

INHERITANCE STUDIES IN SOYBEANS.
 III. SEED-COAT COLOR AND SUMMARY OF ALL OTHER
 MENDELIAN CHARACTERS THUS FAR REPORTED¹

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INTRODUCTION

In 1923 the writer began a study of the inheritance of seed-coat colors in the soybean (*Soja max*). From a practical standpoint, the problem is of great interest because seed-coat colors are very important in the selection of pure strains and in the detection of mixtures in commercial varieties. This led to a rather intensive study of the factors concerned in mottling, the results of which are being published in another paper.

The results of previous workers indicated that two varieties may look alike but may behave very differently in crosses. This made it advisable to secure numerous crosses among a large number of varieties in order that the genetic constitutions of different types could be compared. Al-

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though the original plans have by no means been realized, over 300 hybrids, representing 35 different combinations, have been produced.

Some of the parental plants used for hybridization were from well known varieties, but others were from selections made by the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION, and their origin is uncertain. It has been necessary to assign arbitrary numbers to these selections which have not been identified as belonging to any particular variety.

With but few exceptions all factors studied in soybeans have something to do with the color of the seed coat, either directly or through linkage. It seems logical, therefore, to center the discussion on seed-coat colors although a complete list is given of all the genetic factors that have been reported.

In carrying out the problem the writer has been greatly aided by counsel from Professor B. D. LEITH of the Agronomy Department and Professors L. J. COLE and R. A. BRINK of the Genetics Department of the UNIVERSITY OF WISCONSIN. It is a pleasure to acknowledge all the advice and constructive criticisms which have been received.

SEED-COAT COLOR TYPES

NAGAI (1921) and PIPER and MORSE (1923) have given classifications of seed-coat color types for soybeans, but for the purpose of interpreting Mendelian characters the following classification has been found most useful.

- I. Self-color type
 1. Black
 2. Brown (may be of different intensities according to the variety and somewhat according to conditions at the time of maturity)
 3. Imperfect black
 4. Buff
 } (Associated with gray pubescence)
- II. Bicolor type
 1. Black mottling pattern on brown background
- III. Eyebrow pattern with green or yellow background
 1. Black
 2. Brown
 3. Imperfect black
 4. Buff
 } (Associated with gray pubescence)
- IV. Green or yellow seed coat with dark hilum. (Mottling is developed over the remaining portion of the seed coat according to environmental conditions.)

1. Hilum black
 2. Hilum brown
 3. Hilum imperfect black
 4. Hilum buff
- } (Associated with gray pubescence)
- V. Green or yellow seed coat with light hilum. (Mottling is produced according to environmental conditions but pigments are always potentially present.)
1. Mottled with black
 2. Mottled with brown
 3. Mottled with imperfect black
 4. Mottled with buff
- } (Associated with gray pubescence)

PIGMENTS RESPONSIBLE FOR SEED-COAT COLORS

What has been designated as black pigment in the seed coat of the soybean is really a very intense purple and belongs to the general class of anthocyanins. The brown or phlobaphene-like pigments are undoubtedly closely related to quercetin but their exact chemical composition is not known. In the imperfect blacks there is a mixture of the two pigments and even in the self-blacks a considerable amount of brown pigment is masked by the black.

In addition to the pigments mentioned above, which are glucosides and have many properties in common, there is another blackish-brown pigment which behaves very differently. In all probability it belongs to the general class known as "oxidation pigments" because of their connection with the process of oxidation. This is the pigment responsible for the common blackish-brown pod color in black-podded varieties but this same pigment may also find its way into the parenchymatous cells of the seed-coat. Frequently it produces such a smudgy appearance that a classification of a plant in regard to the other pigments is rather uncertain. Unlike the anthocyanins and phlobaphene-like pigments, the production of the oxidation pigment may be greatly increased by injury to the pod and testa.

Another colored condition has been described by MATSUMOTO and TOMOYASU (1925) which they have called "purple speck." The pigment responsible for this abnormal coloration, according to the descriptions, has similar properties to anthocyanins but a fungus (*Cercosporina Kikuchii*) was found to be responsible for its occurrence.

The black and brown pigments, with which the present article is chiefly concerned, are independent of the green and yellow plastid pigments. In self-browns or mottled types not all of the plastid color is concealed,

so it is usually possible to detect the presence of green when it is present but in self-blacks plastid colors are entirely masked.

THE VARIABILITY OF SEED-COAT COLORS

Except in self-colored types there is a great deal of variability in the amount of pigment produced in the seed coat. This has been regarded as objectionable by practical growers, so the subject of mottling has received considerable attention in all the important soybean-growing sections of the United States. Much effort has been spent in selecting for non-mottled strains but all varieties which have been tested in cooperation with the Agronomy Department of the WISCONSIN AGRICULTURAL EXPERIMENT STATION have produced mottling in some degree. Some selections have proved to be much more badly affected than others but mottling seems to be a general phenomenon with all yellow- and green-seeded varieties. Details of this work are being published in another paper.

WOODWORTH and COLE (1924) have given evidence to show that environmental factors may be important in bringing out mottling pigments and their conclusions have been well confirmed during the progress of this study. Varieties which failed to develop any signs of mottling in the greenhouse during winter months were badly mottled in the field.

A rich loam soil has consistently produced much more mottling than a light sandy soil, but the distance of spacing plants has also been found to be very important. Plants grown three feet apart have produced more mottled seed than plants grown closely together, but on certain types of soil the distance of spacing has not been a determining factor. These results are confirmed by HOLLOWELL'S (1924) findings at Iowa.

Although it seems probable that there may be many factors which affect mottling, perhaps the most important is the maturation of the seed. An extreme amount of mottling was found to be associated with plants which remained green after the normal time for the seeds to mature.

Environmental factors have no apparent influence in the case of self-colored varieties, because no difficulty is experienced in getting these varieties to breed true; but there are noticeable differences in intensity of color due to environment. The imperfect-black type is extremely variable in its appearance. Sometimes seeds are nearly black and in other cases some experience is required to distinguish this type from light brown. NAGAI (1924) gives evidence to show that this variability is not independent of inheritance but the present writer has no data on the relative importance of environment and heredity on this particular coloration.

The self-browns and buffs are also variable in regard to the intensity of color and conditions at maturity seem important in this connection.

For a genetic study of seed-coat colors it is usually desirable to have the plants growing in an environment that will produce the maximum amount of pigment. This together with an intimate knowledge of the variability that might be expected due to environment is necessary in order to classify intelligently certain F_2 progenies.

RESULTS OF PREVIOUS INVESTIGATIONS

PIPER and MORSE (1923) found black seed coat dominant to brown. In the particular example cited a single factor was responsible for the difference. NAGAI (1921) also found black to be dominant to brown but he distinguished between different types of blacks and browns. He demonstrated the presence of a factor R which produced anthocyanin pigment and a factor C which increased the intensity of pigmentation. According to NAGAI's hypothesis, with CR present the seed coat is black; with cR it is an imperfect black; with Cr it is brown; and with cr it is a very light brown or "buff."

In addition to C and R , NAGAI assumed that another factor O must be present in order to explain the inheritance of certain browns. A rich reddish brown was recessive to the common dull brown. Therefore, with CO present the pigment was a dull brown, and with Co , reddish brown; but the relation between the two factors, C and O , was not entirely clear.

WOODWORTH (1921) observed that two factors, B and H , were necessary for the production of black pigment in the hilum, but H was found to be completely linked to T , the factor for tawny pubescence. Plants homozygous for ht always produced gray pubescence and a seed coat with a brown hilum. In Part II of this series another factor, E , was added to this linkage group. This factor E is a partially dominant factor for early maturity but since the expression was not always clear-cut it was only through the linkage relation that its effect could be attributed to a single factor. There was evidently a small percentage of crossing over but this could not be accurately measured.

