complete analysis of the modifiers affecting white spotting."

This scheme may be represented as follows. The data supporting it have not been published.

S – – –	Self
ss Fa – *Na* –	Narrow facial streak only
ss Fa – *na na*	Extensive facial white
ss fafa –	White on body as well as face

This scheme resembles that presented by WRIGHT in assigning one main factor to spotting but differs in treating this as completely recessive.

The greatest difference is with respect to non-genetic variability. IBSEN finds so little that definite formulae can be assigned certain phenotypes, while we have found so much in all inbred spotted stocks that such an assignment would be quite impossible.

New crosses between inbred strains

Thus since 1932 there have been three widely different interpretations of the genetics of white spotting of the guinea pig. It seemed desirable to make a new series of tests. The following experiments involve only three closely inbred strains.

Strain D has been closely inbred since 1906 first by Prof. W. E. CASTLE since 1916 by WRIGHT. It has never thrown white spotting of any sort.

Strain 2, as used here, is entirely descended from a single mating in the 15th generation of brother-sister mating (U. S. D. A strain, p. 759). It has

TABLE 6

Distributions of spotting in strains 2, 13, in reciprocal crosses between them and in F_2.

	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂	♀
Strain 2				0.2	2.0	5.8	20.8	68.2	3.0	1650	93.2	94.6
Strain 13					0.1	1.2	12.5	62.4	23.8	1688	97.0	98.6
2×13							28.6	71.4		28	94.0	93.8
13× 2						3.3	13.3	73.3	10.0	60	95.9	96.4
Total F_1						**2.3**	**18.2**	**72.7**	**6.8**	**88**	**95.2**	**95.4**
F_2					1.9		28.8	69.2		52	94.4	96.9

consisted wholly of high grade spotted (median percentage of white 93.2 in males, 94.6 in females). There has been, however, considerable variability in grade as shown in table 6. The correlation of $+.064 \pm .025$ between parent and offspring (here made without any transformation of scale, but arbitrarily treating black eyed white as a grade higher than grade 20 with a trace of color) shows that this variability is largely non-genetic (see table 16).

Strain 13 as used here traces to a single mating in the 18th generation of brother-sister mating. Two substrains are distinguished, 13 and 13E, the former but not the latter from continued brother-sister mating. There is, however, no recognizable difference between them in amount of white, which is the greatest of any inbred strain we have had. The median grades are 97.0 in males, and 98.6 in females. As with strain 2, the absence of appreciable correlations between parent and offspring (table 16) indicates that practically all variability is non-genetic.

Crosses have been made between strains 2 and 13 which indicate that

TABLE 7

Crosses involving only strain 2 (ss with median about 94 per cent white, table 6) with strain D (SS, always self colored).

MATING	AMOUNT OF D ANCESTRY	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO.	MEDIAN IN *ss*	
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂	♀
2×D	1/2 D	*ss*×*SS*	91.5	8.5								59		
F_1×D	3/4 D	*Ss*×*SS*	95.7	4.3								207		
1 BC×D	7/8 D	(*Ss*, *SS*)×*SS*	99.0	1.0								305		
2 BC×D	15/16 D	(*Ss*, *SS*)×*SS*	100.0	0.0								182		
3 BC×D	31/32 D													
F_1×F_1	1/2 D	*Ss*×*Ss*	46.2	23.1		3.8		23.1	3.8			26		
2 BC×2 BC	7/8 D	*SS*×*S*-	100.0									21	6.8	5.0
		Ss×*Ss*	71.0	22.6	6.4							31		
2 BC×7/8	7/8 D	*Ss*×*ss*	45.9	45.9	5.4		2.7					37		
7/8×7/8	7/8 D	*ss*×*ss*	0	69.7	20.7	6.9	0.7	1.4	0.7			145	6.5	12.1
7/8×2	7/16 D	*ss*×*ss*			14.1	18.7	26.6	20.3	12.5	7.8		64	47.5	55.8
(7/8×2)×7/8	21/32 D	*ss*×*ss*		45.4	18.2	27.3	9.1					11	12.5	30.0

they are closely similar genetically. The distributions of F_1 and F_2 are shown in table 6. The medians (95.2 and 94.4 per cent for F_1 and F_2 males respectively and 95.4 and 96.9 per cent for F_1 and F_2 females respectively) give no indication of any complementary effect and the doubtful increase of variability in F_2 gives indication of only minor differences.

Strain 2 (*ss*) had been mated with strain D (*SS*) for another purpose and repeated backcrosses had been made to D (WRIGHT 1935a). F_1 (table 7) consisted of 59 animals (all *Ss*) of which 54 were completely self while 5 had a trace of white (one or both hind feet). This result from a cross to a strain as white as 2 indicates that D must have a rather exceptional stock of modifiers which repress white. Among 207 backcross animals (about half *Ss*, half *SS*) 198 showed no white while 9 had traces consisting in most cases of a few white hairs between the ears. Among 305 of the second generation of backcrossing to D (about 25 per cent *Ss*, 75 per cent *SS*) only 3 showed a few white hairs. 182 animals which were 15/16 or 31/32 blood of strain D were entirely self although it is probable that some were still heterozygous. Evidently *S* is almost invariably fully dominant over *s* where there is a preponderance of modifiers from strain D.

Some of the 7/8 blood animals (all self colored) were tested by mating with strain 13. Eight of these produced only self or low grade spotted among 83 young and were considered to be *SS*. The distribution of grades is shown in table 8. Two males and two females gave some high grade spotted, presumably *ss*, demonstrating the tested self parent to be *Ss*.

These tested heterozygotes were now mated *inter se* in order to extract a spotted line deriving its spotting from strain 2 but 7/8 of its modifiers from strain D (table 7). Their young consisted of 22 fully self and 9 low grade spotted. Matings of tested heterozygotes with these spotted segregants produced 17 fully self and 20 low grade spotted. Matings among these spotted animals and their descendants have produced 145 all spotted and, with few exceptions, of low grade. These results indicate complete recessiveness of spotting in animals which are 7/8 blood of strain D. Most of them were of what PICTET calls the localized type (where spotted at all on the trunk). The results are thus close to those which he has reported for his recessive spotting factor except that white on the head (few white hairs to extensive white on nose and cheeks) nearly always accompanied the localized spotting of the trunk, indicating that only a single main factor was involved.

