INHERITANCE STUDIES IN SOYBEANS. II. GLABROUSNESS, COLOR OF PUBESCENCE, TIME OF MATURITY, AND LINKAGE RELATIONS[1]

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INTRODUCTION

Through the courtesy of Professor ISABURO NAGAI the writer obtained a glabrous mutant variety of soybeans (*Soja max*) the origin and description of which has been reported (NAGAI and SAITO 1923). This variety was given the arbitrary number J 1 in our records. Another glabrous variety from Japan was also furnished by W. J. MORSE of the UNITED STATES DEPARTMENT OF AGRICULTURE and given the arbitrary number J 5.

Many crosses were attempted with these glabrous varieties because the linkage relationships seemed particularly interesting. Due to difficulties in the mechanics of artificial hybridization only a few crosses were successful but the breeding results show much of interest. The F_2 progenies were started in cold-frames in March 1925 and later transplanted in order that they might reach maturity. A limited number of F_3 progenies were also grown at the Arlington Farm during the 1926 season through the courtesy of W. J. MORSE. It would be very desirable to grow more F_3 progenies but it is unlikely that this can be done at the present time.

The crosses to be discussed are as follows:

Black Eyebrow ($p_1R_1TEI^k$) ♀ × Glabrous No. J5 ($P_1r_1teI^i$) ♂
Green No. C4 ($p_1mR_1LI^i$) ♀ × No. J1 (P_1Mr_1li) ♂

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RELATION OF GLABROUSNESS TO GROWTH AND VIGOR

The dominant factor P_1 as described by Nagai and Saito (1923) explains the behavior of the glabrous character in the No. J5 selection as well as in the No. J1 selection obtained from Professor Nagai. The character is fully dominant in each case but the use of the term "glabrousness" may be somewhat misleading because there are short bristles on the stems and petioles of glabrous plants.

Nagai and Saito (1923) also called attention to a decrease in vigor of glabrous plants and the writer has observed the same phenomenon. Glabrous plants grow much more slowly than pubescent plants and are inclined to send out more side branches. The stems are also very woody and tough as compared with those of pubescent plants.

A very similar type of glabrousness to that studied by Nagai and Saito (1923) has been reported by Stewart and Wentz (1926), but instead of being inherited as a dominant character it is strictly recessive. In this case a factor p_2 for glabrousness is assumed to be recessive to a factor P_2 for pubescence. The relation between P_2 and P_1 is naturally of interest because their effects seem to be exactly opposite. Crosses between the two glabrous types would be very desirable to study this relationship.

Another peculiar behavior of the crosses with glabrous varieties has been the occasional occurrence of abnormal plants. In all F_2 populations there has been a close approach to a 3:1 ratio of glabrousness to pubescence, respectively, but a small number were intermediate (4 out of a total of 442 F_2's grown in 1925). The growth of these intermediates was very much reduced, even more than that of the fully glabrous plants. The leaves were somewhat glabrous but the pods were as fully pubescent as those of pubescent plants. These appeared to be abnormalities that cannot be explained by genetic factors. An abnormal chromosome number seems more likely, so the four abnormal plants are not included in the genetic analyses which follow.

BLACK EYEBROW × GLABROUS SELECTION NO. J5

In the F_2 progeny numbers 10, 11, and 80 from crosses between Black Eyebrow and glabrous (No. J5), 251 plants were grown which were normal for either glabrousness or pubescence. The parental varieties in this combination differed in the following pairs of factors:

Black Eyebrow	*Glabrous (No. J5)*
Pubescent, p_1	Glabrous, P_1
Black seed-coat pigment, R_1	Brown seed-coat pigment, r_1
Eyebrow pattern, I^k	Pigment restricted to hilum, I^i
Tawny pubescence, T	Gray pubescence, t (potentially present)
Early maturing, E	Late maturing, e

Factors I^k, I^i and T will be more fully discussed in Part III of this series but brief mention should be made of the effect of T to clarify the classification in table 2. This factor (T) is known to intensify the pigment in the seed-coat as well as in the pubescence. In the F_2 populations, therefore, "imperfect black" and "buff" (see NAGAI 1921) in gray-pubescent plants correspond to black and brown respectively in tawny-pubescent plants, and indicate the presence and absence respectively of the factor R_1.

TABLE 1

The single pairs of factors in progenies 10, 11 and 80 considered separately.