NAGAI and SAITO (1923) found self-blacks or self-browns recessive to yellow or green and they have described three restriction factors. According to these writers, the factor K inhibits the production of pigments forming a "patched" or "eyebrow" pattern; I inhibits nearly completely the formation of chromogenic substance; and H inhibits it entirely. The recessives of these factors have no effect.

NAGAI and SAITO also found a single factor M responsible for the production of a black mottling pattern upon a brown background. They assumed that M is linked to the factor P which inhibits the formation of pubescence, giving a crossover value of approximately 18 percent. In Part II of this series the writer also reports a linkage between P and R_1 , a factor for anthocyanin pigment. This linkage appears to be nearly complete with only occasional crossovers. Mention is also made of the factor p_2 for glabrousness reported by STEWART and WENTZ (1926) which was recessive to P_2 , a factor for pubescence. Thus we have a character (glabrousness) which is dominant in one case and recessive in another case; apparently due to different factors.

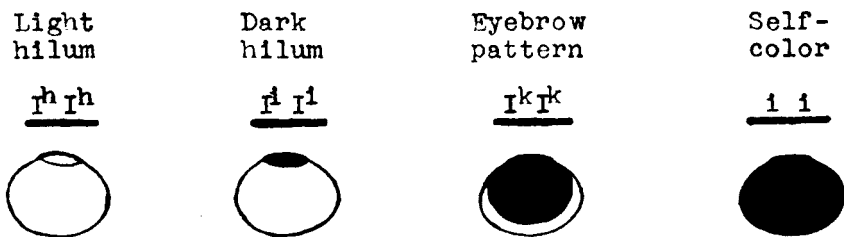
TAKAHASHI and FUKUYAMA (1919) have given results obtained in F_2 from five different crosses but no attempt was made to give an interpretation, due to the small amount of data available. Their results at least indicate that varieties are green or yellow because of the presence of factors that inhibit pigment formation. More blacks than browns were secured in the F_2 's but the data do not warrant conclusions in regard to the factorial relationships.

NAGAI (1921) found that black and brown pigments are formed regardless of whether the plastid pigments in the seed coat are green or yellow. Green, however, was dominant over yellow. WOODWORTH (1921) obtained identical results but found that V , the factor used for green seed coat, was linked with D , a factor for the production of yellow cotyledons, giving about 13 percent crossing over; but V was assumed to be independent of another factor I which is a duplicate of D and also produced yellow cotyledons. These findings have been well confirmed by the writer and the results are briefly presented in Part I of this series.

TERAO (1918) found a single factor responsible for green seed coat color when the maternal parent was yellow, but when the maternal parent was green there was no segregation, the F_1 's and F_2 's being the same color as the female parent. PIPER and MORSE (1923) confirm TERAO's findings, and both writers have postulated that two types of green are responsible for the odd results. Although the inheritance of the green seed coat was independent of the paternal parent yet two types of greens were distinguished in the F_2 progenies of green seed-coat ♀ × yellow seed-coat ♂. One type was more intense than the other and when counts were made it was found that the results were approximately in accord with a ratio of 3 dark green to 1 light green. One type of green was therefore inherited according to Mendelian expectation while the other type was a clear case of maternal inheritance.

ALLELOMORPHIC SERIES FOR THE RESTRICTION OF PIGMENT IN
THE SEED COAT

NAGAI and SAITO (1923) postulated that the three restriction factors which they described as H , I , and K , were allelomorphous. This assumption has been well confirmed in the present study, so there seems to be no good reason for not making this clear in the terminology. Four allelomorphs may, therefore, be described according to their inhibiting effect upon the production of pigment in the seed coat. The following figure illustrates the action of the factors which have been found in this allelomorphous series.



As the above figure suggests I^h , under the proper environmental conditions, inhibits all pigment formation in the seed coat; I^i inhibits pigments to the hilum; I^k inhibits the formation of pigment in such a way as to form the "eyebrow" or "patched" pattern; and i , the recessive allelomorph, has no effect.

Although it is necessary to define these restriction factors rather definitely, it should be clearly kept in mind that environmental conditions unfavorable for pigment formation are necessary in order for these restriction factors to fully express themselves. Varieties with the genetic constitution $i i$ come under Class I or II of the "Seed-coat Color Types." In these two classes there is no restriction of the pigment and environment has practically no effect but in classes III, IV, and V, which $I^k I^k$, $I^i I^i$, and $I^h I^h$ represent respectively, environmental conditions are very important in addition to the hereditary factors. Thus, varieties which come in Class V are of constitution $I^h I^h$, but may develop considerable mottling. From individual seeds it would frequently be impossible to distinguish the three types because of the variation due to environment but when all the seeds from a plant are available for classification there is usually no difficulty. It should be mentioned, however, that these restriction factors are only partially dominant. A cross between $I^h I^h$ and $i i$ invariably

produces an F_1 that is mottled more than plants homozygous for I^h and in such cases it is sometimes difficult to distinguish the color types.

Unfortunately the writer was not successful in obtaining a cross between varieties of constitution ii and I^kI^k but NAGAI and SAITO (1923) have already described this relationship. All other combinations, however, have been made and the results from these crosses constitute a critical test for the theory that I^h , I^i , I^k , and i are allelomorphic. The best evidence for this relationship rests upon the results of the three combinations of crosses between Mandarins (I^hI^h), Soysotas (ii) and Manchus (I^iI^i). The parental plants of the Mandarin and Soysota varieties were from strains selected which were known to be homozygous, and the Manchu selection (No. 59), which will be referred to here, was also known to be homozygous.

The distribution of dominant and recessive types in all F_2 progenies closely approached a 3:1 ratio and the crucial proof for the allelomorphic series rests upon this simple but consistent behavior. The following tabulation indicates this relationship.

	Dominant	Recessive	Deviation
Mandarin (I^hI^h) \times Soysota (ii)	15	5	0.0 ± 1.3
Manchu (I^iI^i) \times Soysota (ii)	250	92	6.5 ± 5.4
Mandarin (I^hI^h) \times Manchu (I^iI^i) ¹	223	75	2.0 ± 5.0

Any explanation other than that of an allelomorphic series is obviously precluded because this is the only way in which a 3:1 ratio can be secured for each of the combinations. If the Mandarin variety were of constitution $HHii$ and if the Manchu were $hhII$ (H being synonymous with I^h and I synonymous with I^i) the crosses with the Soysota variety could be explained because it may have the constitution $hhii$. Such a theory is shattered when the Mandarin is crossed with the Manchu because with two factors involved there would be a dihybrid ratio and 1/16 of the plants in F_2 would be self-colored, but none were observed. The probability of this happening when 412 plants are grown would be $(15/16)^{412}$ or the odds would be over $10^{11}:1$, and results from other similar crosses are also against a two factor difference.

The following crosses were also obtained in which the factor I^k is involved. In the cross Black Eyebrow (I^kI^k) \times Glabrous No. 5 (I^iI^i), 186 plants produced seed with the pigment restricted to the hilum and 65 produced seed having the eyebrow pattern. Based on a 3:1 ratio the

¹ There were 412 F_2 plants in this cross but only tawny-pubescent plants were classified for reasons which will follow in the text.

deviation is 2.3 ± 4.6 . If I^k were not an allelomorph of I^i then the Glabrous No. 5 parent would necessarily have had the two dominant factors I^i and I^k . Such an assumption is not in accord with the breeding facts from crosses between varieties with pigment restricted and self-color because in every case a single factor difference explains the results.

In order to furnish further proof for the allelomorphic series it would have been desirable to have backcrossed an F_1 plant of constitution $I^h I^i$ or $I^h I^k$ to the self-colored $i i$ variety. Such crosses should bring out only the two dominant types in the next generation, and the proof for the allelomorphic series would be complete. Due to the difficulty in crossing soybeans, however, this task would be difficult to carry out and the indirect evidence which is already available is practically conclusive that I^h , I^i , I^k , and i occupy identical positions on the chromosomes.