These animals have necessarily derived their white spotting from strain 2 (with generalized white). The great difference in average grade of white must therefore be due to independent modifiers. Matings back to strain 2 have produced 64 young with median grade about half way between the parents.

TABLE 8

Crosses between strain 13 and animals which were 7/8 blood strain D, 1/8 blood strain 2.

	BLOOD OF 13	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO.	MEDIAN % W			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
7/8 D×13	1/2	*SS*×*ss*	19.3	72.3	6.0	2.4						83	0.9	1.7		
		Ss×*ss*		46.2		7.7	7.7	7.7	23.1	7.7		13			42.5	72.5
		ss×*ss*			20.0	20.0	40.0	20.0				5				

TABLE 9

Repeated backcrossing of near self segregants to strain 13, illustrating persistence of gene S in spite of continued attempts at dilution. Reciprocal backcrosses are combined in this table.

Ss×*ss*	BLOOD OF 13	GRADES OF *Ss* PARENTS	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
1/2 (13)×13	3/4	All X	—	43.6	5.1	2.6	2.6	6.4	11.5	24.4	3.8	78	1.6	7.2	87.8	95.6
3/4 (13)×13	7/8	X–6	—	17.9	14.9	14.9	—	6.0	9.0	25.4	11.9	67	16.2	24.2	93.1	98.2
7/8 (13)×13	15/16	X–6	—	18.9	16.8	6.3	3.2	2.1	12.6	38.9	1.1	95	14.1	17.0	93.0	93.1
15/16(13)×13	31/32	3	—	16.7	25.0	8.3	—	—	8.3	25.0	16.7	12				
31/32(13)×13	63/64	2–6	—	5.9	11.8	21.6	2.0	7.8	11.8	29.4	9.8	51	16.7	31.2	96.2	96.7
63/64(13)×13	127/128	8–9	—	22.7	9.1	4.5	—	9.0	18.2	18.2	18.2	22				

TABLE 10

Tests of 15/16 blood segregants from backcross matings to strain 13 and of their inbred descendants. Low grades (Ss) produce a ratio which can be interpreted as 1 SS: 2 Ss: 1 ss while high grades (ss) breed almost like strain 13.

SOURCE	MOTHER CONSTITUTION AND GRADE	FATHER CONSTITUTION AND GRADE	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
15/16 backcross	*ss* (16–20)	*ss* (16–20)					0.8	2.3	29.0	56.5	11.5	131			92.5	96.5
Descendants	*ss* (14–20)	*ss* (17–19)						8.2	20.4	53.1	18.4	49			93.9	95.0
15/16 backcross	*Ss* (1–9)	*Ss* (X–8)	12.5	31.6	11.8	10.3	2.2	2.9	11.0	13.2	4.4	136	12.0	28.5	88.4	97.7
Descendants	*Ss* (1)	*Ss* (X)	22.2	50.0	11.1			5.6		5.6	5.6	18				
	S- (0–1)	*SS* (0–X)	26.3	69.7	3.0		1.0					99				
	SS (0–1)	*ss* (15–20)		50.0	30.0	10.0	10.0					10				
15/16 backcross	*ss* (16)	*Ss* (9)			20.0			20.0		60.0		5				
Descendants	*ss* (19)	*Ss* (14)		30.0				10.0	40.0	20.0		10				

We will now turn to the descendants of the test crosses to strain 13 (table 9). Animals with only a trace of white were selected and backcrossed to 13 (*ss*). The process was repeated for several generations. No fully self-colored animals have appeared in these backcrosses. A markedly bimodal distribution of grades of spotting has, however, persisted throughout. In those 7/8 blood or more of strain 13, the median of the lower group (*Ss*) was, however, higher than that of the extracted spotted (*ss*) with 7/8 blood of strain D, while the median of the higher group is nearly the same as that of pure 13. As in the previously reported experiments of this type, there can be little doubt of the segregation of a single major unit factor. As a critical test, however, matings were made within each of the groups of supposed segregants (table 10). The matings between high grade spotted (grades 16 to 20) gave an array almost like that of pure 13. These were clearly *ss*×*ss*. Supposed heterozygous segregants (grades X to 9) of these same generations were also mated *inter se*. The progeny were utterly different. Of the 136 young, 17 were fully self and 24 more had only a trace of white. About 66 per cent had more color in the coat than the most colored of the young from the high grade parents. This result leaves no doubt of the conclusion that there is here segregation of a single spotting factor showing incomplete dominance over self. The results from the crosses between 2 and 13 show that it is the same spotting factor that was completely recessive where there was 7/8 blood of strain D.

As noted 17 of the 136 young from *Ss*×*Ss* were recorded as self. This is only 12.5 per cent or half the proportion of *SS* expected, indicating that a small amount of white may be brought out by the "modifiers" of strain 13 even in the absence of the main spotting factor. Unfortunately strain 13 is silvered as well as spotted. The above 17 animals showed more or less silvering but no white in the regions (nose, forehead and feet) where piebald white is most likely to appear. Those recorded as having a trace or more of piebald white all showed white in one or more of these regions.

Tests of grading

It seemed desirable to test the reliability of the grades. As noted, these were based on drawings made in rubber stamp outline. Most of the drawings were made by the senior author at the time of recording births. The estimates of grades were made by the junior author, using an outline on tracing cloth, divided into squares. In 306 cases (all from backcrosses of *Ss* to *ss* of strain 13 and including all grades from a trace of white (x) to black eyed white (w) estimates were made later by the senior author without knowledge of Mr. CHASE'S grades. This comparison tests the accuracy of grading but not, of course, of the drawings. The statistical

analysis of the two series of grades (not using Shepard's correction) gave the following results.

