STUDIES ON THE TRICOLOR PATTERN OF THE GUINEA PIG.[1] II. THE DISTRIBUTION OF BLACK AND YELLOW AS AFFECTED BY WHITE SPOTTING AND BY IMPERFECT DOMINANCE IN THE TORTOISE SHELL SERIES OF ALLELES

HERMAN B. CHASE[2]
The University of Chicago, Chicago, Illinois

Received March 3, 1939

GENETICS OF THE TRICOLOR PATTERN

THE tricolor pattern of the guinea pig can be analyzed into two components: (1) the pattern of color and white and (2) the pattern of black and yellow. The former has been considered in the previous paper (CHASE 1939).

Pattern of black and yellow

The tortoise shell pattern consists of yellow hairs sprinkled lightly among black hairs in a few irregular, asymmetrical regions over the body. This is the typical pattern of family D, a highly inbred strain which never shows white. Occasionally a foot has a small area of segregated yellow at the tip. Large areas often appear to be solid black.

The tricolor pattern of black, yellow, and white, was a feature of the earliest scientific description of the guinea pig. According to CUVIER this variety was described by ALDROVANDUS about 1550 (CASTLE 1912). Compared with the tortoise shell, the tricolor has a tendency to greater segregation of black and yellow hairs (WRIGHT 1917), giving typically in addition to some brindling clear areas of black, yellow, and white. The individual irregularity of the black-yellow pattern is even greater than for the color-white pattern.

In 1916 IBSEN proposed the symbol e^p (partial extension of black) for the gene determining the tortoise shell pattern. Later (1919) he reported on the inheritance of the three alleles at this locus, E (self black) dominant over e^p and e (self yellow), and e^p dominant over e. The question of dominance in multiple allelic series is interesting and a quantititve study has been one of the objects in the present investigation.

No statistical analysis of the array of modifiers of this pattern has been made but the existence of such modifiers will be shown by differences in average number of yellow hairs in certain experiments.

[1] Submitted in partial fulfilment for the degree of Doctor of Philosophy at the University of Chicago.

[2] The author is grateful to PROFESSOR SEWALL WRIGHT of the University of Chicago for the suggestion of this problem, the use of pattern records, the facilities for breeding experiments, and the constant stimulating guidance throughout the work. The guinea pig colony has been maintained under a grant to the University of Chicago from the Rockefeller Foundation.

One modifier, however, has been a principal object of the present paper. This is white spotting. WRIGHT (1917, 1923) observed the relation between these two types of spotting, the presence of white tending to decrease the amount of brindle, to separate the pigmentation into clear-cut areas of black and yellow, and where brindling persists to organize the spots so that black tends to be central and yellow peripheral. The effect of white spotting on tortoise shell has also been discussed by ILJIN (1928). WRIGHT found no change in the ratio of yellow to black areas in relation to increased amounts of white in tricolors of the same stock. In animals of low grade spotting, much brindling is generally still present as well as the segregated colors; in animals of high grade spotting, brindling may occur but is comparatively rare. Frequently in the case of the latter, only one color is present in the spots. These occasional bicolors (black-white, yellow-white) breed, however, as normal tricolors. Whether these effects depend on white spotting as such or on the specific factor *s* is a question which will be considered later.

Of the non-hereditary factors involved in the black-yellow pattern, no statistical analysis has been made but the great irregularity and asymmetry demonstrate clearly their existence.

That sex modified both aspects of the tricolor pattern was shown by WRIGHT (1917, 1920). His studies indicated that males have seven percent more color than females and four percent more black in the colored spots.

Of tortoise shell patterns in other mammals, that of the cat is of particular interest here. The heterozygous condition of the sex-linked black and yellow factors produces a mixture of black and yellow. WHITING (1919) found that the presence of white tended to decrease the amount of brindle and to separate the coat into clear areas of black and yellow. He was unable to discover any increase in the amount of yellow relative to black. Black and yellow spotting in the rabbit (Japanese variety) was reported by CASTLE (1924) and PUNNETT (1924) and is due to an allele in the black series as is the case for the guinea pig. An additional case in mammals is that in swine (WRIGHT 1918). The Berkshires and Poland-Chinas are black with white largely at the extremities. Analysis of crosses indicated that the white of this pattern represented tortoise shell yellow diluted to white. Crosses between Berkshires or Poland-Chinas and red breeds produce well-defined tortoise shells. The data of LUSH (1921) and WARWICK (1926, 1931) indicate that tortoise shell differs from self red by one major factor whereas the extent of black in tortoise shells depends on multiple factors. The black-white extended tortoise shell of Berkshires and Poland-Chinas contrasts with the black-white pattern of Hampshires which resembles somewhat that of the Dutch rabbit and probably belongs in the same category with the spotting patterns of color and white.

Analysis of cross between tricolor and self-yellow

The tricolor stock used was DS (figure 1, CHASE 1939). Its origin from strains D and 2 has been described in Part I. It should be noted that strain D, constituting seven-eighths of its ancestry, had been closely inbred since 1906 (first by PROFESSOR W. E. CASTLE and later by PROFESSOR SEWALL WRIGHT). While considerable fixation might be expected from

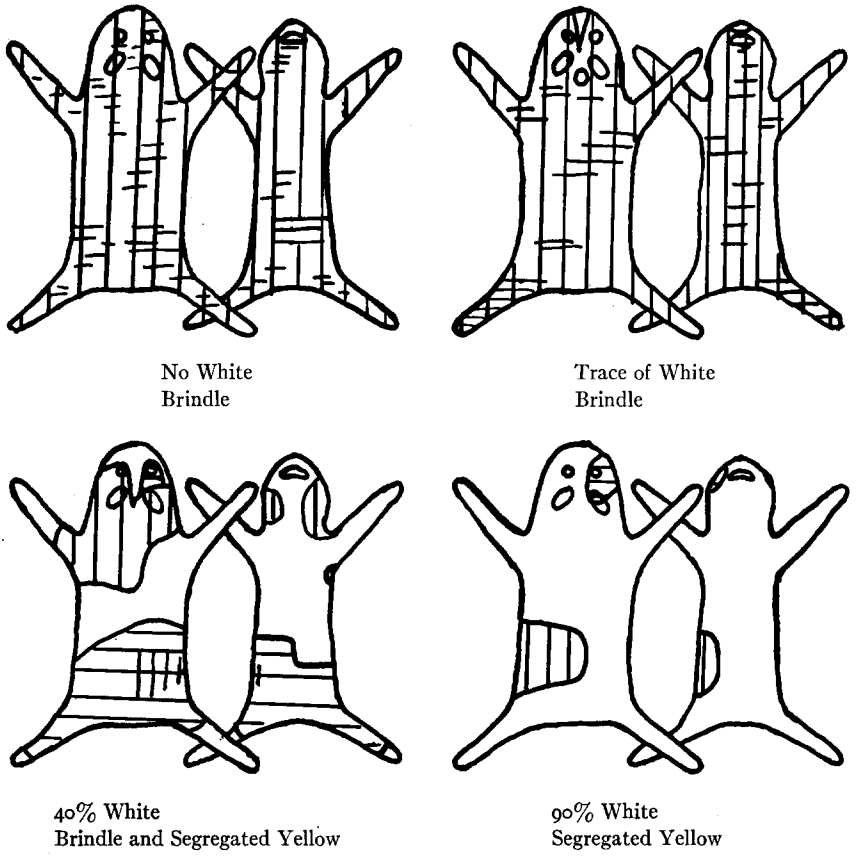

FIGURE 1.—Typical patterns of e^{p} animals. Areas are represented by vertical lines for black, by horizontal lines for yellow, and by clear regions for white.

the procedure involved in deriving DS, persistent heterozygosity was shown by a parent-offspring correlation of +.182 as compared with the similar correlation of a random bred stock, +.191. The average in five inbred stocks was only +.012 (WRIGHT and CHASE 1936).