Restriction of pigment to the hilum

It is impossible to assert definitely that a factor identical with NAGAI and SAITO's factor I has been found, but at least it is convenient to use such a designation. This factor I^i , as it is being defined here, is responsible for restricting pigments to the hilum.

The Manchu variety has a yellow seed coat with a black hilum and results have shown that the reason for the seed coat being yellow instead of black is due to the presence of I^i , the dominant restriction factor. Environmental factors produce great variations in the amount of mottling developed; but the hilum remains colored in spite of the fact that environmental conditions may be very unfavorable for the development of mottling.

With no restriction factors other than I^i present the hilum is always black or brown, but the intensity of the color may depend on other factors. In tawny-pubescent varieties the hilum color is prominent but in gray-pubescent varieties the color may be very faint. Varieties differ also in regard to size of hilum and this likewise seems to determine the prominence of the color. Of course, we can not be absolutely sure from the data now available that the factor responsible for restricting pigments to the hilum has been the same one in every case, but neither have we proof for the contrary. It seems logical, therefore, to assume that I^i is responsible for restricting pigments to the hilum.

Three different strains of Manchus were crossed with self-browns but each of these combinations gave very similar results. Table 4 gives the detailed behavior of these crosses but the combined results obtained in F_2 are the following:

Phenotypes	Black hilum R_1I^i	Brown hilum r_1I^i	Self-black R_1i	Self-brown r_1i
Observed	584	169	171	71
Calculated	559.7	186.6	186.6	62.2
Difference	+24.3	-17.6	-15.6	+8.8

The value of χ^2 is 5.26 assuming independence between I^i , the restriction factor, and R_1 the factor for black pigment. This gives P a value of 0.16.

Another cross already mentioned was made between the Black Eyebrow variety (I^kI^k) and a glabrous Japanese variety (I^iI^i) (No. J5) with a brown hilum. The Black Eyebrow variety is characterized by the eyebrow pattern that has been described so we assume that the restriction factor I^k is present. The factor I^i has the same action with the eyebrow pattern as with the self-color, inhibiting the development of pigment outside of the hilum in both cases. The results are as follows:

Phenotypes	Black hilum R_1I^i	Brown hilum r_1I^i	Black eyebrow R_1I^k	Brown eyebrow r_1I^k
Observed	136	50	47	18
Calculated	141.2	47.1	47.1	15.7
Difference	-5.2	+2.9	-0.1	+2.3

It may be safely assumed that I^i and R_1 were independent because of the small deviations from the theoretical 9:3:3:1 ratio and the value for χ^2 is 0.329 and $P=0.9+$.

Restriction of pigment over the entire seed coat

NAGAI and SAITO (1923) have described a factor H which completely inhibits the development of brown and black pigments. They neglect to state that mottling is possible when H is present but there are many reasons to believe that such is the case. The writer's experience has been that the expression of this factor, I^h according to the new usage, may be greatly influenced by environmental conditions. All pigment may be entirely restricted or there may be considerable mottling. The Mandarin, Aksarben, and Ito San varieties have such restriction factors and these three varieties have been crossed rather extensively.

When a self-color (brown or black) is crossed with a variety possessing the restriction factor I^h , the F_1 has a strong tendency to be intermediate. Even in the greenhouse, where conditions extremely unfavorable for the production of mottling were provided, heterozygous plants produced seeds slightly speckled or blotched with pigment. This condition was not apparent, however, when the cross was made with the Manchu variety

which possessed the factor I^i for the restriction of pigment to the hilum. It is to be assumed, therefore, that with both I^i and I^h present the tendency for a restriction of pigment is stronger than if only one I^h is present.

In order to have a convenient method of referring to pigment patterns the designations used in the classification of color types have been adhered to. "Dark hilum," therefore, means that the hilum is colored as in the Manchu variety but the pigment may be black or brown. The factor I^i is responsible for inhibiting the pigment to the hilum in this manner. "Light hilum" means that I^h is present and no pigment is formed except that which may be due to mottling.

No difficulty was experienced in classifying tawny-pubescent plants according to whether their seeds had dark or light hilums, but with gray-pubescent plants the distinction has not always been clear because of the faint development of pigment. In some way the presence of T seems to intensify seed-coat pigments and this helps materially in classifying plants for the presence or absence of I^h .

Four different combinations between self-browns and yellows have been made. The detailed results are given in Table 5 but the combined results are the following:

<i>Phenotypes</i>	<i>Tawny mottled</i> $T I^h$	<i>Tawny self-brown</i> $T i$	<i>Gray mottled</i> $t I^h$	<i>Gray, Buff</i> $t i$
Observed	137	48	38	22
<i>Calculated</i>	<i>137.8</i>	<i>45.9</i>	<i>45.9</i>	<i>15.3</i>
Difference	-0.8	+2.1	-7.9	+6.7

A comparison with calculated results, on the basis of independence, gives deviations that may be accounted for as being due to chance; the value of χ^2 is 4.39 and $P=0.23$.

Six combinations between black-seeded varieties and varieties possessing the factor I^h were also secured (table 1). The combined F_2 data from these crosses are as follows:

<i>Mottled or colorless</i>	<i>Self-colored</i>	<i>Dev.</i>	<i>P. E.</i>	$\frac{\text{Dev.}}{\text{P. E.}}$
518	186	10.0	7.75	1.3

These data again show a fairly good agreement between observed figures and the theoretical 3:1 ratio.

A cross, Black Eyebrow ($I^k I^k$) \times Ito San ($I^h I^h$), was also made which gave an actual ratio of 18 with pigment restricted to 4 with the eyebrow pattern. These data are almost in perfect agreement with theoretical figures, the deviation being 1.5 ± 1.4 .

A cross between a Manchu No. 1 ($I^i I^i$) and an unidentified yellow variety ($I^h I^h$), designated as No. 12 in the records, gave the following results in F_2 .

	LIGHT HILUM		DARK HILUM	
	Black	Brown	Black	Brown
Observed	120	36	51	14
Calculated	124.4	41.4	41.4	13.8
Difference	-4.4	-5.4	+9.6	+0.2

Comparing the observed data with the calculated, assuming that I^h is independent of R_1 , $\chi^2=3.10$ and $P=0.38$. The behavior of F_3 progenies also corroborates the results obtained in F_2 and these data are given in table 6.

Six combinations between light hilum and gray pubescence ($t I^h$) \times dark hilum and tawny pubescence ($T I^i$) were secured. From a general observation of these F_2 's one would naturally assume that I^i is completely linked with T , because no plants with gray pubescence bore seed with the typical dark hilum. If complete linkage is assumed we have the following results compared with the theoretical 2:1:1 ratio.

	Tawny, Light hilum	Tawny, Dark hilum	Gray, Light hilum	Gray, Dark hilum
Observed	709	235	299	
Calculated	621.5	310.7	310.7	
Difference	+87.5	-75.7	-11.7	

With this comparison $\chi^2=31.20$ and $P=0.000001$, so it is unlikely that the deviations could be due to chance. Since T , the factor for tawny pubescence, has a marked effect on both the quantity and quality of pigments, one may assume that some gray-pubescent plants really had dark hilums but could not be easily distinguished. Recalculating with this assumption we get the following comparison with the 9:3:4 ratio.

	Tawny, Light hilum	Tawny, Dark hilum	Gray, (Hilum color not distinguished)
Observed	709	235	299
Calculated	699.2	233.1	310.7
Difference	+9.8	+1.9	-11.7

In this case $\chi^2=0.59$ and $P=0.9$, showing a very good agreement between observed and expected results.

A very similar cross between the dark and light hilums has been described by WOODWORTH (1921) but he was unable to classify his F_2 plants, because of the irregular pigment which was probably due to environmental conditions.