	Mean	*Standard Deviation*	*Correlation*
Chase	11.41	7.96	.9978 ± .0003
Wright	11.36	7.93	

There is no indication of any differential systematic errors and random errors are of negligible importance. Only about 0.2 per cent of the variance is determined by random errors.

In another test, Mr. CHASE made wholly independent records of the patterns of 119 miscellaneous animals, distributed rather uniformly from grade x to 20, but including no black eyed whites. The two sets of drawings were also graded independently.

	Mean	*Standard Deviation*	*Correlation*
Chase	9.99	6.53	.989 ± .002
Wright	10.11	6.52	

There can be no important differential systematic errors either in drawing or grading. Random errors are naturally considerably increased but yet account for only one per cent of the variance. As the standard deviation in this population was nearly twice that of an isogenic stock with mean near grade 10, it may be estimated that about 3 or 4 per cent of the non-genetic variance may be attributed to the combined errors of drawing and grading.

Aberrant results

Results which at first seemed rather aberrant have been obtained from crosses between two stocks which are not related to any of the inbred strains of the preceding experiments. Strain A was built up to be dominant in a large number of genes. It consists typically of self agoutis (*SEACPFB*) with smooth fur except on hind toes (*RM*). Strain B was made up to be recessive in most of these respects. It consists of smooth-furred pink-eyed, dilute brown-yellow-white tricolors (*se^{v}ac^{k}pFbrm*). The results of a large backcross generation have been described in a previous paper (1928). We are indebted to Dr. STRANDSKOV for use of recent data from these stocks and their crosses.

The earlier records of strain A naturally revealed heterozygosis in many respects including *S,s*. Since 1926, however, only low grade spotting has appeared. The complete records, 1926–1934 inclusive, are given in condensed form in table 11. The records for strain B are given for the years 1924–25 as reported previously and separately for the period 1926–34. The previous paper reported F_1 (B×A) only from 3 males of strain A, demonstrated to be homozygous *SS*. These records, it turns out, give a

far from adequate picture of the situation. The total records for F_1 (B×A) and (A×B) 1926–1934, are reported here. The backcross data (F_1×B) are merely repeated (in condensed form) from the previous paper.

The first question raised by these data is whether *ss* can ever be completely self-colored. No such case was present in the data described earlier in this paper although the median grade of white was very low in certain strains (39,7/8D) and occasional animals had only a few white hairs. The records of 851 animals from strain B from 1926 to 1934 agree in this respect, but in the earlier records of B, 4 animals out of 454 (about 1 per

TABLE 11

White spotting in strains A, B and crosses. All A's are believed to be SS in spite of some low grade spotting (up to grade 2), all B's ss in spite of 4 selfs (grade 0). Note segregation of self in F_2 from strongly spotted F_1's.

SOURCE	REMARKS	GRADE OF SPOTTING									NO.
		0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
A	20 Matings (0×0)	100.0									96
	17 Matings (0×0)	86.2	13.8								217
	7 Matings (X×0)	73.2	26.8								41
	Total	**88.4**	**11.6**								**354**
B	1924–25	0.9	5.7	9.0	7.7	13.2	20.7	18.3	24.4	—	454
	1926–34	0	5.9	9.4	10.9	15.9	20.0	17.0	20.7	0.2	851
F_1	A×B low	36.9	63.1								160
	A×B high		87.5	12.5							16
	B×A low	29.8	70.2								242
	B×A high	16.7	69.8	5.2	3.1	2.9	1.2	1.0	0.2		420
	Total	**24.0**	**69.0**	**2.9**	**1.6**	**1.4**	**0.6**	**0.5**	**0.1**		838
F_1×B		9.8	37.8	12.4	8.9	6.6	9.4	8.7	6.4	0.0	437
F_2	From selected F_1's (grades 9–12)	17.2	41.4	6.9	3.4	10.3	6.9	3.4	10.3	0.0	29

cent) were recorded as completely self colored although from homozygous spotted parents. Unfortunately this question was not definitely in mind at the time of record and there may have been less care in looking for traces of white than in later experiments. We think it probable that plus modifiers may occasionally bring about complete absence of white spotting in *ss* but hesitate to affirm this positively until a case has been examined with this point in mind and has been tested genetically.

Other questions are raised by the traces of white in strain A itself and the very high grade white spotting (up to 95 per cent white) found in some of F_1 (B×A). In strain A only self-colored males have been used since 1926, but in 7 cases females were used which had traces of white. Six of

these females produced at least one offspring each, with traces of white. The other had only 2 young. The total ratio was 30 self to 11 with traces of white. The remaining matings of strain A (all self by self) may be divided into two groups: 20 matings with 96 young all self-colored, and 17 matings with at least 1 white-marked offspring each and a total ratio of 187 self to 30 with white. Two of the latter had white belts across the shoulders (grade 2).

If white spotting were due to a simple recessive in this stock, we should expect more than 50 per cent from matings classified as $Ss \times ss$ by the occurrence of at least one spotted young one, but only 27 per cent were observed. We should expect more than 25 percent from matings similarly classified as $Ss \times Ss$, but only 14 per cent were observed. This hypothesis is ruled out unless *ss* is frequently self-colored, which is contrary to all previous experience. The traces of white in strain A are clearly not due to *ss*.