Strain 2 has high grade spotting (median percentage white 93.2 in males, 94.6 in females), strain DS has low grade spotting (6.5 in males, 12.0 in females), the difference being due solely to the seven-eighths of its modifiers which came from D. It was desirable to learn something of

the nature of these modifiers and although the lack of homozygosity in DS made it rather unsatisfactory material for this purpose, some data were obtained. Strains DS and 2 were crossed giving 80 animals in the F_1 generation and 72 in the F_2 generation. The ranges were similar (table 1), the slightness of the increase of variability of F_2 over F_1 indicates multiple factors.

TABLE 1

Strain 2 by strain DS. Distribution of 2, DS, F_1, F_2, and backcross to DS. Males, females, and reciprocal crosses combined.

CROSS	GRADE OF WHITE SPOTTING*											NUMBER	MEDIAN PERCENT
	o	x	1	2	3–5	6–8	9–11	12–14	15–17	18–20	W		
2	—	—	—	—	—	3	33	96	343	1125	50	1650	93.90
DS	—	29	37	35	30	10	1	2	1	—	—	145	9.30
F_1	—	—	—	—	12	15	17	18	12	6	—	80	52.50
F_2	—	—	2	2	8	7	17	16	16	4	—	72	57.50
Backcross to DS	—	1	5	4	7	6	3	1	—	—	—	27	21.25

* o—no white, x—trace of white; 1 = 2.5–7.5% white; 2–20 = 5% intervals up to all white (W).

Strain DS (tricolor) was crossed with yellows to obtain data on the dominance of e^p over e and on the relation of tortoise shell and white spotting. A stock of yellows having the dilution factor c^kc^k (WRIGHT 1925) was used. Strain DS of constitution *CC* shows intense yellow (or red). Phenotypically the parental types were black-red-whites (sse^pe^pCC) and yellows (mostly $SSeec^kc^k$). The distribution of intense (*C*) and dilute (c^k) young seems to present nothing of interest and is not considered.

Table 2 gives the total F_1 and F_2 progeny according to sex, color, and the amount of white. The F_1 generation from the usual self yellow parent, consisted of black-reds with no white (61 percent) or a little white (D modifiers frequently repressing the appearance of white in these *Ss* animals). The segregants in the F_2 generation from the matings *inter se* of the F_1 heterozygotes were the animals used in this analysis. The original animals (grandparents) from which all the F_2 descended were three tricolor males, two tricolor females, two yellow males, and seventeen yellow females. Having about 31 pairs of chromosomes (60 to 65 chromosomes, LEAGUE 1928, MOLS 1928), F_2 segregants with respect to any one locus should have, on the average, at least 97 percent of the same modifiers. There is thus little chance that results have been due to linkage to any important extent.

TABLE 2

Distribution of F_1 and F_2 from cross between tricolor and yellow.

MATING	TYPE	AMOUNT OF WHITE IN PERCENT						NUMBER
		0	TRACE	5	10–20	25–50	55–90	
ssᵉᵖeᵖ	*eᵖe* ♂	23	11	1	—	—	—	35
F_1 ×	*eᵖe* ♀	26	19	—	—	—	—	45
SSee								
ssᵉᵖeᵖ	*eᵖe* ♂	2	—	—	—	1	2	5
F_1 ×	*eᵖe* ♀	1	2	—	1	1	2	7
Ssee								
Sseᵖe	*eᵖ*— ♂	71	19	5	12	9	3	119
F_2 ×	*eᵖ*— ♀	43	15	3	4	7	3	75
Sseᵖe	*ee* ♂	17	10	1	5	2	2	37
	ee ♀	19	9	4	5	6	3	46
Sseᵖe	*eᵖ*— ♂	5	—	—	2	—	—	7
F_2 ×	*eᵖ*— ♀	7	2	2	—	5	2	18
ssᵉᵖe	*ee* ♂	1	1	—	—	—	—	2
	ee ♀	—	1	—	—	1	—	2

For 277 F_2's (those from $Ss \times Ss$) the expectation is 25 percent *SS*, 50 percent *Ss*, and 25 percent *ss*. For the remaining 29 F_2's (from $Ss \times ss$) the expectation is 50 percent *Ss* and 50 percent *ss*. Combining, the expectation for the total F_2 is found to be 22.6 percent *SS*, 50 percent *Ss*, and 27.4 percent *ss*. Assuming that in this cross *SS* is always self and *ss* is always at least 2.5 percent white, the total F_2 can be analyzed as in table 3. In

TABLE 3

Percentage analysis of S, s in F_2.

GENOTYPE	AMOUNT OF WHITE IN PERCENT				TOTALS
	0	TRACE	5	10–90	
SS	22.6	—	—	—	22.6
Ss	30.7	18.6	0.7	—	50.0
ss	—	—	4.2	23.2	27.4
	53.3	18.6	4.9	23.2	100.0

the F_2 generation over half (58 percent) of the self animals were probably heterozygous for the white spotting factor. From another viewpoint, about 61.3 percent of *Ss* in F_2 were self. This agrees well with the 61 percent self of the F_1's that were *Ss*. The total F_2 distribution indicates that the line of cleavage between *Ss* and *ss* is in the category of five percent

(grade 1). The overlap may extend beyond this category (2.5 to 7.5 percent white) but from table 3 as well as from breeding tests and the F_1 distribution there is probably very little overlap. In this experiment males and females agree so closely in the amount of white that for each the line of cleavage falls within this category of five percent (males at the line between trace and five percent). Of the *ss* animals most are in the region from 10 to 50 percent, only one having as much as 90 percent white. With possibly rare exceptions the genotypes and phenotypes are thus: *SS* self; *Ss* self or a trace of white, rarely in the class 2.5 to 7.5 percent white; *ss* rarely in the class 2.5 to 7.5 and ranging up to 90 percent.

TABLE 4

Tests of the F_2 tortoise shells and tricolors.

MATING	PROGENY		
	SEX	e^pe	ee
♀ e^pe^p (18)×♂ ee	♂	43	—
	♀	44	—
♀ ee×♂ e^pe^p (27)	♂	115	—
	♀	119	—
♀ e^pe (40)×♂ ee	♂	25	29
	♀	34	29
♀ ee×♂ e^pe (49)	♂	85	74
	♀	53	75

The results in F_2 (see table 2) from *Ss* by *Ss* are decidedly irregular both with respect to sex ratio (of tortoise shells and tricolors) and with respect to the segregation of yellow (in females). The probability that chance might yield such a series of deviations is only .003 ($\chi^2 = 14.0$, three degrees of freedom). Nevertheless, this probably is merely an extreme chance deviation since on adding the small number of F_2's from *Ss* by *ss*, which happen to show a compensating deviation, χ^2 falls to 7.6 and P rises to .06.