Restriction of pigment to form an eyebrow pattern

Although the three restriction factors I^h , I^i , I^k seem well established, there are some facts which indicate a larger series of allelomorphs. It has already been mentioned that some strains of Manchus have been selected which mottle much more badly than others, but selection within any one strain has not been successful. In other words these strains must have been homozygous to begin with, and if mutations occur they have not been with sufficient frequency to be selected. In progeny tests with selections from the Black Eyebrow variety, however, heritable variations from the normal eyebrow pattern have been observed, indicating the presence of another factor which restricts the pigment less than I^k , but it is possible that modifying factors were involved.

One selection (No. 84) bred true for the normal eyebrow pattern for four generations and the uniformity was very remarkable. Another selection (No. 82) bred true for a very dark pattern, and in this progeny plants with half of their seeds completely black were not uncommon, but no plant produced 100 percent black seeds. A third progeny (No. 83) was apparently heterozygous for factors affecting the eyebrow pattern because selections were made which bred true for a normal eyebrow pattern; others bred true for a very dark pattern; and still other selections continued to produce some plants with normal patterns and others with dark patterns.

RELATION BETWEEN BLACK AND BROWN PIGMENT

It has been pointed out that self-black is dominant to self-brown in the seed coat and that ratios of 3 blacks to 1 brown are secured in F_2 . The first cross made by the writer was of this type and the following results were obtained.

<i>Black</i>	<i>Brown</i>	<i>Des.</i>	<i>P. E.</i>
72	28	3	2.92

In this cross (Black, No. 3 \times Brown, No. 30)¹ the F_1 plant bore seed fully as black as the maternal parent, and in the F_2 's there was no visible

¹ This progeny also segregated for an unusual type of hilum peculiar to the Soysota variety, the paternal parent. Ordinarily there is a clean separation between the hilum and the funiculus in soybeans, but in this abnormal type the Malpighian cells are arranged in such a way that the funiculus is torn, leaving a rough surface that is very characteristic. This condition is recessive

difference between the intensity of blacks, that is, black was fully dominant to brown.

In 1923 a natural hybrid (Selection No. 60) was accidentally found that segregated according to a ratio of 3 blacks to 1 brown in the progeny that was grown, but all heterozygous plants bore seed slightly speckled with brown. The results were as follows:

	<i>Black</i>	<i>Dilute black</i>	<i>Brown</i>
Observed	7	14	8
Calculated	7.25	14.50	7.25

It so happened that the observed distribution was exactly in accord with the calculated except for an extra brown. Results in F_3 proved that all of the 14 plants with dilute black were actually heterozygous. The peculiar behavior of the black pigment is noticeable only in the heterozygous condition. In homozygous plants the black is apparently just as intense as that produced by any other variety. It is, therefore, a simple case of incomplete dominance. This kind of partial dominance is well known, and 1:2:1 ratios are perhaps as much to be expected as 3:1 ratios. BLAKESLEE and AVERY (1917) have reported a case of incomplete dominance of mottling in the Adzuki bean (said to be *Phaseolus Mungo*) that is very similar to this lack of dominance in the seed-coat color of the soybean.

The difference between the two kinds of blacks appeared interesting, so crosses were made using the hybrid F_2 plants for parental material. Crosses with types possessing the dominant inhibiting factor I^h were secured. Actually this was equivalent to back-crossing because in some cases heterozygous plants were used as parents. Therefore, combinations with r_1 were secured as well as with R_1 .

Two combinations with R_1 are given in table 1, Mandarin and Aksarben varieties being the other parents. Without considering the details of the table at this time it may be of interest to state that out of 17 self-blacks grown in 1925 from these crosses 10 were dilute black and were undoubtedly heterozygous, as was previously found to be the case.

FLOWER AND PUBESCENCE COLORS IN RELATION TO SEED-COAT PIGMENTS

In table 1 six crosses are compared between blacks with a factor present for the production of anthocyanin pigment and yellows with I^h present for the restriction of all pigment. It has already been pointed out that

to the normal hilum and in all crosses it has proved to be independent of all other perceptible characters. Tables 9 and 10 give the evidence for this statement, the factor for normal hilum being designated as N and that of the Soysota variety as n .

black \times brown has usually given 3:1 ratios, black being dominant. WOODWORTH'S (1921) example, where complementary factors were involved giving a 9:7 ratio, is the only exception reported.

In the crosses reported in table 1 three strains of blacks (No. 3, No. 29,¹ and No. 60) and four strains of yellows (Mandarins, Ito Sans, Aksarbens, and No. C3) were used. No. C3 is an unidentified Chinese variety, and it resembles the Mandarin variety very much but the flowers are white. It may also be said that this is the only white-flowered variety used in the crosses referred to in table 1. The other six parents had the ordinary purple flowers which are dominant to white.

Black No. 60 was a selection from the natural hybrid that has been described, the peculiar thing about this selection being the incomplete dominance of the black seed-coat color in the heterozygous condition.

Table 1 affords an opportunity for a study of pubescence colors in relation to seed-coat colors. Ito Sans have tawny pubescence while the other three yellow-seeded varieties have gray pubescence. When Ito Sans were crossed with blacks a 9:3:3:1 ratio resulted in F_2 and the results can be explained due to the presence of R_1 in the blacks and I^h in the Ito Sans, but these results are quite different from those obtained when gray-pubescent varieties were used as parental stock. When Mandarins and the Aksarbens were crossed with blacks a 27:9:9:3:9:3:3:1 ratio was found to explain the results. It will be noted that rather high values for χ^2 are obtained for these crosses in table 1 but the numbers are rather small and the fact that all classes are represented may be of some significance. Combining progenies, 133, 139, 172, 175, 171, and 164 $\chi^2 = 14.52$ and $P = 0.04$. Both the black and the brown pigments were influenced in some way by the presence of T . On plants with gray pubescence, of constitution tt , there was an incomplete development of pigment. The black and brown correspond with what NAGAI (1921) has described as "imperfect black" and "buff," respectively. It is therefore convenient to continue the use of these terms.

NAGAI does not state that imperfect black was associated with gray pubescence but this has been the case in progenies 133, 172, and 164 (table 1). The logical assumption, therefore, is to consider T identical with NAGAI'S factor C . If these factors are not identical they are at least completely linked, because tawny pubescence has always been associated with a more intense development of both the anthocyanin and phlobaphene-like pigments. These are the exact properties which NAGAI has attributed to his factor C .

¹ Selection from Wisconsin Black variety. Nos. 3 and 60 could not be identified as to variety.

TABLE 1
*F*₂ progenies of black×yellow seed coats.

	PROGENY NUMBERS	TAWNY PUBESCENCE				GRAY PUBESCENCE				
		Black		Brown		Imperfect black		Buff		
		Mottled	Self-color	Mottled	Self-color	Mottled	Self-color	Mottled	Self-color	
Black (No. 3) ×Ito San	150	13	5	4	0					$\chi^2 = 1.25$
<i>Calculated</i>		13	4	4	1					
Difference			+1		-1					$P = 0.74$
Black (No. 29) ×Ito San	127	26	18	11	6					$\chi^2 = 7.24$
<i>Calculated</i>		34.4	11.4	11.4	3.8					
Difference		-8.3	+6.6	-0.4	+2.2					$P = 0.07$
Black (No. 29) ×Mandarin	139 ³	72	21	18	10	10	7	7	1	$\chi^2 = 14.89$
	133	31	7	6	7	8	3	2	2	
<i>Calculated</i>	Total	103	28	24	17	18	10	9	1	
		88.6	29.5	29.5	9.8	29.5	9.8	9.8	3.3	
Difference		+14.4	-1.5	-5.5	+7.2	-11.5	+0.2	-0.8	-2.3	$P = 0.04$
Black (No. 60) ×Mandarin	175	30	11	19	3	6	3	4	0	$\chi^2 = 12.56$
	172	26	6	10	2	5	3	7	3	
<i>Calculated</i>	Total	56	17	29	5	11	6	11	3	
		58.2	19.4	19.4	6.5	19.4	6.5	6.5	2.2	
Difference		-2.2	-2.4	+9.6	-1.5	-8.4	-0.5	+4.5	-0.8	$P = 0.08$
Black (No. 60) ×Aksarben (No. 53)	171 ³	31	13	14	4	9	3	10	2	$\chi^2 = 12.64$
	164	25	11	10	0	8	2	4	2	
<i>Calculated</i>	Total	56	24	24	4	17	5	14	4	
		62.4	20.8	20.8	6.9	20.8	6.9	6.9	2.3	
Difference		-6.4	+3.2	+3.2	-2.9	-3.8	-1.9	+7.1	+1.7	$P = 0.08$
Black (No. 3) ×Yellow (No. C3)	142	70	25			15 ²	4 ²	7 ¹	4 ¹	$\chi^2 = 3.21$
<i>Calculated</i>		70.3	23.4			17.6	5.9	5.9	2.0	
Difference		-0.3	+1.6			-2.6	-1.9	+1.1	+2.0	$P = 0.67$

¹ Plants also had white flowers.