The next possibility is that these white-marked animals are *Ss*. If this is true, many selfs in the strain must also be *Ss*, so many that a considerable number of reasonably high grade spotted (*ss*) should have appeared. But this has not been the case since 1926. More evidence on this hypothesis is provided by the crosses with strain B. Table 11 distinguishes matings which produced at least one offspring above grade 2 (12.5 per cent white) from those which did not and in which the parent from strain A was certainly *SS*. In the former there may be a suspicion that it was *Ss*. However, the young above grade 2 from these matings constitute only 13.5 per cent of the total, a proportion so low as to practically rule out this hypothesis. It may be noted that the low ratio applies not only to the total but to every individual mating. The most extreme spotted (over 50 per cent white) came from 9 males of strain A. These produced the following ratios (making the cleavage at 12.5 per cent): 20:10, 28:7, 22:4, 31:3, 16:1, 25:3, 25:5, 6:1 and 2:1. None of these is in harmony with the mating formula $Ss \times ss$. Thus it is strongly indicated that all of strain A, self and white-marked alike, were *SS* and that these strongly marked F_1's from (B × A) reaching 95 per cent white in one case, as well as the selfs (24 per cent of the total) were all *Ss*.[1]

This is confirmed by a number of tests. Matings between rather strongly marked F_1's (grades 9 to 12) produced the F_2 population in the bottom line of table 11. The appearance of as many as 5 self and 12 with only a

[1] These crosses were in part from an experiment in which Dr. STRANDSKOV was testing the effect of X-radiation of males and many of the highest grade F_1's were from treated males. Fourteen males produced 12 above grade 2 out of 163 young before raying and 29 above grade 2 out of 191 after raying, a difference with a probability of being exceeded by random sampling of only .02. There is some suggestion here of a modifying effect.

trace of white out of 29 young make it certain that the F_1 parents were *Ss* (not *ss*). In other cases F_1's of various grades were mated with strain 13 (the whitest of the inbred strains) (table 12). There is no appreciable difference in the results from low grade and high grade F_1's and both agree with expectation from *Ss*×*ss* and not at all with that from *ss*×*ss*.

TABLE 12

Tests of F_1 (A×B) of widely different grades by mating to the most extreme strain No. 13. Both groups of F_1's breed like Ss.

	GRADE OF SPOTTING									NO.
	o	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
F_1 (2–6)×strain 13	o	32.0	10.0	6.0	4.0	8.0	16.0	24.0	—	50
F_1 (13–17)×strain 13	o	20.4	12.2	4.1	10.2	10.2	14.3	28.6	—	49

These results from (B×A) may be compared with those in table 13 from the mating (B×D). It will be recalled that strain D was wholly self (*SS*) and gave results in crosses with the high grade spotted of strain 2 which indicated an expectional array of modifiers which reduce white.

TABLE 13

Crosses of strains B and D.

		GRADE OF SPOTTING									NO.
		o	X	1–2	3–5	6–8	9–11	12–14	15–17	18–20	W
F_1(B×D)	*ss*×*SS*	77.9	22.1								68
F_2(B×D)	*Ss*×*Ss*	57.3	24.2	4.4	5.2	3.9	2.3	1.0	1.3	0.3	384

The results from (B×D) are quite as expected. The white-suppressing modifiers of strain D have reduced *Ss* to a distribution similar to that of *SS* of strain A, and the *ss* segregants in F_2 must have a distribution much like that of *Ss* of F_1 (B×A).

Analysis of variability

Having established that there is just one major pair of alleles affecting white spotting in a number of diverse stocks, it is desirable to return to the evaluation of the roles of heredity and environment in determining the variation within a spotted stock. In doing this it is desirable to allow for the obvious damping of variability at both extremes of the range from self color to self white. An approximate method of doing this has been discussed in previous papers (WRIGHT 1920, 1926). The assumption that the pigmentation tendencies of the various areas of the coat are distributed normally leads to the transformation $X' = \text{prf}^{-1}(X - .50)$ where X is the proportion of color in the coat and prf^{-1} is the inverse probability function.

For the purpose of calculating the correlations, the grades were grouped in pairs and a transformed grade was found for the mid point of each (table 14). Table 15 shows the correlations between parents, between parent-offspring by sex and between littermates also by sex in a random bred stock and in a portion of strain 35 derived from a single mating in the 12th generation of brother-sister mating, using the above transformation of scale. The records for these two stocks were made during the same period of years (1916 to 1924) and are thus strictly comparable. The same set of correlations but without the transformation of scale has been found for a more recent branch (1926–1934) of strain 35, tracing to a single mat-

TABLE 14

Transformed grades, based on the inverse probability function of the midgrades and used in calculating standard deviations and correlation coefficients.

GRADE	MIDGRADE PERCENTAGE (X)	TRANSFORMED GRADE X' $\left(X' = \text{prf}^{-1}\frac{(X-50)}{100}\right)$
X– 2	6.25	−1.534
3– 4	17.5	− .935
5– 6	27.5	− .598
7– 8	37.5	− .319
9–10	47.5	− .063
11–12	57.5	+ .189
13–14	67.5	+ .454
15–16	77.5	+ .755
17–18	87.5	+1.150
19–20	96.25	+1.780

ing in the 22nd generation (35 D) (table 15) and for strain 2 and the two branches of strain 13 described earlier in this paper (table 16). In the case of 35 D, the correlations among brothers and sisters which were not littermates were also found. The correlations between brothers and between sisters were calculated from symmetrical tables, each pair being entered twice.

In no case was there a significant amount of assortative mating. There are clearly significant correlations between parent and offspring in the random bred stock (average +.191) but none that are significant among the inbred strains (grand average +.016). The littermate correlations (+.282) are larger than the parent-offspring correlations (+.191) in the random bred stock; and though small are clearly significant among the inbred strains (grand average +.079). These results can only be due to environmental factors common to littermates. This interpretation is confirmed by the absence of positive correlation (−.048) between siblings which were not littermates in the inbred strain 35 D.

For analysis of the variability we can use only the data based on the transformed scale. If h^2 represents the portion of the variance due to heredity, e^2 that due to environment common to littermates and d^2 that due to environment not common to littermates; the correlation between parent and offspring should equal $\frac{1}{2}h^2$ if the effects of all genes combine additively (no dominance or epistasis) but should be somewhat less if these conditions are not met (WRIGHT 1920). Under the same conditions the

TABLE 15

Parent-offspring and fraternal correlations in a random bred stock, and in the inbred strain No. 35 at two periods. Transformed grades used in case of random breds and the earlier data from strain 35. Untransformed grades used in 35 D.