	OBSERVED NUMBERS	DEV	P. E.	DEV / P. E.	ACTUAL RATIO
Black versus Brown	183: 68	5.25	4.63	1.1	2.7:1
Glabrous versus Pubescent	182: 69	6.25	4.63	1.3	2.6:1
Mottled versus Eyebrow Pattern	186: 65	2.25	4.63	0.5	2.9:1
Tawny versus Gray	169: 64	5.75	4.46	1.3	2.6:1
Early Maturity versus Late Maturity	144:107	44.25	4.63	9.56	1.3:1

In the F_2 generation the presence of T was evident in the field only in the case of the pubescent plants because the hairs on the glabrous plants were too much reduced in development to reveal the desired information. Fortunately this was not a handicap after the plants were ripe because the intensity of the seed-coat color has proved to be a very good index of the presence of T. The classification was therefore completed at that time and breeding results of 12 F_3 progenies have proved the correctness of the classification for a few cases which were somewhat doubtful. It will be observed, however, that no attempt was made to classify the glabrous plants with a brown "eyebrow" pattern for the intensity of color. This was advisable because of the gradations of colors in this particular class. In this case, therefore, the gray-pubescent class is included with the tawny.

The factors I^k and I^i belong to an allelomorphic series, in which i is the recessive for self-color and I^h is dominant for the restriction of all pigment in the seed coat; I^i restricts pigment to the hilum and I^k produces a saddle effect which has been designated the "eyebrow" pattern.

Linkage between P_1 and R_1

Table 1 gives the ratios obtained when each character is considered separately and table 2 gives the combined results from these crosses. Table 2 shows that there is evidently a linkage between P_1 and R_1 that may be complete, but because of the fact that the progeny segregated for tawny and gray pubescence, the situation is complicated. In the tawny-pubescent class there was no indication of the recessive brown color, but

TABLE 2

F_2 progenies 10, 11, and 80 combined, Black Eyebrow $(p_1R_1I^kT)$ ♀ × *No. J5* $(P_1r_1I^it)$ ♂.

	GLABROUS PLANTS							
	Tawny pubescence				Gray pubescence			
	Yellow mottled		Eyebrow pattern		Yellow mottled		Eyebrow pattern	
	Black	Brown	Black	Brown	Black	Brown	Black	Brown
Phenotypic formula.....	$P_1R_1I^iT$	$P_1r_1I^iT$	$P_1R_1I^kT$	$P_1r_1I^kT$	$P_1R_1I^it$	$P_1r_1I^it$	$P_1R_1I^kt$	$P_1r_1I^kt$
Plants flowering before July 20....	48	26	14	13	5	0	1	..
Plants flowering after July 20....	23	9	3	5	16	15	4	..
Total observed ..	71	35	17	18	21	15	5	..
Calculated	*70.6*	*35.3*	*23.5*	*15.7*	*23.5*	*11.8*	*7.8*	..[1]
Deviation	+.4	−.3	−6.5	+2.3	−2.5	+3.2	−2.8	
PUBESCENT PLANTS								
Phenotypic formula...	$p_1R_1I^iT$	$p_1r_1I^iT$	$p_1R_1I^kT$	$p_1r_1I^kT$	$p_1R_1I^it$	$p_1r_1I^it$	$p_1R_1I^kt$	$p_1r_1I^kt$
Plants flowering before July 20....	23	..	12	..	1	..	1	..
Plants flowering after July 20....	5	..	6	..	15	..	6	..
Total observed ..	28	..	18	..	16	..	7	..
Calculated	*35.3*	..	*11.8*	..	*11.8*	..	*3.9*	..
Deviation	−7.3		+6.2		+4.2		+3.1	

$\chi^2 = 13.005 \quad P = .22$

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[1] This class was very likely represented but because of difficulties in classification it was included with the tawny pubescent class under the same seed coat pattern. Without this discrepancy the theoretical ratio would have been 18:9:6:3:6:3:2:1:9:3:3:1, but with the system used the ratio becomes 18:9:6:4:6:3:2:0:9:3:3:1.

in the gray-pubescent class the distinction was not always clear. Seven plants from the total of 16 in the "yellow mottled, gray pubescent" class (table 2) were apparently buff rather than imperfect black, as classified in the table, but the writer has had considerable difficulty in distinguishing buffs from imperfect blacks in these crosses. However, breeding behavior in F_3 progenies revealed the fact that these plants really had a factor for the "imperfect black" coloration in the seed coat and that the F_2 plants were homozygous for that factor.

