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Lettuce Genetics: Inheritance, Linkage, and Epistasis

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Abstract. **Two new lettuce *(Luctuca sativa* L.) genes are described and named *truncated leaf (tn),* and *sickly (si).* A gene for reflexed involucre is identical to that previously described in wild lettuce (L. *serriola* L.). *Mosaic reaction (me)* and *light green (lg)* are linked, with *P = 0.448.* Six gene pairs tested for linkage are independently inherited. *Sickly* is epistatic to *light green*.**

This paper continues the description of major genes, linkages, epistasis, and other interactive relationships in lettuce. Eventually, the accumulated information of this type will be suitable to be combined with information from RFLP and similar analyses to provide a comprehensive lettuce genome map. I report here two new genes, one linkage and one epistatic combination.

Materials and Methods

Eight experiments were conducted. The study of truncated leaf began with the observation of off-type plants in a field planting. These plants were in a group of BC_2F_3 families from the cross M631 × 'Salinas', which were segregating for mosaic resistance. The off-type plants were darker green and smaller than the normal plants' and had entire leaf margins and less-blistered leaves. The reduced size was partially due to truncation of the leaves. The truncated plant, 78-162-1, was crossed to 'Vanguard 75', a crisphead cultivar. Plant type was observed and recorded in F_1, F_2, and F_3 families.

A slow-growing, sickly appearing plant (85-1174cd) was observed in the F_2 progeny of a cross 82-1061-1 x 'Vanguard 75'. The plant was severely stunted, chlorophyll deficient, and had necrotic areas on the leaves. It survived to produce flowers and was crossed by 'Vanguard 75' and 'Prizehead', a red leaf lettuce. Plant type was observed in F_1 and F_2 populations of both crosses and in F_3 families of the 'Prizehead' cross. The latter cross also segregated for dark vs. light green, which led to a study of epistasis with the sickly character.

'Australian', a light-green leaf lettuce, produces reflexed mature involucres. A study of shattering (reflexed) vs. nonshattering (erect) -was reported by Whitaker and McCollum (1954) in a cross between wild *(L. serriola)* and cultivated lettuce. A single gene controlled the trait, with shattering dominant. The gene was designated as *b* and later renamed *er* (Robinson et al., 1983). Whitaker and McCollum footnoted a personal communication from R.C. Thompson that 'Australian' was the only known cultivated form with ". . . some degree of shattering. . . ." To study the inheritance of reflexed as manifested in 'Australian', an F_2 population from a cross 'Calmar' × 'Australian', planted for a linkage study, was also observed for involucre type. To confirm the involucre observations, a random sample of 80 F_3 families was grown. A reflexed plant from another 'Australian' cross was crossed with *L. serriola* to test for gene identity.

A possible linkage relationship between lettuce mosaic (LMV) reaction and dark- vs. light-green leaves was studied in F_2 populations of three crosses: 'Australian' × 'Vanguard 75', 'Chung Chuk Myun' × 'Vanguard 75', and 'Vanguard 75' × 'Prizehead'. 'Vanguard 75' is dark green and LMV resistant *(Lg*-Lgmomo), while the three other parents are light green and susceptible *(lglgMoMo).* 'Chung Chuk Myun' is a Korean leaf lettuce.

Several other linkage studies were based on F_2 populations of the crosses: M400-27-23 × 'Golden Bibb', yellow vs. salmon, normal vs. virescent, LMV reaction; and 'Calmar' × 'Australian', yellow vs. pale, black seed vs. white, dark vs. light green, reflex vs. erect.

All plantings were in a greenhouse. Plants were grown in flats or plastic pots of various sizes, in soil or sand-soil mixtures. The containers were fertilized and hand-watered as needed. Crosses were made by the method of Oliver (1910), modified to maximize the number of hybrid seeds (Ryder and Johnson, 1974; E. J. R., unpublished).

Genetic ratios were compared by standard x^2 procedure. Maximum likelihood tables were used to calculate recombination values (Allard, 1956). Recombination values are denoted by *p* and probability values by *P*.

Results and Discussion

Inheritance studies

Truncated leaf. Plants of this type grow normally for 45 to 55 days. The 15th or 16th leaf continues to expand normally latitudinally but at a slower rate at the marginal area around the apex, resulting in a truncated or foreshortened appearance. The F_1 was normal. The F_2 segregated ≈3 normal : 1 truncated (Table 1). F_3 families from normal F_2 plants segregated 1 normal :2 segregating, and segregation within the latter group was again 3:1. The evidence indicates that truncation is recessive and the trait is inherited as a single gene. The trait is named *truncated leaf* and designated *tn.*

The gene will probably be of limited use as a marker because the phenotype appears late in the vegetative growth period. It also has incomplete penetrance. The differential leaf expansion may, however, prove interesting in a developmental study.