Tortoise shells and tricolors of the F_2 generation have been tested as far as possible to determine which animals are e^pe^p and which, e^pe. The test involves backcrossing to a yellow (*ee*). Of 129 animals having had test progeny at this time, 89 have had at least one yellow offspring and are e^pe, 40 have had no yellows among from two to 24 offspring and are probably e^pe^p (three exceptions probable). Table 4 gives the test data. Of the 40 classified as e^pe^p, three have had two young, five have had three young, six have had four young, and the remainder have had five or more.

A few of these (expectation 3.0) are no doubt $e^p e$ but there should be no serious statistical error in treating this group as $e^r e^p$.

In order to describe in detail the interrelationships between the white and yellow spotting patterns, a precise and objective method of recording the latter was necessary. The amount of white was calculated by dividing the coat into 20 squares on a tracing cloth and reliable estimates were obtained from the recorded drawings (WRIGHT and CHASE 1936). The measurement of tortoise shell was more difficult. The same 30 points were used as in the correlation studies (figure 6, CHASE 1939). These could be detected objectively on each animal. Such points were the tip of the middle toe on the hind foot, the most dorsal point of the scapula in normal resting position, *et cetera*. By using available points of reference, as umbilicus, mammae, elbows, and so on, the 30 points are sufficiently constant from animal to animal. Hairs were plucked with one movement from the selected point with no regard for the colors present. By this means an average of 50 hairs was obtained, spread out, and the first 25 counted. In this manner the numbers of black, yellow, and white hairs were tabulated for each of the 30 points. If the 25 hairs at a point were of one color, that point was termed "segregated." In a few cases all three colors were plucked at one point, in which case the proportion of colored hairs that were yellow was taken as the grade of brindle. This method, involving 750 hairs for each animal, appears to be a reliable and objective means of making comparisons with respect to the complicated tortoise shell pattern.

Dominance in E series

The problem of dominance is of special interest in multiple allelic series. *E* is completely dominant over the lower alleles, e^p and *e*. From PROFESSOR SEWALL WRIGHT'S unpublished data (included here with his permission), only one case of incomplete extension of black has been found among approximately 900 *Ee* guinea pigs (from matings of *Ee* by *ee*). This animal (♀ 9942 of experiment EC-92) was black with the right hind leg red (SSC^r). It bred like *Ee* in successive matings with two yellow (*ee*) males.

The uniformity of *ee* (no extension of black) is shown by the occurrence among over 3,000 *ee* animals with no e^p in the ancestry of only two with black spots. These animals, also from WRIGHT'S unpublished data, were ♂9807 of experiment ED-107 and ♀ 7167 of experiment Yd-f-45. Male 9807 was cream (SSc^dc^r) with a small sepia spot on the left shoulder at the mid-dorsal line. Female 7167 was yellow and white (Ssc^dc^d) with a dark sepia spot between the ears.

Now the question arises as to whether e^p is completely dominant over *e*. As noted above, the F_2 tortoise shells and tricolors in my experiment have

been tested as far as possible by mating to yellows to determine if they are homozygous or heterozygous for e^p. The amount of segregation of colors and the percentage of yellow hairs in the colored areas vary greatly among individuals similar in genotype and in amount of white. The ranges of the 40 possible homozygotes and the 89 heterozygotes overlap greatly as shown by the percentages of colored hairs that are yellow (table 5 and figure 2).

TABLE 5

Distribution of tortoise shells and tricolors of F_2 according to the percentage of colored hairs that are yellow, the amount of white, and the genotypes.

	AMOUNT OF WHITE																			
	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%	0	TRACE	5%	10–20%	25–90%
91–100																				
81– 90		1										1								
71– 80	1		1	1	3					1	1			1	1			1		1
61– 70	1	1		1	3						1	1			1				1	2
51– 60	7	3		3	4		1				6			2	2	1	2		1	2
41– 50	11	3	3	3	3	1					6	2	2	2		4	1	1	1	3
31– 40	17	6	3	4	2	2		1			9	6	2	3	1	6			1	1
21– 30	18	9	2	3	5	4			1	1	9	6		1	2	5	3	2	1	2
11– 20	28	5	1		4	9	1	1		3	9	2			1	10	2			
1– 10	30	3		1	1	10	1		1		6	1			1	14	1			
0	2			1	2	1				1					1	1			1	
PERCENTAGE OF COLORED HAIRS	TOTAL e^p— (200)					TESTED e^pe^p (40)					TESTED e^pe (89)					UNTESTED e^p— (71)				

In analyzing the distributions of table 5, the total distribution will be considered first with no separation according to the amount of white. The calculations are based on the actual individual percentages rather than on the class percentages. Table 6 indicates that there is no real difference between tested and untested but that there is a clearly significant difference between e^pe^p and e^pe. The standard error of the difference is calculated from the standard deviation of the total distribution ($\sigma = 19.0$) in order to avoid any argument in a circle which might come from the use of the separate standard deviations of e^pe^p and e^pe. This gives a conservative test.

It has been previously discussed that the break between *S-* and *ss* falls approximately between a trace and five percent white. Table 6 gives the analysis for the *S-* animals. Here again, basing the standard error of the difference on the standard deviation of the total ($\sigma = 17.3$), the difference between tested and untested is not significant but the difference between

$e^p e^p$ and $e^p e$ is clearly significant. No appreciable modification results from the consideration of selfs alone (no white). The difference between the means of $e^p e^p$ and $e^p e$ is 4.8 times the standard error (16.2 ± 3.4) if the calculation is based on the separate standard deviations of each class. The difference is 4.05 times the standard error (16.2 ± 4.0) if the calculation is based on the extremely conservative test (σ of the total number of

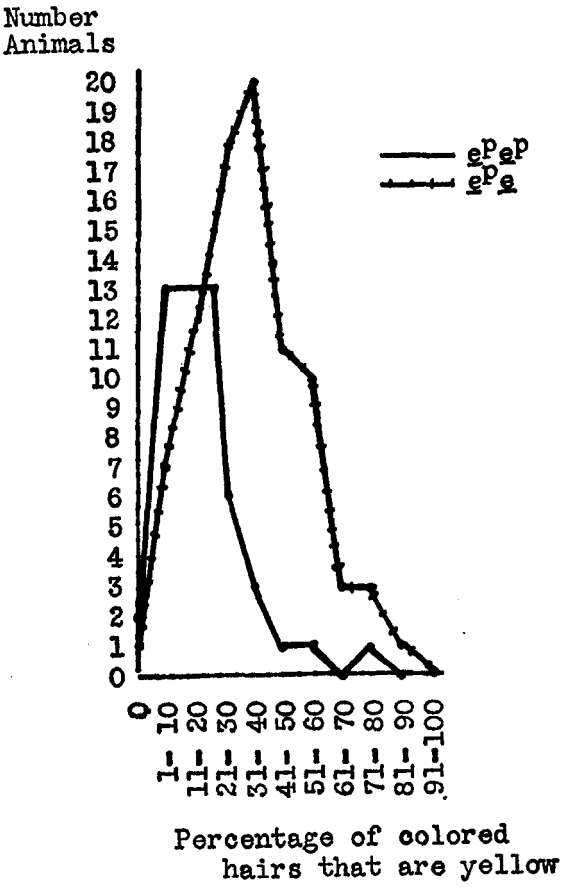

FIGURE 2.—Graphic distribution of the 40 F_2 animals which are tested $e^p e^p$ and of the 89 which are tested $e^p e$.

selfs, 115). Selfs which were *SS* (23) and selfs which were *Ss* (41) indicated no essential difference.