		RANDOM BRED STOCK		STRAIN 35 (1916–24)		STRAIN 35 D (1926–34)	
		NO.	r	NO.	r	NO.	r
Father-Mother		143	+.060	140	+.064	72	−.047
Parent-offspring	♂-♂	973	+.244	738	+.015	200	−.068
	♂-♀	929	+.187	688	+.074	190	+.149
	♀-♂	1014	+.217	738	+.013	196	+.052
	♀-♀	965	+.116	688	−.004	185	−.031
	Average	**3881**	**+.191**	**2852**	**+.024**	**771**	**+.026**
Littermates	♂-♂	537	+.355	340	+.128	91	+.024
	♂-♀	1050	+.288	722	+.089	180	+.109
	♀-♀	493	+.190	305	+.107	89	+.218
	Average	**2080**	**+.282**	**1367**	**+.103**	**360**	**+.115**
Sibs not littermates	♂-♂					335	−.061
	♂-♀					509	−.049
	♀-♀					202	−.021
	Average					**1046**	**−.048**

correlation between littermates would be $\frac{1}{2}h^2+e^2$. The assumption of complete dominance and equal frequencies of alleles reduces the parent-offspring correlation to $1/3h^2$ and that between littermates to $5/12h^2+e^2$. In all cases, dominance reduces the genetic component of the fraternal correlation just half as much as it does the parent-offspring correlation (PEARSON 1909; WEINBERG 1910; FISHER 1918). Any non-additive effects among genes which are not alleles (epistasis) also reduces the correlations between relatives (WRIGHT 1935).

The average standard deviation of males and females in the random bred stock was .757, that in strain 35 was .583 (transformed scale). The

variance of strain 35 is thus 59 per cent $\left(\frac{.583^2}{.757^2}\right)$ of that of the random bred stock indicating that about 41 per cent of the latter variance was due to heredity, and had been lost after long continued inbreeding of strain 35. The correlation between parent and offspring in the random-bred stock (+.191) indicates 38 per cent (=2×.19) of the variance as due to additive gene effects. This differs so little from the 41 per cent estimated from the actual variances that little complication from non-additive gene

TABLE 16

Parent-offspring and fraternal correlations in 3 inbred strains, characterized by very large amounts of white. Untransformed grades.

	STRAIN 2 (1926–34)		STRAIN 13 (1926–34)		STRAIN 13 E (1926–34)		TOTAL	
	NO.	r	NO.	r	NO.	r	NO.	r
Father-Mother	94	+.019	86	+.220	105	+.027	285	+.089
Parent-offspring ♂-♂	402	+.035	486	+.007	368	−.078	1256	−.012
♂-♀	437	+.149	436	+.036	375	−.132	1248	+.018
♀-♂	408	+.044	488	+.041	366	−.019	1262	+.022
♀-♀	433	+.029	436	−.049	375	+.029	1244	+.003
Average	**1680**	**+.064**	**1846**	**+.009**	**1484**	**−.050**	**5010**	**+.008**
Litter-mates ♂-♂	179	+.098	250	+.096	188	+.011	617	+.071
♂-♀	372	+.074	451	+.090	358	+.005	1181	+.059
♀-♀	215	+.068	213	+.025	166	+.054	594	+.049
Average	**766**	**+.078**	**914**	**+.077**	**712**	**+.018**	**2392**	**+.060**

effects (dominance or epistasis) is indicated. It should be said, however, that another method of calculating the transformed standard deviations of the inbred and random bred strains was made by fitting the distributions by the function $\text{prf}^{-1}(\Sigma f - .50) = 1/s\,[\text{prf}^{-1}(X - .50) - a]$ where a and s are the mean and standard deviation on the transformed scale and Σf the running sum of the fractional frequencies (WRIGHT 1926). This yields 54 instead of 59 per cent as the ratio of the variances (s=.782 in random bred stock, s=.574 in strain 35). A somewhat greater role of non-additive gene effects is indicated by this method. The difference was due mainly to irregularities in the distribution of the random bred animals.

The females exceeded the males in amount of white by .241 on the transformed scale in the control stock. This is 31.3 per cent of the standard deviation of the total population (.768). In strain 35, the difference was

.209 or 35.2 per cent of the standard deviation of the total population (.593). The proportion of the total variance due to sex is given by the ratio of the square of the half difference to the total variance, or one fourth the square of the above fractions. It appears that 2.5 per cent of the variance in the random bred stock and 3.1 per cent in strain 35 was due to sex. The analysis into genetic and nongenetic portions applies of course to the 98–97 per cent not due to sex.

The littermate correlation in strain 35 (+.103) can be taken as indicating the portion of the variance (within each sex) which is due to environment common to littermates. This is on the assumption that the

TABLE 17

Mean percentages of white in males and females of strain 35, at two periods, in relation to age of mother.

AGE OF MOTHER (MONTHS)	STRAIN 35 (1916–24)				STRAIN 35 D (1926–34)			
	MALES		FEMALES		MALES		FEMALES	
	NO.	AV.	NO.	AV.	NO.	AV.	NO.	AV.
3– 5	182	56.3	153	60.5	25	56.0	31	65.0
6– 8	195	59.5	187	67.6	45	67.8	41	70.2
9–11	152	60.6	160	66.5	40	71.7	21	78.1
12–14	150	61.3	124	70.6	33	69.8	30	76.3
15–20	174	63.2	149	69.6	27	61.9	34	74.7
21–46	138	66.9	144	73.3	23	78.2	25	77.6
Total	**991**	**61.1**	**917**	**67.8**	**193**	**67.8**	**182**	**73.1**

parent-offspring correlation (+.024) can be ignored. If the latter is accepted, the estimate is reduced to +.079. The figures from the other inbred strains confirm the existence of an effect of the order of these figures as does the difference (+.091) between littermate and parent-offspring correlations in the control stock. It is indicated that the greater portion of the variance (89.7 per cent in strain 35) is due to environmental factors so local in incidence that they do not affect littermates alike.