² All plants had purple flowers.

³ Progenies 139 and 171 are reciprocals of 133 and 164, respectively.

Progeny No. 142 [black No. 3 (r_1R_2TW) \times yellow No. C3 (r_1r_2tw)] is of special interest because no browns appeared in the tawny-pubescent class, but in the gray-pubescent class there were both imperfect blacks and buffs. This gives a ratio of 15:1 for the presence versus absence of anthocyanin pigment. In the gray-pubescent class the imperfect blacks and buffs were very similar to seed similarly classified in the progenies 133, 172, and 164. F_3 progenies were also grown from F_2 plants of progeny 142. The original hypothesis was confirmed by these results. Although only 10 progenies from plants of phenotypic constitution ($T R_2I^h$) were grown yet every possible genotypic arrangement was secured. Two segregated the same as the previous F_2 generation, five segregated for I^h but not for T , and three segregated for T but not for I^h . In the later progenies, R_2 was linked completely with T and there were no signs of crossing over.

It is of special interest to note that the imperfect blacks in progeny 142 were also associated with purple flowers and that buffs were associated with white flowers. Therefore, it seems entirely probable that W , the factor for purple flowers (WOODWORTH 1923), may also be responsible for the development of anthocyanin pigment in the seed coat in this particular case. To obtain further evidence F_3 progenies of phenotypic constitution $T R_2iw$ were grown in 1926 and two of these proved to be heterozygous for T . The linkage between T and R_2 was again complete and all gray-pubescent plants produced buff seed-coat color. This evidence corroborates the previous breeding behavior in showing the necessity of the presence of W before anthocyanin pigment can be produced in the seed coat of gray-pubescent plants.

WOODWORTH found that purple flower color was associated with purple hypocotyls but no effect on the seed coat has been reported; yet this condition is not surprising because in other plants the development of anthocyanin pigment has often been found to be associated with flower color. COMBES (1912) working with a species of *Spiraea*, found that coloration in the bark of twigs was easily brought about by decortication on species with colored flowers but this was not possible with species having white flowers.

As already stated, the crosses Black \times Mandarin or Black \times Aksarben can readily be explained by the three factors, R_1 , T , and I^h . The restriction factor I^h is independent of the other two factors, so there is no need of considering it at this time. The expression of the other two factors is as follows:

R_1T	Black, tawny
R_1t	Imperfect black, gray
r_1T	Brown, tawny
r_1t	Buff, gray

In the cross, Black (No. 3) \times Yellow (No. C3), the situation is also simple but there is difficulty in explaining why the behavior is so different from other crosses. It cannot be said that R_1 is responsible for the production of anthocyanin in this case because of the complete linkage. Perhaps we can postulate that another factor R_2 , not allelomorphic to R_1 , is the factor for anthocyanin formation and that it is completely linked with T . This factor may be identical with Woodworth's factor H because H also produced black pigment and was completely linked with T . The factor W has no apparent effect when T is present but when the constitution was tt it produced an imperfect black. The question then arises—why is it possible that W can have this effect? If it had produced the same effect in Mandarins and Aksarbens the seed coats on these varieties should have been mottled with imperfect black instead of light brown because these are purple-flowered varieties. The genetic constitution of the yellow selection No. C3 must, therefore, differ from the genetic constitution of Mandarins in such a way that when W is present a certain amount of brown pigment is converted into anthocyanin. Perhaps r_2 , an allelomorph to R_2 , makes this possible; and if this is the case a cross between the Mandarin (with W) and selection No. C3 (with r_2) should give imperfect blacks in F_1 ; i.e., the complementary factors W and r_2 are necessary to explain the results.

On the basis of the above assumption the black selection No. 3 (r_1R_2TW) must have a factor R_2 which is different from the factor R_1 in No. 29 and No. 60, and selection No. C3 (r_1r_2tw) must also have an additional factor r_2 that is not present in Mandarins and Aksarbens. Then the results in progeny 142 can be explained as follows:

r_1R_2TW	} Black, tawny
$r_1R_2T'w$		
$r_1r_2'W$	 Imperfect black, gray
$r_1r_2'tw$	 Buff, gray

Since r_2 was completely linked with t , the single factor T may be assumed to be responsible for the production of black seed coats as well as tawny pubescence, but results from other crosses show that T does not always have this effect. When selection No. 3 (r_1R_2T) was crossed with the Ito San ($r_1r_2'T$) there were plants with brown seed coats and tawny pubescence in F_2 (table 1). Furthermore, r_2' of the Ito San variety must be considered different from r_2 of selection No. C3 because r_2 was a complement of W for the production of anthocyanin in the imperfect black seed-coats of progeny 142 (table 1), but r_2' does not have this effect. From

these results it seems possible that an allelomorphic series may be situated at the *t* locus. If this is true the following combinations have now been identified:

- R_2T Tawny pubescence, black seed-coat (selection No. 3)
 r_2t Gray pubescence, seed-coat imperfect black with *W* but buff with *w* (selection No. C3).
 r'_2T Tawny pubescence, seed-coat brown with either *W* or *w* (Ito San variety)
 r'_2t Gray pubescence, seed-coat brown with *W* or *w* (Mandarin and Aksarben varieties).

The identity of the factor R_2 for the production of anthocyanin pigment in the seed coat is well established, but it has been found in only one selection (No. 3). In selection Nos. 29 and 60 the factor (R_1) responsible for the production of anthocyanin pigment in the seed coat is clearly distinct from R_2 , because, unlike the latter, it is not linked with *T*. There is also good reason for using the designation R_1 in selection No. C4 (table 3) but a number of other combinations were made between varieties both of which had tawny pubescence. In these crosses the factor for the production of anthocyanin may have been either R_1 or R_2 but for convenience R_1 has been used in case of doubt.

DUPLICATE FACTORS FOR TAWNY PUBESCENCE

The assumptions that have just been made depend upon *T* being the same factor for tawny pubescence in each case. It might have been assumed that there is only one factor *R* for black seed-coat color and two factors for tawny pubescence. A convenient designation would be T_1 and T_2 . The factor T_1 could be independent of *R*, but *R* and T_2 would be completely linked. Such an assumption would explain the results as well as the method which has been chosen.

Further work is necessary to decide these relationships but the results from one F_2 population give a real indication for the presence of two factors for tawny pubescence. This was progeny No. 71 (No. Sp 3-9 × No. 29) which has been discussed under Part I of this series, as evidence for maternal inheritance of cotyledon color. Both paternal parents were homozygous for tawny pubescence but there was segregation in F_2 giving 71 tawny to 15 gray pubescent plants. Assuming duplicate factors the deviation is 10.6 ± 4.8 . It is very evident that this is a rather wide deviation from a 15:1 ratio but since the odds are only about 6:1 it does not seem entirely improbable. F_3 progenies from this cross are to be grown and results reported at a future date.