Fortunately there is a very convenient method of growing F_3 progenies to determine the relative amount of crossing over between P_1 and R_1. The glabrous character is easily recognized as soon as the seeds are germinated and a germination chamber suffices for this work. If the linkage were complete between P_1 and R_1 the genotypes $P_1r_1p_1R_1$, $P_1r_1P_1r_1$ and $p_1R_1p_1R_1$ should represent all the possible combinations in the F_2 progeny, that is, all glabrous plants with brown seed-coat color should be homozygous for P_1 and all glabrous plants with black seed-coat color should be heterozygous for P_1. To test this hypothesis F_3 progenies were germinated, giving the following results:

Phenotype in F_2	P_1R_1	P_1r_1
Number of progenies homozygous for P_1	3	15
Number of progenies heterozygous for P_1	20	2

These figures show that the linkage was not complete because 5 progenies from the total of 40 belong to crossover classes which indicates about 12 percent crossing over. This estimate, however, does not include plants that may have been of genotypic constitution $P_1R_1P_1R_1$, which represents a crossover class but which could not be recognized at the time of germination because R_1 has its effect on the seed-coat pigments. One progeny from a total of 10 which were grown to maturity in 1926 from the F_2 phenotype P_1R_1 proved to be of this constitution, that is, it was homozygous for R_1.

The evidence from the breeding behavior in F_3, therefore, indicates that crossing over between P_1 and R_1 is of rather frequent occurrence. These results were somewhat surprising because according to the seed-coat colors of the 46 tawny-pubescent plants in the F_2 generation there was no indication of crossing over. This inconsistency seems very odd but larger numbers must be grown before one can ascribe any reason other than chance. It shows very clearly, however, that a small percentage of crossing over has really taken place.

A single factor for time of maturity

The Black Eyebrow variety matures much earlier than the glabrous paternal parent. The differences and the comparison with the F_1's and F_2's were approximately as follows:

	Date planted	*Time of flowering*	*Time of maturity*
Black Eyebrow	March 13	July 10	Sept. 15
Glabrous (No. J5)	"	Aug. 3	Oct. 15
F_1'*s*	"	July 20	Oct. 5
F_2'*s*	"	(July 5–Aug. 10)	Sept. 10–Oct. 20)

An attempt was made in the F_2 population to make a careful record of the time of flowering but this was done only at certain periods so an exact quantitative record is not available for each plant. The only available method, therefore, was to classify the progeny according to general classes and, to simplify the results, a single division seems advisable. An arbitrary date was set at July 20 to distinguish early-flowering plants from late-flowering plants. At this time the distinction seemed clearer than at any other time because many of the early-maturing plants were setting pods but there were no signs of flowering on most of the late-maturing plants.

There was not a clear-cut 3:1 ratio for time of maturity because the expression of this character was rather variable and a large number of factors for the difference in the parental varieties might as well have been assumed as one, were it not for the high correlation that was found to exist between late maturity and gray pubescence. Table 2 shows this correlation.

If there were no correlation between time of maturity and color of pubescence there should be a simple 3:1 ratio for tawny- and gray-pubescent plants in both the early- and late-maturing classes. These theoretical results in comparison with the observed figures are as follows:

	Early-maturing		*Late-maturing*	
	Tawny	*Gray*	*Tawny*	*Gray*
Observed	123	8	46	56
Calculated, assuming no correlation between T and time of maturity	*98.2*	*32.8*	*76.5*	*25.5*

Applying the χ^2 test, $\chi^2 = 75.6$. This shows that there is a very significant correlation between the time of maturity and color of pubescence.

The data are so much out of agreement with Mendelian expectation that a calculation of crossing over means very little but COLLINS's (1924) modification of YULE's method gives 18 percent crossing over between *T* and *E*. By comparing the observed and theoretical figures on this basis the following results are obtained.

	Tawny-pubescent		*Gray-pubescent*	
	Early	*Late*	*Early*	*Late*
Observed	123	46	8	56
Calculated, *On basis of 18 percent crossing over*	*155.2*	*19.5*	*19.5*	*38.8*

The goodness of fit is obviously very poor but there are reasons to believe that it would be better to consider the gray-pubescent class alone. Since heterozygous plants are intermediate for time of maturity, it would be natural, even with complete linkage between *T* and *E*, for a considerable proportion of the heterozygous tawny-pubescent F_2 plants to intergrade into the late-maturing class. Calculating on the basis of the gray-pubescent class alone gives a crossover value of approximately 6 percent. This seems a much more logical value.