Finally, to complete the analysis, animals with 5 to 90 percent white (presumably nearly all *ss*) were considered (table 6). The difference between $e^p e^p$ and $e^p e$ is as great as in the near-self class but is of less certain significance because of small numbers. There is a considerable difference between tested and untested but it is in the opposite direction from that in the near-self group and therefore probably accidental.

TABLE 6

Percentage of yellow hairs in F_2 tortoise shells and tricolors.

CLASS	NUMBER	MEAN	STANDARD DEVIATION	DIFFERENCE OF MEANS
Tested $S-e^pe^p$	30	16.2		
Tested $S-e^pe$	66	31.9		15.7±3.8
Tested S—total	96	27.0		
Untested $S-e^p-$	50	21.1		5.2±3.0
Total $S-e^p-$	146	25.0	17.3	
Tested *ss* e^pe^p	10	21.3		
Tested *ss* e^pe	23	38.9		17.6±7.8
Tested *ss* total	33	33.6		
Untested *ss* e^p-	21	43.5		9.9±5.8
Total *ss* e^p-	54	37.4	20.5	
Tested e^pe^p	40	17.5		
Tested e^pe	89	33.7		16.2±3.6
Tested total	129	28.7		
Untested e^p-	71	27.8		0.9±2.8
Total $S-e^p-$	146	25.0		
Total *ss* e^p-	54	37.4		12.4±3.1
Total e^p-	200	28.4	19.0	

The effect of *ss* on the amount of yellow is rather inconsistent as shown by the great difference in the untested animals and it seems to be less than the effect of heterozygosis in *e*.

The dominance relationships in the black extension series of the guinea pig are then: *E* dominant over e^p and *e*, e^p not completely dominant statistically over *e*. Incomplete dominance would be expected if e^p had a mere quantitative effect, complete dominance would be expected if e^p had a specific effect, one dose of e^p being sufficient to produce this effect.

Relation of tortoise shell and piebald

The black extension series (*E*, e^p, *e*) has been thought not to have any effect on the amount of white. An analysis of the F_2 data, however, suggests that there may be some effect. The genotype *S*-e^pe has more tendency to have a trace of white than *S*-e^pe^p; and *S*-*ee* tends to have more white than *S*-e^p-. Within tricolors the amounts of white seem not to differ between e^pe^p, e^pe, and *ee*. Before reaching any conclusions concerning the

effect of this series on white, a study involving E as well as e^p and e would be necessary.

White spotting as a modifier of the black-yellow pattern has been mentioned earlier. Tricolors of high grade spotting show an extreme of the segregation of color, frequently even to the point of being bicolors (black-white or red-white). Tabulations were made for two inbred strains, 13E (761 spots) of median 98 percent white, and strain 2 (724 spots) of median 94 percent white. The results presented in table 7 show that most of the spots were completely black or completely red (88 percent in strain 13E, 86.5 percent in strain 2). There were a few spots of brindle. For the less evenly mixed of these, there was a tendency for black to be either at the center as a brindle, at the side on the mid-dorsal line, or at the center as a clear black region. This illustrates the observation first mentioned by WRIGHT (1917) that yellow tends to be peripheral, black, central. One difference between the two strains is evident, the greater tendency for strain 2 to have red spots.

TABLE 7

Color of spots (percentage).

STRAIN	SEGREGATED COLOR					BRINDLE						
	BLACK	RED	BLACK AND RED			EVENLY DISTRIBUTED	RED BRINDLE AT CENTER BLACK AT BORDER	RED SOLID AT CENTER BRINDLE AT BORDER	RED BRINDLE AT MID-DORSAL LINE BLACK LATERALLY	BLACK BRINDLE AT CENTER RED AT BORDER	BLACK SOLID AT CENTER BRINDLE AT BORDER	BLACK BRINDLE AT MID-DORSAL LINE RED LATERALLY
			BOTH AT MID-DORSAL LINE	BLACK AT MID-DORSAL LINE	RED AT MID-DORSAL LINE							
13E	54.1	33.9	1.7	—	—	3.9	0.3	0.5	0.1	1.7	1.4	2.2
2	33.3	53.2	1.9	1.8	—	2.2	0.3	0.1	0.4	1.7	3.6	1.5

A more detailed study of the relation between the two types of spotting has been made on the 200 tortoise shell and tricolor animals of the F_2 generation from the cross of tricolor by yellow considered earlier. Using the hair sample method, 25 hairs at each of 30 points, the animals were tabulated as to solid black, brindle, solid yellow, and solid white at each of these points. In figure 3, the white spotting pattern is indicated. The numbers refer to the actual number of animals having color at the points in question. All except one of the 200 animals had color immediately behind the left eye. Only 145 had color on the tip of the nose, the point least frequently colored. Contours were then made showing levels of frequency. Color is present most frequently in the eye-ear regions and posterior half of the animals, least frequently on the feet, nose, lower lip, throat, chest,

and forehead. A belt across the shoulders shows intermediate frequency. This pattern is similar to the other composite patterns and agrees closely with that of strain DS (figure 1, CHASE 1939), the example of low grade

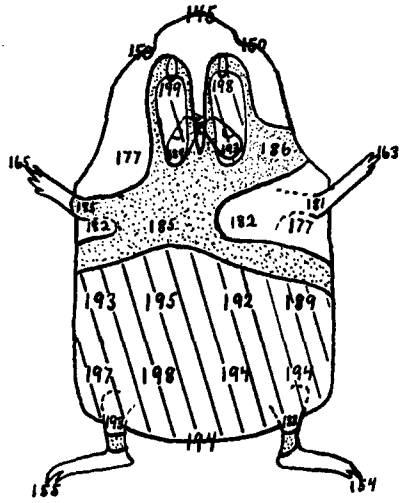

FIGURE 3.—Actual frequency of color at the 30 points. Note: 164 at point between ears.

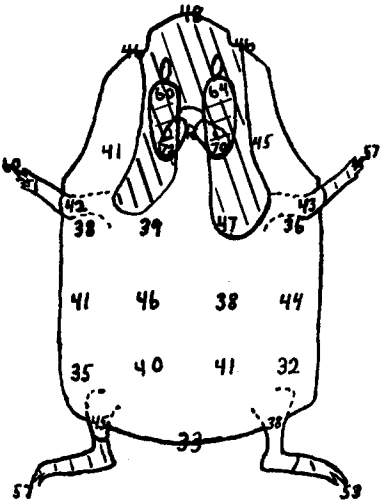

FIGURE 4.—Solid black.