The only direct evidence on the nature of the environmental factors common to littermates is given by tabulations of grade of spotting in relation to age of mother. Such tabulations have been made in strains 35, 35 D, 2 and 13. In the last two cases no significant relations were found, but the highly asymmetrical distributions banked up against the limit of 100 per cent white make these unfavorable material for detecting slight differences. The results in 35 and 35 D are shown in table 17. In strain 35, there is a fairly regular increase in percentage of white with increasing age of the parents (usually littermates) at a rate of 0.68 per cent per month

TABLE 18

Joint frequencies of parent and offspring in inbred strain 35. Correlation (broad categories) +.026

PARENT	OFFSPRING GRADE OF SPOTTING X-	3-	5-	7-	9-	11-	13-	15-	17-	19-	TOTAL
19–20	—	1	5	6	8	7	22	18	10	4	81
17–18	1	7	28	22	51	68	82	73	53	14	399
15–16	6	8	24	43	38	80	97	96	57	17	466
13–14	2	7	25	35	74	90	103	121	72	14	543
11–12	6	8	22	44	59	102	105	99	77	21	543
9–10	3	7	19	24	34	60	66	58	62	10	343
7– 8	3	6	14	29	34	64	51	63	45	8	317
5– 6	—	2	6	13	22	15	28	20	16	1	123
3– 4	1	—	—	2	1	1	1	1	1	—	8
X– 2	2	—	1	2	5	5	7	3	3	1	29
Total	24	46	144	220	326	492	562	552	396	90	2852

up to 15 months of age. This factor determines about 3.6 per cent of the total variance and thus approximately half of that common to littermates. In 35 D, the results are more irregular as expected from the smaller numbers but the correlation of $+.252 \pm .069$ for females, $+.203 \pm .069$ for males give clear evidence of significance (the average $+.227 \pm .049$ is 4.6 times its standard error). From the square of this figure it appears that about 5 per cent of the variance (untransformed scale) was due to this factor. This would be slightly reduced by transformation of the scale. The regression, 0.76 percent white per month (up to 15 months) agrees reasonably well with the earlier data. It should be noted that in case (35 D) the effect is definitely one of age of mother, since most matings were intentionally

TABLE 19

Comparison of correlations obtained by the broad category method with the averages of those given in tables 15 and 16.

STRAIN	PARENT-OFFSPRING NO.	CORRELATION TABLES 15, 16	CORRELATION BROAD CAT.	LITTERMATES NO.	CORRELATION TABLES 15, 16	CORRELATION BROAD CAT.	SIBS, DIFFERENT LITTERS NO.	CORRELATION TABLE 15	CORRELATION BROAD CAT.
35	2852	+.024	+.026	1367	+.103	+.075			
35D	771	+.026	+.020	360	+.115	+.083	1046	−.048	−.045
2	1680	+.064	+.059	766	+.078	+.101			
13	1846	+.009	+.008	914	+.077	+.090			
13E	1484	−.050	−.066	712	+.018	+.005			
Av. 5 inbred strains	8633	+.016	+.012	4119	+.079	+.072			
Random bred-stock	3881	+.191	+.199	2080	+.282	+.260			

made between males and females of widely different ages (WRIGHT 1935, p. 526).

It has seemed desirable to check the parent-offspring and littermate correlations by a different method of allowing for the distortion of the scale. For this purpose the tabulations by sex were combined into single tables and correlations calculated by PEARSON'S method for broad categories. The parent-offspring distribution for strain 35 is shown in table 18; those for littermates in this stock and those in the random bred stock have been deposited with "Genetics." In the broad category method, the mean

TABLE 20

Deviations of class limits from the median in the random bred stock and in strain 35 in terms of their standard deviations assuming normality. See fig. 1.

CLASS LIMIT (% WHITE)	RANDOM BRED STOCK		STRAIN 35	
	100 q	$\frac{x}{\sigma}$	100 q	$\frac{x}{\sigma}$
0	0	$-\infty$	0	$-\infty$
12.5	1.37	−2.206	0.84	−2.391
22.5	3.46	−1.817	2.45	−1.969
32.5	7.09	−1.469	7.50	−1.439
42.5	13.17	*−1.118*	15.21	*−1.027*
52.5	21.62	− .785	26.64	− .624
62.5	32.88	− .443	43.89	− .154
72.5	47.90	− .053	63.60	+ .348
82.5	66.09	+ *.415*	83.95	+ *.952*
92.5	80.98	+ .877	96.84	+1.858
100	100.00	$+\infty$	100.00	$+\infty$

of each class on a hypothetical normal scale, is calculated by the formula $x=\frac{z_1-z_2}{q_1-q_2}$ where q_1 and q_2 are the observed tail frequencies (fractions of 1) cut off by the class limits and z_1 and z_2 are the theoretical ordinates of the unit normal curve at these points. The approximate correlation is given by the formula $r_{x_1x_2}=\frac{\Sigma x_1x_2f}{n\sigma_{x_1}{}^2\sigma_{x_2}{}^2}$. The results are given in table 19. Comparisons are made with the averages of the correlations for separate sexes from tables 15 and 16. The latter might be expected to be slightly larger because of the elimination of sex differences but the different scales prevent exact comparison. On the whole the differences are unimportant.

In this method, the scale is adjusted in each case so as to yield a corrected standard deviation of 1. Thus each distribution yields a separate scale. The transformed location of each class limit of the observed scale can be found by taking $X/\sigma=prf^{-1}(q-.50)$. This is done for the random bred stock and for strain 35 in table 20 and the results plotted against each other in figure 1. With the exception of the boundary between classes 18

and 19 (92.5 per cent white) the values fall rather closely along a straight line. Taking the values at 42.5 and 82.5 as those closest to the standard deviations, considering both scales (fig. 1) we see that a scale interval of 1.533σ in the random bred stock corresponds to one of 1.979σ for strain 35. The corrected standard deviation of strain 35 is thus 77.5 per cent $= \frac{1.533}{1.979}$ of that of the random bred stock and its variance is 60.1 per cent $(= .775^2)$