It should also be mentioned that 4 of the 8 early-maturing gray-pubescent plants were on the border line in the classification for time of flowering. It is possible, therefore, that they may not have been the result of crossing over. The other 4 plants in this class, however, were among the earliest in the progeny to mature, so there seems to be no doubt that these plants were a result of crossing over between the factors *T* and *E*.

A detailed study of the correlation between time of maturity and color of pubescence in this cross would be very desirable in connection with physiological studies as well as genetic. Since the effect of the relative length of day and night on the time of maturity has been so intensively studied in soybeans, it may be of interest to know the effect of a single Mendelian factor.

Further investigations are necessary to accurately determine the percent of crossing over between *E*, a factor for early maturity, and *T*, a factor for tawny pubescence, but there is little doubt that the factor *E* really exists. This knowledge has been made possible by the linkage between a rather indefinite quantitative character and a well known qualitative character. Examples of a very similar nature in Pisum are summarized in WELLENSIEK'S (1925) excellent monograph. The work of LOCK, TSCHERMAK and HOSHINO is particularly interesting in showing the correlation between time of flowering and flower color.

These examples of correlation between time of maturity and qualitative factors are very similar to cases where linkages have been reported with size factors (SAX, 1924 and LINDSTROM, 1926). The desirability of investigating such linkages cannot be overemphasized because of the application of Mendelian analyses to quantitative characters. By means of linkage relations it is possible to study the effect of a single factor where the data would otherwise result in nothing but confusion.

CROSS BETWEEN GREEN (NO. C4) AND GLABROUS (NO. J1)

The contrasted factors in progenies 91 and 101 are indicated by the following differences in the parental varieties.

Green (No. C4)	*Glabrous (No. J1)*
Pubescent, p_1	Glabrous, P_1
Black pod color, L (Woodworth, 1923)	Yellowish-brown pod color, l
Black seed-coat color, R_1	Brown seed-coat color, r_1
Pigment restricted to hilum, I^i	Self-color, i
Absence of mottling factor, m	Mottling factor, M (Black mottling on brown background)
Green plastid color in seed-coat, G	Yellow plastid color in seed-coat, g
Green cotyledon color, d_1d_2	Yellow cotyledon color, D_1D_2

In these crosses the inheritance of glabrousness was dominant and probably due to the same factor P_1 as in the foregoing crosses with the other glabrous variety (selection No. J5). This gives added interest to the linkage relationship with R_1.

The factor for blackish-brown pod color has been described by WOODWORTH (1923); D_1, D_2 and G are described in Part I of this series; and the factors I^i and R_1 have been discussed in connection with the preceding crosses (Nos. 10, 11 and 80). NAGAI and SAITO (1923) have described the factor M and report a linkage with P_1. About 18 percent crossing over is reported.

Table 3 gives the detailed behavior of this cross for progeny 91 but the cotyledon color and plastid color in the seed coat may be neglected at this time. The main point of interest is the linkage between P_1 and R_1 which has apparently been complete. Table 4 shows this relationship.

Progeny 101 was grown in 1926 at Arlington Farm but the detailed classification given in table 3 for progeny 91 was not made. Table 4, however, shows the relationship between P_1 and R_1 for both progenies.

This evidence indicates complete linkage between P_1 and R_1 but when F_3 progenies were tested in the germination chambers as previously described one case of crossing over was brought to light. Only 5 progenies from glabrous plants were tested but the single crossover shows that P_1 and R_1 were not completely linked. It is logical to suppose, therefore, that the same factor P_1 has been responsible for glabrousness in both of the

TABLE 3

F_2 progeny from Green (No. C4)×Glabrous (No. J1).

	GLABROUS							PUBESCENT		
	Black			*Brown*				*Black*		
	Pigment restricted		Self-Color	Pigment restricted		Self-color		Pigment restricted		Self-color
	Green	Yellow		Green	Yellow	Black mottled	Non-mottled	Green	Yellow	
All yellow cotyledon	16	6	8	1	3[1]	3	1	4	1	2
15 yellow : 1 greeen cotyledon......	4		2	2		1		3		
3 yellow : 1 green cotyledon......	8		5	2				5		2
All green cotyledon.	1					1				
Total	29	6	15	5	3	5	1	12	1	4

[1] Two with black mottling visible.

TABLE 4

F_2 progenies Green No. C4 ($p_1R_1I^i$)×Glabrous selection No. J1 (P_1r_1i).