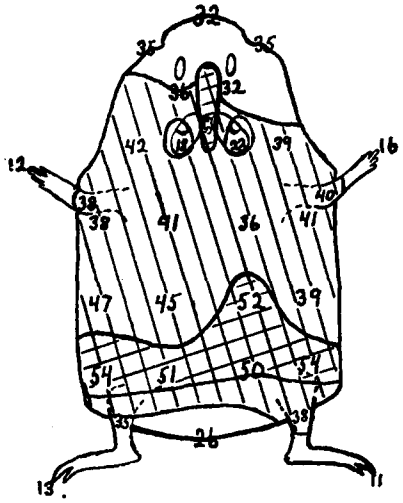

FIGURE 5.—Brindle.

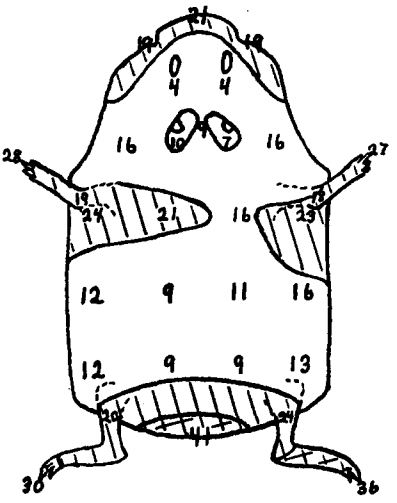

FIGURE 6.—Solid yellow.

FIGURES 4, 5, 6.—Black, brindle, and yellow respectively as percentages of color at the 30 points. Contours separate percentages above and below the average (46 percent for black, 36 percent for brindle, and 18 percent for yellow). A contour at higher frequencies is also shown. Note: for point between ears, 37, 54, and 9 percent respectively.

spotting (making allowance for the fact that F_2 includes *SS*, *Ss*, and *ss*, whereas DS is wholly *ss*).

Figures 4, 5, and 6 show the distribution of segregated black, of brindle, and of segregated yellow in the colored areas. For example, of the 145 cases out of 200 which have color at the tip of the nose, 48 percent are solid black (fig. 4); 32 percent, brindle (fig. 5); and 21 percent, solid yellow (fig. 6). For all points, 46 percent are solid black, 36 percent are mixed, and 18 percent are solid yellow. The nose therefore exceeds the average of the coat as a whole in respect to both segregated colors but is below the average of the coat in respect to brindle. One contour is made at the average percentage, separating in that way those regions of more than average from those of lower than average. Of the former regions those especially high are indicated by an additional contour line. The exceptionally high forehead frequency of the brindle pattern is balanced by lows in black and yellow, the low ear frequencies of brindle are balanced by highs in black, and the low caudal frequency of brindle is balanced by high in yellow. The eye-ear regions are usually black, rarely yellow. Considering the low average percentage of solid yellow, the caudal region is especially high (41 percent). At the extremities, nose and feet, the solid colors occur with higher than average frequency, brindle being especially unusual on the feet. The ventral side, except for the region of the mammae, and the shoulder belt tend to have the remainder of the solid yellow.

A comparison of figures 3 and 6 (nose, feet, and shoulder belt) supports the impression that yellow tends to occupy surface frequently white. Solid black is also frequent on the nose and feet but in addition is most frequent around the ears, regions least frequently white. Brindle is most nearly like the color pattern as a whole (comparison of figures 3 and 5).

Passing from the relationship of white and tortoise shell in locality, there arises the question of the relationship with the amount of white in the animal as a whole. The 200 F_2 animals were tabulated for each point as to solid black, three classes of brindle, one to eight, nine to sixteen, seventeen to twenty-four yellow hairs, and solid yellow. The animals were then divided into five categories each for males and females: no white, a trace of white, five percent white, 10–50 percent white, and 55–90 percent white. In each category the percentages of *colored* points showing solid black, et cetera, were found. The results are presented in figure 7. Selfs have a large amount of black and brindle, a small amount of segregated yellow. Animals with a trace of white show no change in the amount of brindle but a decrease in black and an increase in yellow. At five percent white, a decrease in brindle occurs with both black and yellow increasing. At 10 to 50 percent white (*ss*), the brindle is still decreasing, the black and yellow, increasing. A change other than total amount has appeared for brindle, a relative decrease in the dark brindle (one to eight yellow hairs in the sample of 25). In the last category the numbers are too small to

be relied upon. Sex apparently makes some difference, females having more segregated yellow for a given grade of white than males.

Figure 8 shows the distributions of the tortoise shells and tricolors of F_2 with respect to the percentages of points which are solid black, brindle, and solid yellow. Males and females are combined. The animals are grouped in five classes with respect to white but not all the same classes

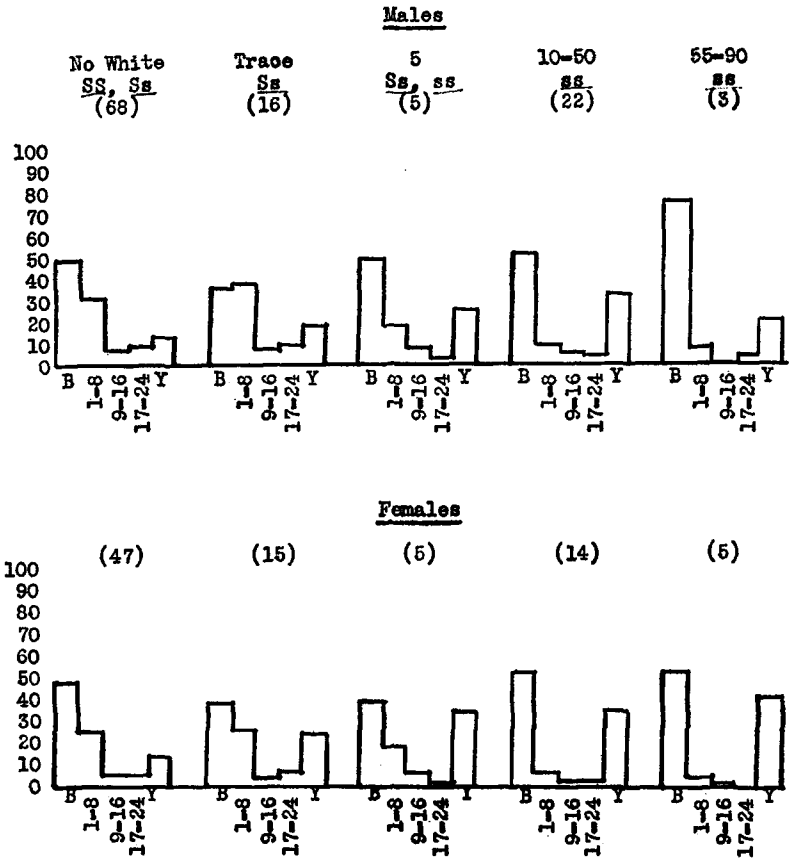

FIGURE 7.—Percentage of *colored* points having all black (B) hairs, 1–8 yellow hairs, 9–16 yellow hairs, 17–24 yellow hairs, and all yellow (Y) hairs in five categories with respect to amount of white. Numbers in parentheses refer to the number of animals (200 in all).

as in figure 7. The figure brings out the great range of individual variability as well as the trend in relation to increasing white. For solid black a decrease occurs with the first appearance of white but subsequently a continued increase. For brindle little difference occurs with the first white but subsequently a continued decrease. For solid yellow a continued increase occurs. A comparison can be made between the frequencies of the colors at specific points (figs. 4, 5, and 6) and the frequencies of the colors at all points in relation to white (figs. 7 and 8). Roughly the comparisons

are: the ears with selfs in which black is frequent, yellow rare; the loin with a trace of white in which brindle is the most frequent; and the "tail," nose, and feet with 10–90 percent white in which brindle is rare, the segregated colors frequent.