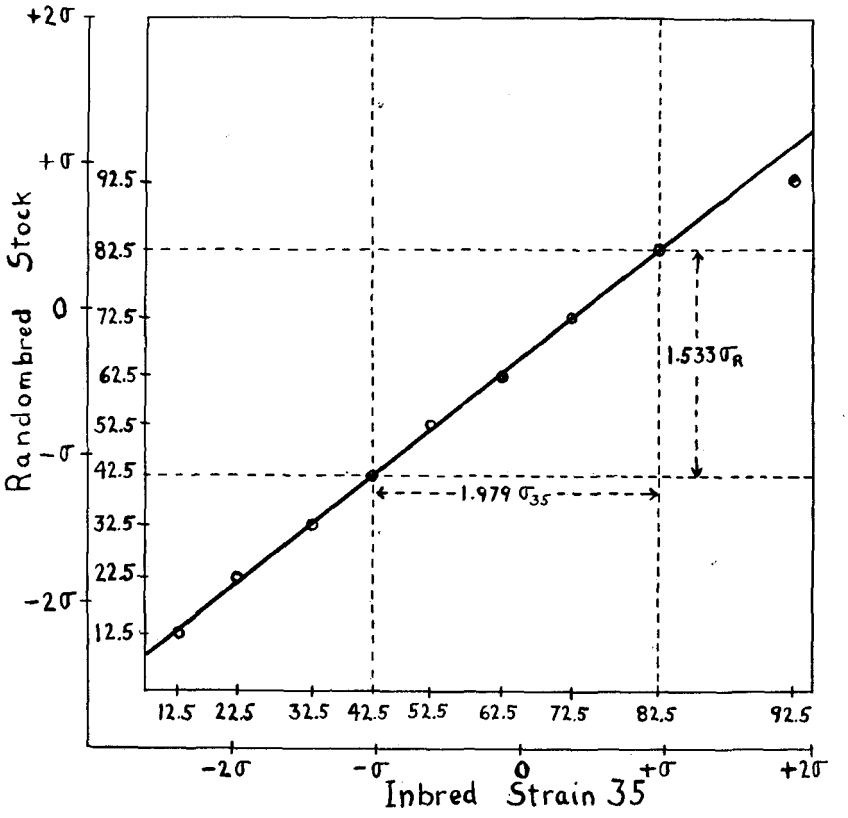

FIGURE 1. Transformation of class limits to give normal distribution of class frequencies in random bred stock plotted against similar transformation for inbred strain 35. The range between 42.5% white and 82.5% white includes 1.979σ in the inbred strain but 1.533σ in the random breds. The variance of the inbred stock is thus 60.1% $(= 1.533^2/1.979^2)$ of that of the random bred stock on a scale based on these two points.

of that of the random bred stock. Since the correlation between parent and offspring in the random bred stock was +.199 by the broad category method, the genetic variance (assuming no dominance or epistasis) should be 39.8 per cent and the variance to be expected in an isogenic stock should be 60.2 per cent of that in the random bred stock, or almost exactly the

value actually found in strain 35 by the above method. The agreement with results from the previous method is as close as can be expected. There can be little or no dominance or epistasis among the minor factors which affect spotting.

Putting all of these results together we reach the following approximate analysis of variance in the random bred stock and an isogenic inbred strain.

	Isogenic Inbred Strain	Random Bred Stock
Heredity	0	40
Sex	3	2
Environment		
Age of mother	4	6
Other factors common to littermates	4	
Factors not common to littermates	89	52
	100	100

DISCUSSION

The results fall into a consistent picture. Four classes of factors are indicated.

First is a major pair of alleles, *S,s*. *SS* is usually self but under exceptional conditions shows traces of white up to a small shoulder belt; *Ss* may be self colored or any grade of spotted up to at least 95 per cent white; *ss* is usually well marked with white, often self white, but may have only a trace of white and perhaps may be completely self-colored under very unusual conditions.

Second is a multiplicity of minor genetic factors with additive effects (little or no dominance or epistasis). The median grade of *ss* can be shifted from about 10 to 97 per cent white by the appropriate combination. The median grade of *Ss* may similarly be shifted from self color to about 30 percent white. Finally a little white may appear in *SS* with an extreme array of white modifiers.

Third, there is an enormous amount of non-genetic variability responsible for a range extending from a trace of white to 100 per cent white in isogenic strains whose median grade is near 50 per cent. This can be subdivided into a small portion composed of factors common to littermates, in which is included an effect of age of mother in at least one stock, and a large portion not common to littermates and hence to be interpreted as due to developmental accidents. In a typical random bred stock, all *ss*, about 58 per cent of the variance was due to non-genetic factors, including only 6 per cent common to littermates.