LINKAGE *versus* INDEPENDENCE OF SEVEN PAIRS OF FACTORS

Eleven crosses between Mandarins and an unidentified Chinese variety, given the arbitrary number C4, proved successful. These varieties differ in seven pairs of characters. These contrasted characters and the factors which correspond to them are as follows:

<i>Mandarin</i>	<i>Green No. C4</i>
Purple flower color, <i>W</i>	White flower color, <i>w</i>
Yellow pods, <i>l</i>	Black pods, <i>L</i>
Gray pubescence, <i>t</i>	Tawny pubescence, <i>T</i>
Yellow seed-coat, <i>g</i>	Green seed coat, <i>G</i>
Light hilum, <i>I^h</i>	Dark hilum, <i>I^s</i>
Brown mottling pigment, <i>r₁</i>	Black mottling pigment, <i>R₁</i>
Yellow cotyledon color, <i>D₁D₂</i>	Green cotyledon color, <i>d₁d₂</i>

The factor pairs *Gg*, *I^hI^s*, and *R₁r₁* have the same meaning here as in NAGAI and SAITO'S (1923) descriptions; and *Ww*, *Ll*, *Tt*, and *D₁d₁*, have been described by WOODWORTH (1921 and 1923). The factor *D₂* is a duplicate of *D₁* for the production of yellow cotyledons, and is identical with WOODWORTH'S factor *I*, the only reason for the change being the use of *I* in designating a restriction factor.

The action of *R₁* in this cross was the same as has been previously described. The dark hilum was easily distinguished from light hilum on tawny-pubescent plants, but the intensity of pigment was reduced on gray-pubescent plants, making it difficult to detect the presence of the restriction factor. In fact, all the gray-pubescent plants bore seed so much lighter in color than the tawny-pubescent plants that all were classified as having light hilums. Therefore only 3/16 of the total number of plants should come in the dark hilum class, and a 13:3 ratio should be expected.

The seed-coat color due to plastid pigment was perhaps the most variable character. The *F₁* seed coats produced a green color but it was somewhat intermediate. *F₂* results were in very close agreement with a 3:1 ratio but there were all gradations in intensity of green. The pod color was no doubt responsible for much of this variation because light-colored pods were always associated with a "faded-out green." Perhaps this can be explained as a result of the plants remaining in the field in the presence of bright sunlight for a considerable length of time after ripening. Black-podded plants always produce a normal green or yellow seed coat, and it seems possible that this can be accounted for by attributing to the black color the absorption of the sunlight which changes normal green seed

coats to a yellowish green. In the light-colored pods the same effect was also produced on green cotyledons as described in Part I of this series.

There was a close approach to a 15:1 ratio for cotyledon color in all F_2 seed. The total numbers were 1686 with yellow cotyledons to 118 with green cotyledons, so the deviation is only 5.25 ± 6.9 from the expected numbers. Usually the seeds were easy to classify because the difference was clear-cut. Both of the parental varieties matured well in Wisconsin and no cotyledons were green due to immaturity. The F_1 plants also produced black pods, so there was no tendency for the cotyledon color to fade as it did on certain F_2 plants with yellow pods.

The parental variety No. C4 has very dark brown or black pods. As has been stated the pigment responsible for this coloration has no relation to the anthocyanin or the brown phlobaphene-like pigment; yet very frequently, on black-podded plants, much of this pigment finds its way into the seed coat and produces a smudgy-brown coloration. This condition has often masked other pigments to such an extent that the classification of seed-coat colors has been very difficult. Fortunately the F_2 's were grown on soil which brought out considerable mottling, but it was frequently necessary to treat the seed coats with acid in order to be sure of the classification. This test proved a great aid because a small amount of anthocyanin can be detected by the red color produced in acid solution.

TABLE 2
F₂ progenies of Mandarin × Green No. C4.

	OBSERVED NUMBERS	DEVIATION	DEV. P. E.	ACTUAL RATIO
Green <i>versus</i> Yellow seed-coat	252:85	0.75	0.14	2.96:1
Tawny <i>versus</i> Gray pubescence	262:75	9.25	1.73	3.49:1
Anthocyanin <i>versus</i> Brown pigment	251:86	1.75	0.33	2.92:1
Black <i>versus</i> Yellow pods	238:99	14.75	2.75	2.40:1
Purple <i>versus</i> White flowers	251:86	1.75	0.33	2.92:1
Light <i>versus</i> Dark hilum (calculated on basis of 13:3)	265:72	8.8	1.82	11.04:3
Yellow <i>versus</i> Green cotyledons (corrected figures)	321:16 315.9:21.1	5.1	1.70	20.06:1

Table 2 gives the observed data for each of the contrasted characters of 337 F_2 plants. There is good agreement with expectation among all seven of these contrasted characters when considered separately except black versus yellow pods. This discrepancy perhaps can be accounted for by the fact that the classification was made from a few pods after thresh-

ing. Since the character is somewhat variable, further tests would be necessary to prove that there is any other reason for the results not being in accord with a 3:1 ratio.

In order to determine whether or not there was any linkage, each contrasting pair of characters has been considered separately with every other pair. Table 3 gives these data in a condensed form. Twenty-one combinations are possible and the actual ratios as well as the theoretical are given in the table. The values of χ^2 and P are also included.

TABLE 3
F₂ progenies of Green No. C4 × Mandarin—total of 337 plants.

		THEORETICAL RATIO WITH INDEPENDENCE	ACTUAL RATIO	χ^2	P
<i>D₁D₂</i>	<i>versus d₁d₂</i> ¹	45:15: 3:1	44.3:15.7: 3.5:0.5	2.07	0.56
"	and <i>T versus t</i>	45:15: 3:1	46.2:13.8: 3.8:0.2	4.68	0.20
"	and <i>R₁ versus r₁</i>	45:15: 3:1	44.7:15.3: 3.0:1.0	0.05	0.9+
"	and <i>L versus l</i>	45:15: 3:1	41.9:18.1: 3.5:0.5	6.45	0.09
"	and <i>I^h versus Iⁱ</i>	195:45:13:3 ²	192.2:51.7: 9.1:3.0	1.83	0.61
"	and <i>G versus g</i>	45:15: 3:1	44.1:15.9: 4.0:0.0	7.44	0.06
<i>G</i>	<i>versus g</i>				
"	and <i>W versus w</i>	9: 3: 3:1	8.7: 3.2: 3.2:0.9	1.21	0.75
"	and <i>T versus t</i>	9: 3: 3:1	9.1: 2.9: 3.3:0.7	2.69	0.45
"	and <i>R₁ versus r₁</i>	9: 3: 3:1	9.3: 2.7: 2.6:1.4	4.54	0.21
"	and <i>L versus l</i>	9: 3: 3:1	8.7: 3.3: 2.6:1.4	5.58	0.14
"	and <i>I^h versus Iⁱ</i>	39:13: 9:3 ²	37.6:12.7:10.3:3.4	1.52	0.68
<i>I^h</i>	<i>versus Iⁱ</i>				
"	and <i>W versus w</i>	39:13: 9:3	36.8:13.4:10.8:2.8	2.71	0.44
"	and <i>T versus t</i>	9: 3: 4:	9.0: 3.4: 3.6:	2.25	0.33
"	and <i>R₁ versus r₁</i>	39:13: 9:3 ²	36.6:13.6:11.0:2.7	3.50	0.33
"	and <i>L versus l</i>	39:13: 9:3	35.5:14.8: 9.7:4.0	4.95	0.18
<i>L</i>	<i>versus l</i>				
"	and <i>W versus w</i>	9: 3: 3:1	8.4: 2.9: 3.5:1.2	3.48	0.33
"	and <i>T versus t</i>	9: 3: 3:1	8.7: 3.8: 2.6:0.9	5.30	0.15
"	and <i>R₁ versus r₁</i>	9: 3: 3:1	8.3: 3.6: 3.0:1.1	3.89	0.28
<i>R₁</i>	<i>versus r₁</i>				
"	and <i>W versus w</i>	9: 3: 3:1	8.6: 3.3: 3.3:0.8	2.17	0.54
"	and <i>T versus t</i>	9: 3: 3:1	9.4: 3.0: 2.5:1.0	2.07	0.56
<i>T</i>	<i>versus t</i>				
"	and <i>W versus w</i>	9: 3: 3:1	9.3: 3.2: 2.6:0.9	1.41	0.71

¹ The observed ratio was corrected for a 15:1 ratio for cotyledon color in making comparisons with the calculated figures. This is legitimate and advisable because no effort was made to grow these seeds to maturity in exactly that proportion.