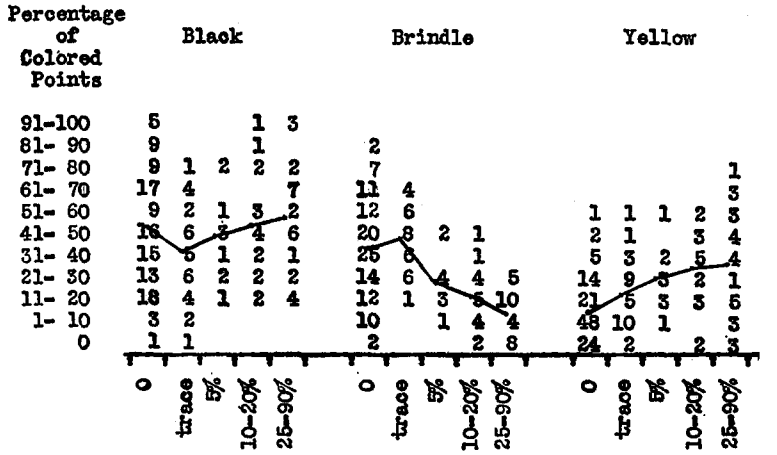

FIGURE 8.—Percentage of colored points that are solid black, brindle, or solid yellow in relation to amounts of white. The 200 animals (males and females) are represented. Mean points found and the trend indicated.

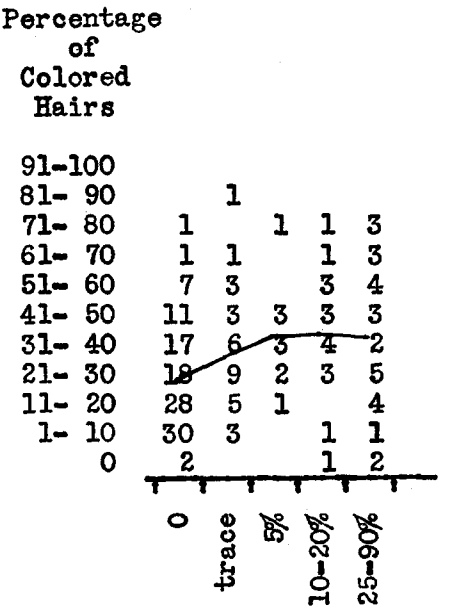

FIGURE 9.—Percentage of colored hairs (750 in selfs) that are yellow in relation to amounts of white. The 200 animals (males and females) are represented. Mean points found and the trend indicated.

Figure 9 shows the percentage of colored hairs (750 in self animals) that are yellow, combining the points. This proportion increases from 23

percent in selfs to 31 percent in animals with a trace of white and to 38 percent in animals with five percent white but increases no further with greater amounts of white. White spotting thus definitely affects both the nature and extent of the yellow spotting pattern.

DISCUSSION

In attempting to interpret gene action in relation to pattern there are a number of alternatives that at once present themselves.

We will consider here both the pattern of color and white on which data has been presented in Part I of this series and the pattern of black and yellow discussed in Part II.

1. Is the pattern of color and white essentially morphological, one of presence or absence of specialized pigment cells; or is it essentially physiological, a pattern of physiological differences in cells similar morphologically?

2. Is the pattern of color and white determined in place, under which there are the alternatives of a local differentiation of morphologically or physiologically distinct cells or a local destruction; or is it a matter of a migration stream which has not reached all parts of the coat? In the latter case the observed pattern might be due in part to local differences in the region of determination and in part to mere distance from the latter.

3. If there is a process of local differentiation either where observed or elsewhere, does it involve nuclear change (somatic mutation) or is it merely cytoplasmic?

4. If there is cytoplasmic differentiation, is it irreversible or is it maintained by local conditions?

Similar questions arise in connection with the pattern of black and yellow.

Embryology

As determined by histologic examination of skin of guinea pig embryos from timed matings (black by black), no pigment was observed up to 41 days 20 hours. Some pigment was found at 42 days 12 hours and at all later ages. Presumably, however, the determination of the pattern was considerably earlier. In Fundulus STOCKARD (1915) showed that chromatophores, at first unpigmented, originated from the wandering mesenchyme cells of the embryo. GOODRICH (1927) describes a similar migratory activity occurring early in another fish, *Oryzias latipes*. For amphibia DUSHANE (1935) has demonstrated the origin of dermal melanophores, not initially pigmented, from the neural crest. Although he was unable to trace epidermal melanophores from that source, he found that they were dependent on neural crest material either for origin or for pigment induction. Although comparable experimental evidence is lacking for mammals, it is possible that here also pigment cells originate from, or are dependent

on, the neural crest and that the color-white pattern is due to an incomplete migration. Observations of the patterns (color-white and black-yellow), the correlation studies, and examples of pigment invasions in transplant experiments suggest a migration stream in general ventrally from the mid-dorsal region and thus in harmony with DUSHANE's observation of a migration of melanophores from the neural crest in amphibia. The pattern would then be due in part to local differences in the region of determination and in part to the extent of migration from the latter.

Transplantation

The results of transplantation experiments bear on some of the questions of gene action in pattern formation. In the guinea pig, skin transplants in the ear have been performed by CARNOT and DEFLANDRE (1896), LOEB (1897), and SAXTON, SCHMECKEBIER, and KELLEY (1936). These were all autoplastic except for some by the first authors. All found spreading of black skin grafted on white and gradual replacement of white skin grafted on black. Hair color, however, was unchanged. SEEVERS and SPENCER (1932) made autoplastic transplants of considerably larger areas (two square centimeters) between regions on the back and belly. In every case the graft continued to produce hair of its original color as long as observed (six to nine months). These grafts included black on red and on white, red on black and on white, and white on black and on red. They found only slight invasion of the skin at the edges of white grafts from the surrounding colored regions. They found complete autonomy of the hair follicles. REED and SANDER (1937) using black-and-tan mice and grafting embryonic tissue on new-born young were able to demonstrate that the color (black or tan) was determined by the seventeenth to the eighteenth day which is before either pigment granules or hair follicles appear.

In fowls, color and pattern were found by DANFORTH and FOSTER (1929) to be fully autonomous (except for sex differences) in transplants between different breeds. WILLIER, RAWLES, and HADORN (1937) transplanted skin ectoderm reciprocally between chick embryos of colored and dominant white breeds at about 75 hours incubation. At 15 days colored down was found on the implants from the colored breed but colored down was also found on the implants from the white breed. The authors note that the latter result might be due either to diffusion of chemical substances or to invasion from the colored host. EASTLICK (1939) has transplanted limb buds in chicks at 40 hours incubation and the evidence suggests a migration of dorsally located cells, possibly from the neural crest, to bring about pigmentation of the limb.