Sickly. In the greenhouse, these plants emerge as early as normal ones. Cotyledons are smaller than normal and pale green. The first true leaf is yellow-green, slightly malformed, and small.

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Table 1. Inheritance of truncated leaf in lettuce. Segregation in F_2 population and F_3 families of the cross 78-162-1 × Vanguard 75.

	No. observed (expected)				
Population	Normal	Segregating	Truncated	χ^2	*P*
F_2	70 (68)		21 (23)	0.24(3:1)	0.5-0.7
Among F_3 families	15 (13)	24 (26)		0.47(1:2)	0.3-0.5
Within seg. families	199 (207)		77 (69)	1.24 (3:1)	0.2-0.3

Table2. Inheritance of sickly in lettuce. Segregation in F_2 populations and F_3 families.

	No. observed (expected)				
Population	Normal	Segregating	Sickly	χ^2	*P*
F_2					
85-1174cd x Prizehead	127 (126.75)		42 (42.25)	0.003 (3:1)	0.95–0.98
85-1174cd x Vanguard 75	166 (165)		54 (55)	0.03 (3:1)	0.8–0.9
F_3					
85-1174cd x Prizehead					
Among families	43 (36.5)	80 (73)	33 (36.5)	1.38 (1:2:1)	0.5–0.7
Within seg. families	1417 (1407)		459 (469)	0.28 (3:1)	0.5–0.7

Table 3. Inheritance of reflexed involucre in lettuce. Segregation in F_2 population and F_3 families of the cross 'Calmar' × 'Australian'.

	No. observed (expected)				
Population	Reflex	Segregating	Erect	χ^2	*P*
F_2	537 (530)		170 (177)	0.35 (3:1)	0.5–0.7
Among F_3 families	21 (20)	43 (40)	16 (20)	1.08 (1:2:1)	0.5–0.7
Within seg. families	425 (449)		174 (150)	5.12 (3:1)	0.02–0.05

Table 4. Evidence for linkage between genes for leaf color and mosaic reaction in lettuce, repulsion phase. S = susceptible, R = resistant.

	No. observed (expected)					
	Dark green		Light green		Linkage	
F_2 population	S	R	S	R	χ^2	*P*
Australian x Vanguard 75	361 (364)	134 (121)	125 (121)	26 (40)	5.43	0.01–0.02
Chung Chuk Myun x Vanguard 75	227 (248)	91 (83)	97 (83)	26 (28)	2.54	0.1–0.2
Vanguard 75 x Prizehead	517 (526)	181 (175)	185 (175)	52 (59)	1.72	0.1–0.2
Pooled	1105 (1138)	406 (379)	407 (379)	105 (126)	8.67	<0.01
Test for heterogeneity[z]						
Total χ^2 (9 df) = 13.38						
Pooled χ^2 (3 df) = 8.79						
Heterogeneity χ^2 (6 df) = 4.59						0.5–0.7

[z]Based on x^2 values for 9:3:3:1 ratio.

Three weeks after seeding, the first true leaf on a sickly plant is ≈1 cm long and on a normal plant 2 to 3 cm long. The sickly died during 90 days. The F_1 was normal. The F_2 populations plants produced a leaf every 6 days, about half the rate for the normal plants. Sickly type is partially lethal: 15 of 54 plants segregated ≈3 normal :1 sickly (Table 2). A sample of 156 F_3

Table 5. Linkage detection tests for lettuce showing no evidence for linkage. F, phenotypes: 1 = A-B-, 2 = A-bb, 3 = aaB-, 4 = aabb[z].

Loci[y]	Observed no. in each phenotype 1	2	3	4	Total	Linkage χ^2	*P*
sa;mo	513	170	156	64	903	1.34	0.2–0.3
vi;mo	492	168	183	69	912	0.45	0.5–0.7
lg;sa	525	175	170	70	940	1.59	0.2–0.3
pa;er	408	120	129	50	707	1.89	0.1–0.2
w;er	404	129	133	41	707	0.02	0.8–0.9
lg;er	421	129	116	41	707	0.62	0.3–0.5

[z]Comparison based on 9:3:3:1 F_2 ratio. Expected numbers for each test: *sa;mo* (508,169, 169, 56); *vi, mo(513,* 171, 171, 57); *lg,sa(529,* 176, 176, 59); *pa,er; w,er, lg,er* (398, 133, 133, 44).

[y]Gene symbols: *sa* = salmon flower; *mo* = lettuce mosaic resistance; *vi* = virescent leaf; *lg* = light green leaf; *pa* = pale flower; *er* = erect involucre; *w* = white seed.

families from the cross with 'Prizehead' segregated ≈1 normal :2 segregating :1 sickly. Within the segregating families, segregation was again 3:1. The sickly type is recessive, and the trait is inherited as a single gene, which is named *sickly* and symbolized *si*.

