References

Raghuveer, K. G. and E. G. Hammond. 1967. The influence of glyceride structure on the rate of autoxidation. J. Am. Oil Chem. Soc. 44: 235-243.

Fatemi, S. H. and E. G. Hammond. 1977a. Glyceride structure variation in soybean varieties: I. Stereospecific analysis. Lipids 12: 1032-1036.

Fatemi, S. H. and E. G. Hammond. 1977b. Glyceride structure variation in soybean varieties: II. Silver ion chromatographic analysis. Lipids 12: 1037-1042.

Fehr, W. R., J. C. Thorne and E. G. Hammond. 1971. Relationship of fatty acid formation and chlorophyll content in soybean seed. Crop Sci. 11: 211-213.

S. H. Fatemi
E. G. Hammond

IOWA STATE UNIVERSITY
Department of Agronomy
Ames, IA 50011

1) Evidence for linkage of *G* with one of the *B* loci in soybeans.

In 1963, Tang and Li reported on a study of a cross, *Glycine max* x *G. formosana* (*G. formosana* = *G. soja*), in which the inheritance of a number of qualitative traits was determined. This paper is a reinterpretation of a portion of their data.

Among the genes segregating in the cross were the following:

i^i/i: restriction of dark seedcoat pigments to hilum/self-dark seed

G/g: green seedcoat/yellow seedcoat (obscured in ii genotypes)

$B_2,B_3/b_2,b_3$: two of the three complementary factors for bloom on seedcoat; in the cross studied, both parents were B_1B_1, so that segregation at the other two loci produced F_2 ratios of 9 bloom : 7 smooth.

Parental genotypes were $i^ii^iggb_2b_2b_3b_3$ for *G. max* and $iiGGB_2B_2B_3B_3$ for *G. soja*. When F_2 plants were classified as to seed color (green, yellow, black) and presence or absence of bloom, the observed numbers gave a poor fit (χ^2 probability 0.006) to the ratio expected under independent inheritance. Taken singly, the characters fit the appropriate monogenic and digenic ratios well. Thus, linkage is suspected. The data are consistent with the hypothesis of independent segregation of i^i/i and G/g, and of i^i/i and the genes controlling bloom. There remain for consideration the totals for the combinations of G/g vs B_2-B_3-/$b_2b_2b_3b_3$, as follows:

Phenotype	Observed number	Number expected given independence
bloom-green	125	104.6
smooth-green	57	81.4
bloom-yellow	31	34.9
smooth-yellow	35	27.1

The χ^2 probability for these totals, given independent inheritance, is 0.003, and a deficiency of recombinant classes is apparent. Calculation of linkage intensity is complicated by the complementary action of the B_2 and B_3 genes. Presumably one, say B_3, is linked to G, while the other is independent. We can use the maximum likelihood method, described by Mather (1946), to calculate linkage intensity. With p = the frequency of coupling type gametes ($B_3G + b_3g$), we can derive m_c, the expected frequency of each phenotypic class. There are eight types of gametes produced by the F_1 plant: (1) B_2B_3G; (2) b_2B_3G; (3) B_2b_3g; (4) b_2b_3g; (5) B_2B_3g; (6) b_2B_3g; (7) B_2b_3G; and (8) b_2b_3G. Each of the first four occurs with frequency $\frac{1}{4}p$, the other four with frequency $\frac{1}{4}(1-p)$. The smooth yellow phenotype, for example, is produced by the following gametic combinations:

Female gamete	Male gamete	Frequency
B_2b_3g	B_2b_3g	$(\frac{1}{4}p)^2$
B_2b_3g	b_2b_3g	$(\frac{1}{4}p)^2$
b_2b_3g	B_2b_3g	$(\frac{1}{4}p)^2$
b_2b_3g	b_2b_3g	$(\frac{1}{4}p)^2$
b_2b_3g	b_2B_3g	$(\frac{1}{4}p)(\frac{1}{4})(1-p)$
b_2B_3g	b_2b_3g	$(\frac{1}{4}p)(\frac{1}{4})(1-p)$
b_2B_3g	b_2B_3g	$(\frac{1}{4}[1-p])^2$