² These odd ratios are due to the fact that all gray-pubescent plants were classified as having light hilums. Really $\frac{1}{4}$ of these plants had dark hilums but they could not be readily identified.

It will be noted that five combinations give values of P less than 0.2 but four of these are combinations with black versus yellow pods where

the original ratio was 2.4:1; rather poor fits¹ with other pairs of characters, therefore, would naturally result. One combination alone (plastid color in the seed coat versus cotyledon color) appears to be a case of linkage. In this case the value of P is 0.06 which means that the odds are only about 16:1 against such a fit being due to chance but an examination of the actual results indicates that the odds may be greater. This linkage relation has been discussed in Part I of this series.

LINKAGES

R_2 , factor for black pigment in seed coat (Perhaps identical with WOODWORTH'S H)	} Completely linked.
T , factor for tawny pubescence.	
T , factor for tawny pubescence.	} Since the expression of E is somewhat quantitative the linkage could not be accurately measured, but there is proof for only a small percentage of crossing over.
E , factor for early maturity.	
P_1 , factor for glabrousness.	} Linkage appears to be almost complete with limited data.
R_1 , factor for black pigment in seed coat.	
P_1 , factor for glabrousness.	} About 18 percent crossing over. (NAGAI and SAITO 1923)
M , factor for black mottling.	
G , factor for green seed coat, designated as V by WOODWORTH	} About 13 percent crossing over. (WOODWORTH 1921. Also the present study.)
D_1 , factor for yellow cotyledon color	

Three linkage groups are necessary to explain the above linkages, but the factors cannot be arranged according to their respective positions. These groups are as follows:

$$\begin{array}{c} R_2 - T - E \\ P_1 - R_1 - M \\ G - D_1 \end{array}$$

LIST OF GENETIC FACTORS

The genetic factors used to explain the characters which have been studied in soybeans are as follows:

- T , factor for tawny pubescence. Also increases the intensity of color in the seed coat; t , gray pubescence and a lighter seed-coat color. (WOODWORTH 1921) (Limited evidence indicates that there may be another factor which produces the same results as T but is independently inherited.)
- W , factor for purple flower and hypocotyl color and also a complement of r_2 for the production of black pigment in the seed coat; w , white flowers and green hypocotyls. (WOODWORTH 1923.)

¹ A preferable method of calculating χ^2 as a measure of linkage follows this article.

- R_1 , factor for black pigment in the seed-coat but without T the color is imperfect black; r_1 , brown pigment but without T the color is light brown or buff. (NAGAI 1921.)
- R_2 , also a factor for black pigment in seed coat, but entirely linked with T (possibly this factor is identical with WOODWORTH'S factor H); r_2 , brown pigment (but is a complement of W . With r_2r_2 and W present, the pigment is imperfect black in appearance); r_2' , brown pigment but not complementary to W .
- B , found to be a complementary factor to Woodworth's H for the production of black pigment in the seed-coat. (WOODWORTH 1921.)
- O , factor for dull brown seed-coat color; o , reddish brown. (NAGAI 1921.)
- I , factor which inhibits pigment formation in the seed-coat—an allelomorphous series. i , has no effect.
- I^k , inhibits pigment to form an eyebrow pattern (identical with NAGAI and SAITO'S, 1923, factor K).
- I^i , inhibits pigment to the hilum—dominant to I^k and i . (Possibly the same as the factor I which NAGAI, 1921, describes as nearly completely inhibiting the formation of chromogenic substance in the seed-coat.)
- I^h , inhibits pigments entirely except that which may be due to mottling—dominant to I^i , I^k , and i . (Identical with NAGAI and SAITO'S factor H .)
- M , factor for the production of black mottling upon a self-brown seed-coat; m , no effect. (NAGAI and SAITO 1923.)
- G , factor for green seed-coat; g , yellow seed coat due to the green plastid color fading out at maturity. (NAGAI, 1921, also WOODWORTH, 1921, but V was used for the symbol instead of G .)
- D_1 , factor for yellow cotyledon color; d_1 , no effect. (WOODWORTH 1921.)
- D_2 , duplicate of D_1 , for causing the green cotyledon color to fade out at maturity (identical with WOODWORTH'S factor I); d_2 , no effect.
- N , factor for normal hilum; n , abnormal hilum as in the Soysota variety.
- P_1 , factor for glabrousness; p_1 , pubescence. (NAGAI and SAITO 1923.)
- P_2 , factor for pubescence, p_2 , glabrousness (relationship to P_1 and p_1 not known as yet except that the order of dominance is reversed)—STEWART and WENTZ (1926).
- L , factor for black pods but also causes a black coloration of the seed-coat in certain instances; l , brown pods. (WOODWORTH 1923.)
- S , factor for tall plant; s , short plant. (WOODWORTH, 1923, also reported by the writer in table 8.)
- E , factor for early maturity; e , late maturity.

In the above list of factors reference is made to all original descriptions of previous investigators. Those factors which do not have such a reference are described here for the first time. Of course, there is no definite proof that the factors which are considered synonymous are really identical, but it seems advisable to consider them as such until there is proof for the contrary.

SUMMARY

In all the crosses that have been made black seed coat has been dominant to brown but in one cross the dominance was incomplete. Results also indicate that different factors may produce black pigment; and a genetic interpretation has been given to account for the presence of R_1

and R_2 , each of which is capable of producing anthocyanin pigment in the seed coat.

In one particular cross, the factor W , for purple flowers, was found to be a complement of r_2 for the production of black pigment in the seed coat; but in all other cases purple-flower color was independent of every other character.

Three restriction factors have been described. These are dominant factors but their expression is very sensitive to the conditions under which the plant is grown. An environment unfavorable for the production of mottling is necessary for pigment to be completely restricted; otherwise there may be considerable mottling in spite of the presence of restriction factors.

The factor T , for tawny pubescence, has proved to be very important in increasing the intensity of pigment. This is perhaps due to an actual increase in the amount of pigment.

In black-podded varieties a certain amount of the brownish-black oxidation pigment of the pod was sometimes evident in the seed coat.

Five linkages are postulated. The crossover percentage between G , the factor for green seed coat, and D , a factor for yellow cotyledons, was approximately 13 percent, the same as WOODWORTH (1921) has reported. The factor T , for tawny pubescence was completely linked to R_2 . All other linkages are quite tentative until more data are secured.

A complete list of all the genetic factors studied in soybeans has been given as well as those directly concerned with seed-coat colors.

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APPENDIX

TABLE 4

*F*₂ progenies of brown (*r*₁*i*) × yellow with black hilum (*R*₁ *I*^h).