This trait may be useful as a marker, as the effect is easily observed in the cotyledon-first true-leaf stage. It is also interesting as a variant to the chlorophyll-deficient genes previously identified in lettuce (reviewed in Robinson et al., 1983; Ryder, 1983). Among those genes, albino genes are lethal, and golden is partially lethal. Expressivity of the golden genotype varies in the seedling stage with regard to the chlorophyll-deficiency aspect; those plants with severe reduction of chlorophyll, approaching albinism, usually die. Those with less-severe reduction survive, grow and develop normally, and produce golden flowers. The other chlorophyll-deficient genes restrict growth and cause varying degrees and patterns of yellowing. Sickly plants all survive the seedling stage but become progressively weaker, severely stunted, and partially necrotic. This trait would also be of interest in a study of growth and development.

Reflexed involucre. Segregation in F_2 was ≈3 reflexed : 1 erect (Table 3). The F_3 families segregated 1 reflexed : 2 segregating :1 erect. Segregation within segregating families was significantly different from 3:1, with an excess of erect plants. This result probably is due to a misreading. There was no visible difference between homozygous and heterozygous reflexed plants. The preponderance of evidence is that reflexed is dominant and controlled by a single gene. These are the same results as found by Whitaker and McCollum (1954). A reflexed plant from another experiment with 'Australian' as a parent was crossed with a reflexed plant of *L. serriola.* The F_1 was also reflexed; therefore, the genes are the same.

Linkage studies

Leaf color-mosaic reaction. The F_2 population from the cross 'Australian' × 'Vanguard 75' segregated for several traits, including dark-green vs. light-green leaf and reaction to lettuce mosaic virus. 'Australian' is light green and susceptible *(lglgMoMo),* while 'Vanguard 75' is dark green and resistant *(LgLgmomo).* There was an excess of parental types and a shortage of recombinant types (Table 4). Linkage in the repulsion phase was indicated. Two additional F_2 populations were grown: 'Chung Chuk Myun' × 'Vanguard 75' and 'Vanguard 75' x 'Prizehead'. 'Chung Chuk Myun' and 'Prizehead' are also light green and mosaic susceptible. F_1 plants from crosses between 'Australian' and the other two light-green cultivars were light green; therefore, the three cultivars have the same light-green allele.

The other two F_2 populations also showed a shortage of re-

Table 6. Segregation for dark vs. light green and normal vs. sickly in F_2 and F_3 families of the cross 85-l174cd × Prizehead lettuce.

Population	No. observed (expected) Dark	Light	Sickly	χ^2 (9:3:4)	*P*
F_2	93(92)	34(32)	42(42)	0.21	0.5–0.7
Among F_3 families	*Observed*	*Expected*			
All dark	11	9.75			
Seg. dark : sickly	15	19.5			
Seg. 9:3:4	40	39.0			
Seg. dark : light	22	19.5			
Seg. light : sickly	25	19.5			
All light	10	9.75			
All sickly	33	39.0			
				4.03	0.5–0.7
	No. observed (expected)				
Within segregating families	Dark	Light	Sickly		
Dark : sickly (3:1)	209 (214)		76 (71)	0.47	0.3–0.5
9:3:4 (A)	426 (440)	184 (147)	173 (196)	12.46	<0.01
9:3:4 (B)	192 (193)	62 (64)	89 (86)	0.30	0.8–0.9
Dark : light (3:1)	317 (330)	123 (110)		2.03	0.1–0.2
Light: sickly (3:1)		344 (349)	121 (116)	0.29	0.5–0.7

combinant types, but the x^2 for linkage was not significant in either test (Table 4). However, the consistent shortage of recombinant in all three populations indicates linkage. When the observed numbers are pooled, x^2 for linkage is highly significant. Heterogeneity x^2 for the 9:3:3:1 ratio is not significant, indicating the populations segregate in the same manner. I conclude that there is loose linkage in the repulsion phase. The recombination value was calculated as $P = 0.44 \pm 0.018$.

Other linkage detection tests. Linkage x^2 and probabilities were calculated for six other combinations. Independent assortment was shown for all pairs (Table 5).

Epistasis

Light green and sickly. The F_2 of the cross 85-1174cd × 'Prizehead' segregated for dark- vs. light-green as well as for the sickly trait. Only three phenotypes were distinguishable—dark green, light green, and sickly-and they occurred in the ratio 9:3:4 (Table 6). This ratio suggests that sickly is epistatic to light green. There were seven categories among 156 F_3 families, as expected, if *LgLgsisi, Lglgsisi,* and *lglgsisi* are all sickly, but otherwise not distinguishable. Segregation among families was as expected (Table 6). Segregation within F_3 families was also as expected, except for those segregating for all three phenotypes. In this group, there was an excess of light green plants and a shortage of the other phenotypes. A second planting of seven three-phenotype families was grown and segregated 9:3:4, as expected. Therefore, *Sisi is* epistatis to *Lglg.*

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