The sum of the frequencies of individual gametic combinations yields the value of m_c for each class. The values are as follows:

Phenotype	m_c	Observed number
bloom — green	$\frac{3(2+p^2)}{16}$	a
smooth — green	$\frac{3(2-p^2)}{16}$	b
bloom — yellow	$\frac{3(1-p^2)}{16}$	c
smooth — yellow	$\frac{3p^2+1}{16}$	d

The likelihood expression, to be maximized with respect to p, is

$$P(a,b,c,d|p) = \frac{n!}{a!b!c!d!}\left[\frac{3(2+p^2)}{16}\right]^a \left[\frac{3(2-p^2)}{16}\right]^b \left[\frac{3(1-p^2)}{16}\right]^c \left[\frac{3p^2+1}{16}\right]^d ,$$

where $n = a+b+c+d$. Taking the logarithm before differentiating, this becomes

$$\frac{d \ln P}{dp} = \left(\frac{a}{2+p^2} - \frac{b}{2-p^2} - \frac{c}{1-p^2} + \frac{3d}{1+3p^2}\right)(2p) .$$

This expression is now set equal to zero, and, with $p^2 = x$, becomes, after simplification

$$3(a+b+c+d)x^3 + (-8a+4b+c-3d)x^2 + (3a-5b-12c-12d)x + (2a-2b-4c+12d) = 0.$$

Now the observed values 125, 57, 31, and 35 are substituted for a, b, c, and d, respectively, and the equation (which has only one solution between 0 and 1) is solved by successive approximation to get $x = p^2 = 0.462466$. Thus, $p = 0.680$, and the recombination frequency, 1-p, is 0.320.

The standard error, s_p, of the calculated recombination frequency is determined using the information concept described by Mather (1946). The formulas are

$$i_c = \left(\frac{1}{m_c}\right)\left(\frac{dm_c}{dp}\right)^2 , \quad I_p = \sum_c i_c , \quad \text{and} \quad s_p = \sqrt{\frac{1}{nI_p}} .$$

Here, $s_p = 0.053$.

Weiss (1970) assigned G/g to Linkage Group 3. Further studies should be carried out in an attempt to verify the loose linkage between G/g and either B_2/b_2 or B_3/b_3 for which evidence has been given.

References

Mather, K. 1946. Statistical analysis in biology, 2nd ed. Interscience Publ., New York.

Tang, W. T. and S. L. Li. 1963. Studies on the genetic behavior of both quantitative and qualitative characters in the progeny of an interspecific cross of soybean, Glycine max x G. formosana (2nd report). J. Agric. Assoc. China N.S. 42: 13-24.

Weiss, M. G. 1970. Genetic linkage in soybeans. Linkage groups II and III. Crop Sci. 10: 300-303.

S. K. St.Martin

2) Soybean linkage tests.

F_2 linkage results are presented in Table 1 with a = XY, b = Xy, c = xY and d = xy for the gene pairs listed in the form of Xx and Yy. Percentage recombination was obtained from the ratio of products following the method of Immer and Henderson (1943).

Results from testing F_3 seeds and seedlings to determine F_2 phenotypes indicate possible linkage between seed coat peroxidase (ep) and root fluorescence (fr). Further studies are in progress to test this hypothesis. All other combinations were inherited independently.

Table 1

F_2 linkage tests

Genes	General phenotypic classes a	b	c	d	Sum	%R ± SE	Linkage phase
	'Minsoy' (T_1 fr ep Pb) x 'Hark' (t_1 Fr Ep pb)						
Pb pb Fr fr	235	89	71	25	420	49.0 ± 3.7	repulsion
Pb pb T_1 t_1	255	96	79	25	455	52.4 ± 3.6	coupling
Pb pb Ep ep	267	81	75	24	447	50.7 ± 3.5	repulsion
Fr fr T_1 t_1	226	81	78	35	420	53.1 ± 3.5	repulsion
Fr fr Ep ep	240	62	76	36	414	41.6 ± 3.3	coupling
	(Ep T_1 w_1 F) x (ep t_1 W_1 f)						
W_1 w_1 F f	738	191	252	71	1252	51.2 ± 2.1	repulsion
Ep ep T_1 t_1	418	123	139	33	713	53.0 ± 2.9	coupling
Ep ep W_1 w_1	393	112	141	32	713	46.8 ± 3.0	repulsion