In all cases there was autonomy of cells with the capacity to produce color. Skin and follicles lacking this capacity were also autonomous at

least in the case of white spotting of the guinea pig. This conclusion is of course not invalidated by observations of gradual changes in color if these are correctly interpreted as due either to displacement by the surrounding pigmented skin or to invasion by pigment cells from the latter. The possibility suggested above that the spotting pattern is maintained by local physiological conditions may be considered to be definitely eliminated.

Dopa reaction

The reaction of melanophores to dioxyphenylalanin (dopa), discovered by BLOCH (1917), has been used to demonstrate their presence. SCHULTZ (1925) working with the rabbit and KRÖNING (1930) and RUSSELL (1937, 1937a) working with the guinea pig demonstrated the existence of numerous dendritic cells, blackened by treatment with dopa, in the yellow varieties and in the yellow parts of the tortoise shell pattern of the guinea pig. It seems clear that the tortoise shell pattern depends on physiological differentiation with respect to the production of chromogen rather than on a morphological pattern of presence and absence of pigment cells. GOODRICH (1933) has found a similar situation in the fish *Oryzias latipes*. The dopa reaction is completely negative for the white areas in white spotted animals (in the rabbit, SCHULTZ 1925; in the guinea pig, KRÖNING 1930, RUSSELL 1937, 1937a). There is an indication here that the white spotting pattern (in contrast with the yellow spotting pattern) is determined by actual absence rather than mere physiological alteration of the dendritic cells.

Somatic mutation

In a study of the tricolor pattern in the guinea pig, it is appropriate to consider another class of mosaics, those which are not regularly transmissible as mosaics. Twenty-three cases have been reported for rodents. Eighteen involved heterozygotes in which part of the coat exhibited a recessive condition. Briefly the cases were as follows: one guinea pig, CASTLE (1912); one rat, CASTLE (1922); three guinea pigs, WRIGHT and EATON (1926); seven rabbits, CASTLE (1929); three mice, PINCUS (1929); one mouse, FISHER (1930); two mice, FELDMAN (1935). In one case the animal was homozygous for the color factor (*AA*) agouti guinea pig, but showed a black spot (WRIGHT and EATON 1926). This may have been due to mutations of both *A* genes or possibly to a mutation to a dominant black. The same authors described two quadricolored guinea pigs, black-sepia-red-white. These were homozygous in the intensity factor (*CC*) but the sepia may have been due to an effect of the tortoise shell factor (e^p) since this factor is known to reduce the intensity of black in brindled areas. Apparently these sepia spots were due to such a dilution not associated with the usual mixture of red hairs. The remaining two of

the twenty-three cases of mosaicism are of particular interest because the germ cells were affected. In a dilute-intense mosaic guinea pig, supposedly $c^d c^d$ from its ancestry, a mutation to C must have occurred in a cell ancestral to part of the gonad, as indicated by breeding tests, and to two separate intensely colored areas of the skin (WRIGHT and EATON 1926). In a mouse of constitution Cc^r (black) there apparently occurred a mutation of C to a new allele, affecting about one-fifth of the gonad and several regions of the skin (DUNN 1934).

In the two germinal-somatic mosaics, in three mosaic guinea pigs, and in one mosaic rat, there was found more than one patch of mutant skin. Assuming that these patches were of common origin, the determination must have been early in development with subsequent separations. The possibility of migratory cells suggests itself.

Regular inheritance of mosaicism as a character has not been indicated in any of these cases but the seven black-maltese mosaic rabbits described by CASTLE (1929) occurred in three successive generations indicating some sort of unstable gene condition.

The possibility that the yellow spotting pattern of the guinea pig, very irregular and at times appearing as single yellow hairs among hundreds of black hairs, is due to the instability of the gene e^p during a certain stage in the development of somatic tissue must be considered. Against this view is the fact that in many generations of brother-sister mating, in several different strains, no evidence has been found that e^p has ever mutated to E or e in the germ line (WRIGHT and EATON 1926). The possibility of stability in the germ line, instability in the soma, is however indicated by DEMEREC'S (1929, 1929a) results with the gene miniature-gamma in *Drosophila virilis* which in the presence of certain modifiers is almost fully stable in the germ line but mutable in the wings causing mosaics in practically all cases. No conclusive evidence, however, has been obtained in mammals comparable to that in Drosophila.

A relationship between mosaicism and white spotting has been pointed out by DUNN (1934) and FELDMAN (1935). Twenty of the twenty-three rodent cases cited above were white spotted and in most cases at least the mutant tissue occupied regions frequently white. There is a certain similarity here to the influence of white spotting on black and yellow in the guinea pig noted earlier. Another probably related observation is that of PUNNETT (1924) that the yellow in Japanese rabbits (black-yellow) tends to occur in a pattern simulating that of the black-white Dutch rabbits.

Destruction

Ordinary goldfish (*Carassius auratus*) of genotype TT acquire their final coloration by a destruction of the melanophores which until about

three months of age give in combination with the xanthophores, which are not usually destroyed, a brown appearance (BERNDT 1925, FUKUI 1927). In the colorless "shubunkins" ($T'T'$) there is an earlier loss of melanophores and a loss of xanthophores as well. In the heterozygote (TT'), known as the common shubunkin, a permanent variegated pattern results from irregular destruction of melanophores and xanthophores (GOODRICH and HANSEN 1931, GOODRICH and NICHOLS 1933). Pattern as a consequence of a destructive process is interesting, but can probably be ignored as a possibility for the tricolor guinea pig in which no progressive effects have been observed.

Realizing that the relation of gene action to pattern determination cannot yet be answered, this series has been concerned with the more precise descriptions necessary for a clearer formulation of the problem. As demonstrated by the composite patterns of white spotting, hereditary modifiers tend to affect the animal as a whole. Non-hereditary modifiers act only locally (correlation studies on inbred strains). Although variability is great for the pattern of black and yellow, evidence is presented that in the multiple allelic series, E, e^p, e, the e^p factor is not completely dominant statistically over e. This suggests a quantitative effect of this intermediate allele. The effect of white on the tortoise shell pattern has been described precisely by the method of hair samples. Segregation of colors increases with the amount of white and the number of yellow hairs increases up to a certain point. That the effect is due to the amount of white rather than directly to the presence of s seems probable since SS and Ss among the selfs apparently do not differ and animals with a trace of white (Ss) do exhibit a noticeable change. The view that solid yellow areas tend to be peripheral and tend to occupy regions frequently white has been corroborated. This appears to be related to the observation that mutant tissue in mutational mosaics also tends to occupy white regions and to occur in white spotted animals (DUNN 1934, FELDMAN 1935).

SUMMARY

A cross between tricolors and yellows is analyzed.

The multiple allelic series for extension of black, E, e^p, e, is considered in respect to dominance. The tortoise shell factor, e^p, is found to be statistically not completely dominant over e. Both e^pe^p and e^pe are highly variable and overlap widely.

A method of using hair samples (750 hairs from each animal) for recording the black-yellow pattern is described. By means of this method an analysis of the relation of tortoise shell and piebald is made. Segregated black and yellow tend to occur on the nose and feet and yellow in particular tends to occupy those localities most frequently white. The segregation

of black and yellow and the relative increase of yellow with the introduction of white are measured. The effects upon the black-yellow pattern vary apparently according to the amount of white, not according to the constitution of the animal with respect to the major pair of alleles, *S*, *s*, alone.