		GLABROUS				PUBESCENT			
	Progeny Nos.	*Mottled*		*Self-colored*		*Mottled*		*Self-colored*	
		Black	Brown	Black	Brown	Black	Brown	Black	Brown
Observed................	91 101	35 44	15 16	8 10	6 5	13 31	.. ..	4 9	.. ..
Total................		79	31	18	11	44	..	13	..
Calculated for independent assortment...........		*82.7*	*27.5*	*27.5*	*9.2*	*27.5*	*9.2*	*9.2*	*3.1*
Deviation		−3.7 $\chi^2=2$	+3.5 8.01	−9.5 $P=0$	+1.8 .0002	+16.5	−9.2	+3.8	−3.1
Calculated for complete linkage between (P_1 and R_1) .		*73.5*	*36.7*	*24.5*	*12.3*	*36.7*		*12.3*	
Deviation..............		+5.5	−5.7	−6.5	−1.3	+7.3		+.7	

$\chi^2=4.65 \qquad P=0.46$

Japanese varieties (Nos. J1 and J5) because this factor has been identified in the same linkage group in both varieties.

The mottled pattern on the brown self-colors showed up as expected. Five of the 6 self-browns (table 3) were mottled and 2 plants badly mottled with brown also showed the presence of the black mottling factor. This indicates that the restriction factor I^i has no effect on the expression of M, providing enough brown mottling is produced. Although M and R_1 are both concerned in the production of anthocyanin pigment (M being responsible for concentric rings and R_1 for self-color) in the seed coat, they appear to be separately inherited; but from NAGAI and SAITO's (1923) results one would assume that they were situated on the same chromosome.

TABLE 5

F_2 progenies, Manchu No. 59 ($p_1R_1I^i$)×Glabrous selection No. J1 (P_1r_1i).

	Progeny Nos.	GLABROUS				PUBESCENT			
		Mottled		*Self-colored*		*Mottled*		*Self-colored*	
		Black	Brown	Black	Brown	Black	Brown	Black	Brown
Observed	83	37	21	17	5	20	1	6	2
	88	27	9	1	2	6	0	2	0
Total		64	30	18	7	26	1	8	2
Calculated for independent assortment		*65.8*	*21.9*	*21.9*	*7.3*	*21.9*	*7.3*	*7.3*	*2.4*
Deviation		−1.8	+8.1	−3.9	−0.3	+4.1	−6.3	+0.7	−0.4

$\chi^2=10.090$ $P=0.18$

The results from a very similar cross (Manchu No. 59, $p_1R_1I^i$ ♀ ×No. J1 P_1r_1i ♂) are reported in table 5. These results also show a linkage between P_1 and R_1. More numbers would be very desirable to increase the accuracy of the estimate of crossing over but the same factors p_1, R_1, and I^i explain the results very well thus far.

SUMMARY

1. A dominant factor P_1 for glabrousness has been described which is linked with R_1 the factor for black seed-coat color, but from the limited data available an accurate estimate of the amount of crossing over can not be given.

2. Attention is called to a description of another factor p_2 (STEWART and WENTZ, 1923) which produces the same effect as P_1 but is inherited as a recessive rather than as a dominant factor.

3. A factor *E* has been assumed responsible for early maturity in the Black Eyebrow variety because of the peculiar correlation with *T*, the factor for tawny pubescence. Both of these factors, however, are inherited independently of P_1.

LITERATURE CITED

Collins, G. N., 1924 Measurement of linkage values. Jour. Agric. Res. **27**: 881–891.

Lindstrom, E. W., 1926 Hereditary correlation of size and color characters in tomatoes. Iowa Agric. Expt. Sta. Technical Bul. **93**.

Nagai, I., 1921 A genetico-physiological study on the formation of anthocyanin and brown pigments in plants. Jour. Coll. Agric., Imp. Univ., Tokyo **8**: 1–92.

Nagai, I., and Saito, S., 1923 Linked factors in soy-bean. Japan. Jour. Bot. **1**: 121–136.

Sax, Karl, 1924 The nature of size inheritance. Proc. Nat. Acad. Sci. **10**: 224–227.

Stewart, Ralph T., and Wentz, John B., 1926 A recessive glabrous character in soybeans. Jour. Amer. Soc. Agron. **18**: 997–1009.

Wellensiek, S. J., 1925 Genetic monograph on Pisum. Bibliographia Genetica **2**: 343–476.

Woodworth, C. M., 1923 Inheritance of growth habit, pod color, and flower color in soybeans Jour. Amer. Soc. Agron. **15**: 482–495.