Table 1 (cont'd)

Genes	General phenotypic classes a	b	c	d	Sum	%R ± SE	Linkage phase
			(Ep T_1 W_1 F) x (ep t_1 W_1 f) (cont'd)				
Ep ep F f	440	120	117	36	713	48.3 ± 2.8	coupling
T_1 t_1 W_1 w_1	704	233	225	90	1252	52.7 ± 2.1	repulsion
T_1 t_1 F f	773	254	266	76	1369	52.0 ± 1.4	coupling
			(Separate crosses)				
T_1 t_1 Ep ep	258	69	84	36	447	> 55.0	repulsion
L_1 l_1 K_2 k_2	113	48	39	12	212	54.5 ± 5.4	coupling
K_2 k_2 T_1 t_1	374	125	119	39	657	49.8 ± 2.9	repulsion
L_1 l_1 T_1 t_1	165	26	37	8	236	54.4 ± 4.6	repulsion

Reference

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28: 419-440.

Steven L. Broich
David M. Stelly
Reid G. Palmer—USDA

3) A possible cytoplasmic mutant.

A chimera plant (A75-1165-117) was observed in 1975 in the F_2 of a cross of Ames ms_1 x 'Clark' homozygous translocation (Table 1). Reciprocal crosses were made with 'Clark 63', using branches from the chimera plant that contained a high percentage of yellow trifoliolates. Selfed seed of the chimera plant (A75-1165-117) and F_1 seed from reciprocal crosses were planted in the field in 1976 (Table 2).

We observed 36 yellow and 17 green seedlings from self-pollination of the chimera plant. Twenty-seven yellow and 3 green plants were killed in a June hail storm. The yellow plants segregated for the translocation and gave all yellow plants in the F_4 and F_5. The green plants segregated for the translocation and 13 gave all green plants in the F_4 and F_5. One green plant, however, was lightly chimeric and in the F_4 segregated 198 green : 13 yellow plants.

Table 1

Pedigree of chimera plant A75-1165-117

A72-T30	Ames $\underline{ms_1}$ (See Soybean Genet. Newsl. 1: 28-30, 1974, and Soybean Genet. Newsl. 2: 16-18, 1975)
A73g-13	F_1 plant (A72-T30 $\underline{ms_1}$ x Clark 63)
A73-131	F_2 segregated 3 fertile : 1 sterile
A74-144	F_1 plant (A73-131-15 $\underline{ms_1}$ x Clark homozygous translocation from G. soja PI 101.404B)
A75-1165	F_2 segregated for both $\underline{ms_1}$ and translocation; plant 117 was a chimera and heterozygous for translocation

At present, 84 green F_4 plants have been progeny tested and all 2539 F_5 seedlings were green. One yellow F_4 plant has been progeny tested and gave all yellow F_5 seedlings (Table 2).

When the chimera plant was female parent with Clark 63, we observed 9 yellow and 1 green F_1 seedlings. Eight yellow seedlings were killed in the hail storm. In the F_2, progeny of the yellow F_1 plant segregated for the translocation and gave all yellow progeny, and progeny of the green F_1 plant segregated for the translocation and gave all green progeny. Since we were using chimera branches for pollinations, we assumed that the green F_1 plant did not receive the factor for yellow plant color in the female gamete because we did not observe yellow plants in the F_2 (Table 2).

When the chimera plant was male parent with Clark 63, we observed 23 green F_1 seedlings. Seven seedlings were killed in the hail storm. In the F_2 the green F_1 plants segregated for the translocation, but gave all green progeny. The absence of segregation in the F_2 suggests that cytoplasmic inheritance is involved.

In the crosses with A75-1165-117 we could not tell if the "hybrids" were cross-pollinations or self-pollinations because we had no genetic markers to observe for segregation (Table 2). Yellow plant color may or may not be carried in the gametes because we were crossing with chimeric branches.