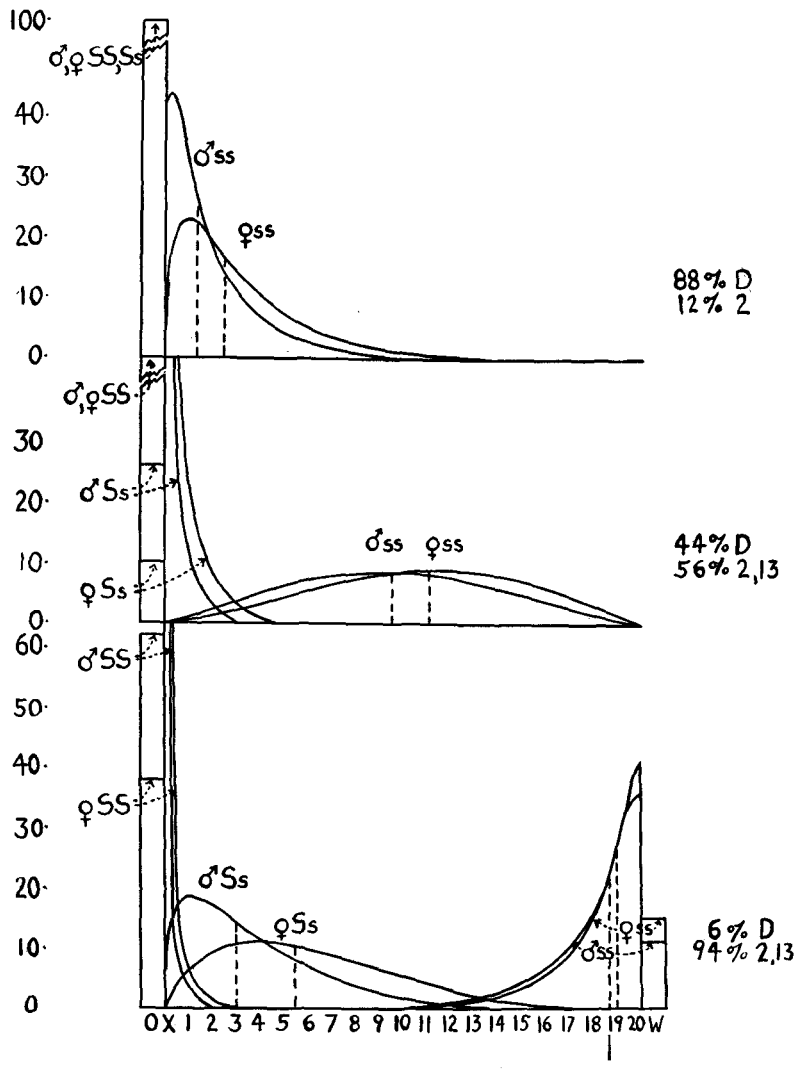

FIGURE 2. Distributions of *SS*, *Ss*, and *ss* according to sex and residual heredity. The abscissas are the classes used in grading. The ordinates are the fitted percentage frequencies in these classes, doubled in the cases of X and 20 to allow for the fact that their ranges are only of half width. Classes O and W are arbitrarily assigned full class ranges. Smooth curves are fitted by the formula $prf^{-1}[\sum f - .50] = 1/.57\,[prf^{-1}(x - .50) - prf^{-1}(M - .50)]$ where prf^{-1} is the inverse probability function, $\sum f$ is the running sum of the fractional frequencies, .57 is the standard deviation (on the transformed scale) of an isogenic stock and M is the median of the distribution to be fitted. The class limits and medians are modified slightly by replacing x by the function $.98x + .0025$ in order to give a class of solid color 0%–.25%) and a class of solid white (98.25%–100%). The medians in the upper series are from 7/8*D* table 7, those in the middle series from (7/8*D*×2), table 7 in the case of *ss* and from (7/8*D*×13), table 8, in the case of *Ss*. The medians in the bottom figure are based on backcrosses (and derivatives) with 7/8 or more of strain 13 (*SS* table 10: *Ss*, *ss* table 9). The distributions so fitted all agree reasonably well with the observed distributions.

Fourth is a sex difference, females having slightly more white than males in all strains, the difference accounting for 2 or 3 per cent of the total variance.

The effects of these four classes of factors are illustrated in figure 2. The variability of males and females of *SS*, *Ss* and *ss*, due to non-genetic factors are shown at the top on a background of modifiers suppressing white, at the bottom with modifiers favoring white, and in the middle with a more typical, intermediate combination of modifiers. The sex difference appears to be greatest in stocks with somewhat less than 50% white in the coat on the average.

We have finally to consider the relation of these results to those of other authors.

Our pair, *S,s*, is doubtless the same as IBSEN'S *S,s* although IBSEN refers to *S* as dominant. IBSEN'S other factors *Fa, fa*; *Na, na*, obviously cannot be used in the interpretation of our results. Judgment as to whether the enormous amount of non-genetic variability of all of our stocks is largely absent from his stocks must await publication of his data.

PICTET'S 4-factor hypothesis also is obviously inapplicable to our data. In our data there is clearly just one major factor with effects on both head and trunk; *ss* typically has much white on both head and trunk, in *Ss* both are reduced while *SS* extracted after many generations of back-crossing to a stock in which both head and trunk are almost wholly white, is usually completely self. The correlation between head and trunk is not perfect, but neither is the correlation between any two areas which could be named, for example, left and right ears, left and right fore feet, shoulder and loin (cf. ILJIN 1928). PICTET has presented no data which justify the conclusion that his stock differs in these respects.

PICTET'S most extensive published data refer to the trunk pattern. How far is our interpretation applicable? It is not possible to give a certain answer. However, it seems possible that the dominant and recessive spotting factors which he found in his two original sets of matings may have been the same factor, *s*, associated with different arrays of modifiers as in our experiments involving 7/8 blood strain 13 and 7/8 blood strain D respectively. With respect to his later generations, derived from crosses of the two foundation stocks, there are so many possible genotypes for every phenotype in both hypotheses and such a wide range of phenotypes for each genotype in ours that it seems not unlikely either could be made to fit. It would require experiments with isogenic stocks to discriminate between them.

Whether there is any non-genetic variability in his stock, that is, a wide range of phenotypes for each genotype, is not considered by PICTET. But the irregularity in localization of spots and frequent asymmetry

which seem to have characterized his stock as well as ours make the existence of such variability probable. If a type of spotting is ever discovered in the guinea pig, which is not subject to non-genetic variability to an important extent, it will probably show something of the orderliness of pattern of hooded rats or Dutch rabbits.

SUMMARY

Analysis of the results of crosses among a considerable number of closely inbred strains of guinea pigs, supplemented by biometric analysis of variability and correlations in random bred and inbred stocks indicate 4 classes of factors as affecting white spotting:

(1) a major pair of alleles *S*,*s* in which *S* (tending toward self) is usually incompletely dominant (statistically) over *s* (tending toward white),

(2) a multiplicity of genes with individually small effects, additive on a suitably transformed scale (no dominance or epistasis),

(3) an enormous amount of non-hereditary variability, not common for the most part even to littermates, but including minor effects of common factors (for example increasing white in young with increasing age of mother),

(4) a sex difference, females having slightly more white than males on the average in all strains.

The interpretations which have been put on white spotting by other recent authors, who have largely ignored the possibility of non-hereditary variability, are shown to be inapplicable to the results described here.

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