	PROGENT NUMBERS	DARK HILUM		SELF-COLORED SEED-COAT			
		Black <i>R</i> ₁ <i>I</i> ^h	Brown <i>r</i> ₁ <i>I</i> ^h	Black <i>R</i> ₁ <i>i</i>	Brown <i>r</i> ₁ <i>i</i>		
Soysoya (Brown No. 30) × Manchu (No. 59)	213	58	14	18	9	$\chi^2=2.53$ $P=0.47$	
	224	53	13	13	6		
	217	84	28	33	13		
	Total	195	55	64	28		
	<i>Calculated</i>		192.4	64.1	64.1		21.4
Difference		+2.6	-9.1	-0.1	+6.6		
Brown (No. 12) × Manchu (No. 1)	186	71	11	18	5	$\chi^2=4.09$ $P=0.25$	
	190	128	41	38	11		
	Total	199	52	56	16		
	<i>Calculated</i>		181.7	60.6	60.6		20.2
	Difference		+17.3	-8.6	-4.6		-4.2
Brown (No. 12) × Manchu (No 16) <i>Calculated</i>	8	190	62	51	27	$\chi^2=4.95$ $P=0.18$	
		185.6	61.9	61.9	20.6		
	Difference		+4.4	+0.1	-10.9		+6.4
Total of <i>F</i> ₂ progenies <i>Calculated</i>		584	169	171	71	$\chi^2=5.26$ $P=0.16$	
		559.7	186.6	186.6	62.2		
	Difference		+24.3	-17.6	-15.6		+8.8

TABLE 5
*F*₂ progenies of yellow (*I*^h)×brown seed coat (*T*_i).

	PROGENY NUMBERS	TAWNY PUBESCENCE		GRAY PUBESCENCE		
		Mottled with brown	Self-brown	Mottled with brown	Self-brown	
Mandarin×Brown (No. 12) <i>Calculated</i> Difference	197	40 37.1 +2.9	10 12.4 -2.4	10 12.4 -2.4	6 4.1 +1.9	$\chi^2=2.04$ $P=0.57$
Mandarin×Soysota (Brown No. 30) <i>Calculated</i> Difference	231	10 11.3 -1.3	2 3.7 +1.7	5 3.7 +1.3	3 1.3 +1.7	$\chi^2=3.61$ $P=0.31$
Yellow (No. C2)×Soysota (Brown No. 30) <i>Calculated</i> Difference	208	68 68	26 22.7 +3.3	18 22.7 -4.7	9 7.6 +1.4	$\chi^2=1.71$ $P=0.65$
Aksarben×Brown (No. 60) <i>Calculated</i> Difference	164a	19 21.4 -2.4	10 7.1 +2.9	5 7.1 -2.1	4 2.4 +1.6	$\chi^2=3.14$ $P=0.37$
Total observed <i>Calculated</i> Difference		137 137.8 -0.8	48 45.9 +2.1	38 45.9 -7.9	22 15.3 +6.7	$\chi^2=4.39$ $P=0.23$

TABLE 6
Dark black hilum (R₁I⁺) × light brown hilum (r₁I^h).

	NUMBER OF PROGE- NIES	LIGHT HILUM		DARK HILUM		
		Black pigment	Brown pigment	Black pigment	Brown pigment	
F ₂ Progeny No. 1 (Manchu No. 1 × Yellow No. 12)		120	36	51	14	$\chi^2 = 3.10$
		124.4 -4.4	41.4 -5.4	41.4 +9.6	13.8 +0.2	$P = 0.38$
F ₃ Progenies from seeds with light black hilum Deviation from calculated	1	45 +2.8	12 -2.1	16 +1.9	2 -2.7	
	1	36 -1.5		14 +1.5		
	2	49 -1.3	18 +1.3			
	1	17 -1		7 +1		
	2	57				
F ₃ Progenies from seeds with black dark hilum Deviation from calculated	1			11 +1.2	2 -1.3	
	2			76		
F ₃ Progenies from seeds with brown light hilum Deviation from calculated	3		71 +1.8		26 -1.7	
	2		38			
F ₃ Progenies from seeds with dark brown hilum	2				44	

TABLE 7
Tawny-pubescent, Dark black hilum (I'R₁) × *Gray-pubescent, Light brown hilum (tI^hr₁)*.

	PROGENY NUMBERS	TAWNY PUBESCENCE				GRAY PUBESCENCE		
		Light Hilum		Dark Hilum		Light-dark Hilum		
		Black Pigment	Brown Pigment	Black Pigment	Brown Pigment	Black Pigment	Brown Pigment	
Green (No. C4) × Aksarben Deviation from calculated	31	20 +1.0	6 -0.3	5 -1.3	3 +0.9	5 -3.4	6 +3.2	$\chi^2=5.75$ $P=0.33$
Yellow (No. C2) × Manchu (No. 1) Deviation from calculated	269	10 -5.6	4 -1.2	6 +0.8	4 +2.3	11 +4.1	2 -0.3	$\chi^2=8.00$ $P=0.16$
Mandarin × Green (No. C1) Deviation from calculated	342	7 -6.9	6 +1.4	9 +4.4	2 +0.5	7 +0.9	2 -0.1	$\chi^2=8.33$ $P=0.14$
Mandarin × Manchu (No. 1) Deviation from calculated	300 292 Total	161 14 175	62 6 68	40 4 44	14 1 15	51 5 56	20 1 21	$\chi^2=11.06$ $P=0.05$
Manchu (No. 59) × Mandarin Deviation from calculated	282 272 Total	134 28 162	47 14 61	48 7 55	17 3 20	71 9 80	23 11 34	$\chi^2=3.84$ $P=0.57$
Green (No. C4) × Mandarin Deviation from calculated	13	140 -2.2	50 +2.6	58 +10.6	14 -1.8	53 -10.2	22 +0.9	$\chi^2=4.44$ $P=0.49$
<i>Calculated on basis of 27:9:9:3:12:4 ratio Difference</i>	Total	514 524.5 -10.5	195 174.8 +20.2	177 174.8 +2.2	58 58.3 -0.3	212 233.1 -21.1	87 77.7 +9.3	$\chi^2=5.60$ $P=0.35$

TABLE 8

Showing independence between *S*, the dominant factor for height of plant and *R*₁ and *G*—Progeny
No. 319—No. 57 (*Sr*₁*g*) × No. C₁ (*sR*₁*G*).

	<i>SGR</i> ₁	<i>SGr</i> ₁	<i>SgR</i> ₁	<i>Sgr</i> ₁	<i>sGR</i> ₁	<i>sGr</i> ₁	<i>sgR</i> ₁	<i>sgr</i> ₁	
Observed	85	29	24	9	33	8	11	3	$\chi^2=1.95$ $P=0.96$
Calculated	85.2	28.4	28.4	9.5	28.4	9.5	9.5	3.2	
Deviation	-.2	+.6	-4.4	-.5	+4.6	-1.5	+1.5	-.2	

TABLE 9

Manchu (*R*₁*I*⁴*N*) × *Soysota* (*r*₁*in*).

	<i>NR</i> ₁ <i>I</i> ⁴	<i>NR</i> ₁ <i>i</i>	<i>Nr</i> ₁ <i>I</i> ⁴	<i>Nr</i> ₁ <i>i</i>	<i>nR</i> ₁ <i>I</i> ⁴	<i>nR</i> ₁ <i>i</i>	<i>nr</i> ₁ <i>I</i> ⁴	<i>nr</i> ₁ <i>i</i>	
Observed	41	11	8	5	17	7	6	4	$\chi^2=9.70$ $P=0.22$
Calculated	41.8	13.9	13.9	4.6	13.9	4.6	4.6	1.5	
Difference	-0.8	-2.1	-5.9	-0.4	+3.1	+2.4	+1.4	+2.5	

TABLE 10

Black No. 3 (*R*₂*N*) × *Soysota* (*r*₂¹*n*).

	<i>R</i> ₂ <i>N</i>	<i>R</i> ₂ <i>n</i>	<i>r</i> ₂ <i>N</i>	<i>r</i> ₂ <i>n</i>	
Observed	49	23	21	7	$\chi^2=2.26$ $P=0.50$
Calculated	56.25	18.75	18.75	6.25	
Difference	-7.25	+4.25	+2.25	+0.75	