In 1977, progeny from the lightly chimeric F_3 plant segregated 198 green : 13 yellow plants. Although this segregation approximated a 15 : 1 ratio, data

Table 2

Evaluation of selfed progeny and crosses with yellow plants derived from self-pollination of A75-1165-117

Parents	F_3 generation	F_4 generation	F_5 generation
Self-pollination of F_2 plant A75-1165-117	36 yellow plants (27 died)	5 F_3 plants segregated for translocation and gave all yellow progeny (73 plants)	6 F_4 plants gave all yellow progeny (152 plants)
		4 F_3 plants did not segregate for translocation and gave all yellow progeny (66 plants)	10 F_4 plants gave all yellow progeny (250 plants)
	17 green plants (3 died)	6 F_3 plants segregated for translocation and gave all green progeny (736 plants)	
		7 F_3 plants did not segregate for translocation and gave all green progeny (931 plants)	
		1 F_3 plant did not segregate for translocation and was lightly chimeric and segregated as follows:	
		- 198 green plants	84 F_4 plants gave all green progeny (2539 plants)
		- 13 yellow plants	1 F_4 plant gave all yellow progeny (21 plants)

Table 2 (cont'd)

Parents	F_1 generation	F_2 generation
A75-1165-117 x Clark 63	9 yellow plants (8 died)	1 F_1 plant segregated for translocation and gave all yellow progeny (4 plants)
	1 green plant	1 F_1 plant segregated for translocation and gave all green progeny (218 plants)
Clark 63 x A75-1165-117	23 green plants (7 died)	10 F_1 plants segregated for translocation and gave all green progeny (961 plants)
		6 F_1 plants did not segregate for translocation and gave all green progeny (887 plants)

collected previously suggested cytoplasmic inheritance; data collected subsequently substantiated the hypothesis of cytoplasmic inheritance. The chimeric condition of the F_3 plant was reflected in the F_4 segregation.

Allelism testcrosses and their reciprocals were made between the new yellow mutant and other yellow mutants ($\underline{y}_9$, $\underline{y}_{10}$, $\underline{Y}_{11}\underline{y}_{11}$, $\underline{y}_{12}$, $\underline{y}_{13}$, $\underline{Y}_{18}$ – and T253). All F_1 progeny behaved as expected if the new yellow mutant was inherited cytoplasmically.

In Table 3, we have presented parental, F_1 and F_2 data for reciprocal crosses with $\underline{Y}_{18}$ $\underline{Y}_{18}$. We have $\underline{w}_1$ as a nuclear genetic marker from $\underline{Y}_{18}$. The data indicate that the new yellow plant trait is inherited cytoplasmically.

Table 3

Evaluation of F_1 and F_2 generations of reciprocal crosses with yellow plants derived from self-pollination of A75-1165-117

Parents	F_1 generation	F_2 generation
$\underline{Y}_{18}$ $\underline{Y}_{18}$ $\underline{w}_1$ $\underline{w}_1$ x yellow $\underline{W}_1$ $\underline{W}_1$	green $\underline{W}_1$	131 green $\underline{W}_1$: 41 green $\underline{w}_1$
yellow $\underline{W}_1$ $\underline{W}_1$ x $\underline{Y}_{18}$ $\underline{Y}_{18}$ $\underline{w}_1$ $\underline{w}_1$	yellow $\underline{W}_1$	126 yellow $\underline{W}_1$: 44 yellow $\underline{w}_1$

Reid G. Palmer – USDA
Kimberly S. Lewers

CELL CULTURE and NITROGEN FIXATION LABORATORY
Beltsville Agricultural Research Center
Beltsville, MD 20705

1) Genetic linkage studies of factors controlling nitrogen fixation.

We are especially concerned with the genetic factors controlling symbiotic nitrogen fixation in soybeans. Four classical Mendelian factors have been identified in the macrosymbiont which regulate nodulation response (Vest et al., 1972). These are: $\underline{rj}_1$, which in homozygous recessive condition produces a non-nodulating phenotype with a broad spectrum of Rhizobium strains (Williams and Lynch, 1954); $\underline{Rj}_2$, a dominant factor conditioning an ineffective response with strains of the Cl and 122 serogroups (Caldwell, 1966); $\underline{Rj}_3$,