Questions are presented which must be solved in any final interpretation of pattern in relation to gene action. Evidence is considered from studies in various fields, embryology, transplantation, dopa reaction, and somatic mutation.

LITERATURE CITED

BERNDT, W., 1925 Vererbungsstudien an Goldfischrassen. Z.i.A.V. **36**: 161–349.

BLOCH, B., 1917 Chemische Untersuchungen über das specifische pigmentbildende Ferment der Haut, die Dopaoxydase. Z. phys. Chem. **98**: 226.

CARNOT, P., and DEFLANDRE, CL., 1896 Persistence de la pigmentation dans les greffes épidermiques. C. R. Soc. Biol. **48**: 178–180.

CASTLE, W. E., 1912 On the inheritance of tricolor coat in guinea-pigs, and its relation to Galton's law of ancestral heredity. Amer. Nat. **46**: 437–440.

1922 On a non-transmissible tricolor variation in rats. Pub. Carnegie Inst. Washington **320**: 51–54.

1924 Genetics of the Japanese rabbit. J. Genet. **14**: 225–229.

1929 A mosaic (intense-dilute) coat pattern in the rabbit. J. Exp. Zool. **52**: 471–480.

CHASE, H. B., 1939 Studies on the tricolor pattern of the guinea pig. I. The relations between different areas of the coat in respect to the presence of color. Genetics **24**: 610–621.

DANFORTH, C. H., and FOSTER, F., 1929 Skin transplantation as a means of studying genetic and endocrine factors in the fowl. J. Exp. Zool. **52**: 443–470.

DEMEREC, M., 1929 Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 834–838.

1929a Changes in the rate of mutability of the mutable miniature gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. **15**: 870–876.

DUNN, L. C., 1934 Analysis of a case of mosaicism in the house mouse. J. Genet. **29**: 317–326.

DUSHANE, G. P., 1935 An experimental study of the origin of pigment cells in Amphibia. J. Exp. Zool. **72**: 1–32.

EASTLICK, H. L., 1939 A study of pigmentation in the chick embryo by means of limb bud transplantation. (Abstract). Genetics **24**: 98–99.

FELDMAN, H. W., 1935 The mosaic (dark-eyed intense—pink-eyed dilute) coat colour of the house mouse. J. Genet. **30**: 383–388.

FISHER, R. A., 1930 Notes on a tricolour (mosaic) mouse. J. Genet. **23**: 77–81.

FUKUI, KEN'ICHI, 1927 On the color pattern produced by various agents in the goldfish. Folia Anatomica Japonica **5**: 257–302.

GOODRICH, H. B., 1927 A study of the development of Mendelian characters in *Oryzias latipes*. J. Exp. Zool. **49**: 261–287.

1933 One step in the development of hereditary pigmentation in the fish *Oryzias latipes*. Biol. Bull. **65**: 249–252.

GOODRICH, H. B., and HANSEN, I. B., 1931 The postembryonic development of Mendelian characters in the goldfish *Carassius auratus*. J. Exp. Zool. **59**: 337–358.

GOODRICH, H. B., and NICHOLS, ROWENA, 1933 Scale transplantation in the goldfish *Carrassius auratus*. I. Effects on chromatophores. II. Tissue reactions. Biol. Bull. **65**: 253–265.

IBSEN, H. L., 1916 Tricolour inheritance. Genetics **1**: 287–309.

1919 Tricolour inheritance. IV. The triple allelomorphic series in guinea pigs. Genetics **4**: 597–606.

ILJIN, N. A., 1928 The distribution and inheritance of white spots in guinea pigs. (Russian). Trans. Lab. Exp. Biol. Zoopark, Moscow **4**: 255–349.

KRÖNING, F., 1930 Die Dopareaktion bei Verschiedenen Färbenrassen des Meerschweinchens und des Kaninchens. Arch. f. EntwMech. Org. **121**: 470–484.

LEAGUE, B. B., 1928 The chromosomes of the guinea pig. J. Morph and Physiol. **46**: 131–142.

LOEB, L., 1897 Über Transplantation von weissen Haut auf einen Defekt in schwarzen Haut und umgekehrt am Ohr des Meerschweinchens. Arch. f. EntwMech. Org. **6**: 1–44.

LUSH, J. L., 1921 Inheritance in swine. J. Hered. **12**: 57–71.

MOLS, G., 1928 Étude comparative de Mitoses chez le Cobaye. Arch. Biol. **38**: 31–86.

PINCUS, G., 1929 A mosaic (black-brown) coat pattern in the mouse. J. Exp. Zool. **52**: 439–441.

PUNNETT, R. C., 1924 On the "Japanese" rabbit. J. Genet. **14**: 231–240.

REED, S. C., and SANDER, G., 1937 Time of determination of hair pigments in the mouse. Growth pp. 194–200.

RUSSELL, W. L., 1937 An analysis of the action of color genes in the guinea pig by means of the Dopa reaction. (Abstract). Genetics **22**: 207.

1937a An analysis of the action of color genes in the guinea pig by means of the Dopa reaction. Unpublished Ph.D. dissertation, Department of Zoology, University of Chicago, pp. 30.

SAXTON, J. A., JR., SCHMECKEBIER, MARY M., and KELLEY, R. W., 1936 Autogenous transplantations of pigmented and unpigmented ear skin in guinea pigs. Biol. Bull. **71**: 453–461.

SCHULTZ, W., 1925 Verhalten der Einzelnen Färbungsgene zur Dopareaktion bei Kaninchenrassen. Arch. f. EntwMech. Org. **105**: 677–710.

SEEVERS, C. H., and SPENCER, D. A., 1932 Autoplastic transplantation of guinea pig skin between regions with different characters. Amer. Nat. **66**: 183–189.

STOCKARD, C. R., 1915 A study of wandering mesenchyme cells on the living yolk-sac and their developmental products: chromatophores, vascular endothelium and blood cells. Amer. J. Anat. **18**: 525–599.

WARWICK, B. L., 1926 Inheritance of black in swine. J. Hered. **17**: 251–255.

1931 Breeding experiments with sheep and swine. Ohio Agric. Expt. Sta. Bull. 480. pp. 37.

WHITING, P. W., 1919 Inheritance of white-spotting and other color characters in cats. Amer. Nat. **53**: 473–482.

WILLIER, B. H., RAWLES, MARY E., and HADORN, E., 1937 Skin transplants between embryos of different breeds of fowl. Proc. Nat. Acad. Sci. **23**: 542–546.

WRIGHT, SEWALL, 1917 Color inheritance in mammals. V. The guinea pig. J. Hered. **8**: 476–480.

1918 Color inheritance in mammals. VIII. Swine. J. Hered. **9**: 33–38.

1920 The relative importance of heredity and environment in determining the piebald pattern of guinea pigs. Proc. Nat. Acad. Sci. **6**: 320–332.

1923 The relation between the piebald and tortoise-shell color patterns in guinea pigs. Anat. Rec. **23**: 393.

1925 The factors of the albino series of guinea-pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

WRIGHT, SEWALL, and CHASE, H. B., 1936 On the genetics of the spotted pattern of the guinea pig. Genetics **21**: 758–787.

WRIGHT, SEWALL, and EATON, O. N., 1926 Mutational mosaic coat patterns of the guinea pig. Genetics **11**: 333–351.