Additional Genes Controlling Flowering Time in *Lactuca sativa* and *L. serriola*

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ABSTRACT. Six genes controlling flowering time or bolting time in *Lactuca* L. have been reported. Several crosses between parents differing in time to opening of first flower were made to ascertain the inheritance of additional flowering time traits in *Lactuca* species. The parents in the crosses were of five flowering classes: very late (VL), late (L), early (E), very early (VE), and very, very early (VVE). Segregation from a cross between C-2-1-1 (VL) (*L. sativa* L.) and ‘Vanguard 75’ (L) confirmed that ‘Vanguard 75’ flowering was controlled by the previously identified gene *Ef-2ef-2*. Mutant line 87-41M-7 (VVE) was crossed by D-3-22M (VE) and segregated 3VVE:1 VE, indicating a dominant allele, *Ef-3*, that decreased flowering time an additional 7 days. Cos-like line 796 (VE) was crossed to cultivars Salinas (VL) and Vanguard 75. Segregation indicated a gene *Ef-4ef-4*, with lateness dominant. PI 175735 (E) (*L. serriola* L.), crossed with C-2-1-1 produced an F₂ population with a bimodal distribution, segregating 3 E:1 VL, indicating a single gene *Ef-5ef-5*. PI 236396 (E) and PI 250020 (E) were crossed to ‘Salinas’ and ‘Vanguard 75’. Segregation and morphological similarity indicated the same gene in both PI lines, *Ef-6ef-6*, with earliness dominant.

Flowering time is closely associated with stem elongation in rosette forming plants such as lettuce (*Lactuca sativa*). Early stem elongation, or premature bolting, is a problem in lettuce production, since it can cause loss of quality or of the crop itself. Information about flowering time, and by extension, stem elongation, may be of use in breeding programs for bolting resistance.

Flowering time is an interesting trait in plants for other reasons. A sufficient degree of earliness enables a researcher to study the genetics and physiology of life cycles in short time periods (Ryder, 1996; Waycott, 1995; Waycott and Taiz, 1991; Waycott et al., 1991, 1995). Very early flowering plants are also useful as educational tools, as the rapid cycling enables the completion of a research project in short time periods (Hawk and Crowder, 1978). Earliness can be useful as a breeding tool, particularly in speeding up the backcross method (Ryder, 1985).

The earliest work on the genetics of earliness characteristics in *Lactuca* species was by Bremer (1931). He crossed a long-day cultivar (‘Kaiser Treib’) with a day-neutral cultivar (‘Rudolph’s Liebling’), and showed that long-day-influenced bolting was dominant and day-neutral was recessive. Bremer and Grana (1935) named the gene *Tt*.

Early flowering time was identified by Ryder (1983) as a single gene trait. Later, a second gene was discovered (Ryder, 1988). The genes were named *Ef-1ef-1* and *Ef-2ef-2*. *Ef-1* is partially dominant to *ef-1*, while *Ef-2* is partially dominant to *ef-2* when *Ef-1* is present, but recessive in the *ef-1ef-1* genotype. Plants that are homozygous dominant for both genes (very early, VE) flower under summer greenhouse conditions in 45 d, while the double recessive flowers in 140 d (very late, VL). The trait is quantitatively photoperiod related; all genotypes are slower to flower in winter than summer. In another study, two quantitative analysis methods were used to identify three partially dominant genes

for flowering time (Kim and Ryder, 1994). This paper describes the continued work with early flowering in lettuce and its relatives. Specifically, we discuss early flowering traits inherited as qualitative characters.

Materials and Methods

From the previous flowering time studies, we were able to identify lines with the *ef-1ef-1ef-2ef-2* (VL) genotype, which could be used as a VL flowering parent in crosses to identify additional flowering time genes (Table 1). One of these lines, 81-1252-C-2-1-1 (C-2-1-1) (Ryder, 1996) was crossed with ‘Vanguard 75’ (L). ‘Vanguard 75’ is a crisphead lettuce grown in the late winter–early spring period in the California and Arizona desert districts. It flowers earlier than other current crisphead lettuces and was postulated to have *Ef-2* in its genotype (Ryder, 1988).

The cross with C-2-1-1 was made in 1986. The F₁ population was grown in the greenhouse in 1987. The F₂ population was grown in 1988 and 1990 and F₃ families were grown in 1990. One segregating F₃ family and parents were also grown in 1995.

Table 1. Seasonal flowering time ranges for early and late flowering parents used in crosses. Number of days from seeding to first open flower.

Item	Spring–summer (d)	Fall–winter (d)	Flower time class
PI 175735	52–80	128–166	Early
PI 236396	45–60	82–124	Early
PI 250020	47–63	97–116	Early
81-1252-C-2-1-1			
(C-2-1-1)	111–156	192–214	Very late
Salinas	125–150	175–210	Very late
Vanguard 75	94–136	163–178	Late
87-41M-7	36–42	NA ²	Very, very early
D-3-22M	45–50	65–69	Very early
09-960796-0 (796)	45–49	NA ²	Very early

²Fall–winter plantings were not made.

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Line 87-41M is an early flowering line (*Ef-1Ef-1Ef-2Ef-2*, VE) (Table 1) that also carries the gene *enen* for *endive-like* leaf. A single plant (87-41M-7) in a small population flowered several days earlier (52 d, VVE) than the other plants (mean: 66 d). The plant was crossed to D-3-22M, which is also *Ef-1Ef-1Ef-2Ef-2* (VE). The VVE plant was lost and the population was regrown. Another VVE flowering plant was identified, assumed to be genetically the same as the original, and the cross was made again. F₁ populations were grown in 1995 from the original cross and in 1998 and 1999 from the second cross. One F₂ population was grown from the first F₁ and two from the second. One group of F₃ families was grown from the first F₂ population and one from the second.

A group of lines was received from A. van der Arend, Leen de Mos Seed Co. (Gravzande, The Netherlands) with various genetic anomalies. Among them was a cos line, 09-960796-0 (796), which flowered early; compared to a line D-3-22M (*Ef-1Ef-1Ef-2Ef-2*), it flowered in about the same number of days (D-3-22M = 55.8 d; 796 = 55.3 d when seeded 29 Mar. 2002) (Table 1). Line 796 was crossed with 'Vanguard 75' and 'Salinas' (VL). F₂ populations were grown in 2000, seeded 14 July 2000, and F₃ families in 2001, seeded 23 Mar. 2001 ('Vanguard 75' cross) and 20 Apr. 2001 ('Salinas' cross). A second seeding of F₃ families from the 'Salinas' cross was made 22 May 2003.

Three Plant Introduction (PI) accessions were identified in greenhouse and growth chamber tests as early flowering. PI 175735 (*L. serriola*) (early, E) has elongated leaves with entire margins, spines on stems and leaf midribs (abaxial), anthocyanin, and it bolts and flowers early in a long-day environment and later in a short-day environment, but not as late as parents designated L or VL (Table 1). It was crossed with C-2-1-1, to produce F₁, F₂, and F₃ family populations. To investigate flowering time variation within the early class of the F₂, two crosses were made between pairs of derived F₄ lines from the original cross: I-B-20-2 (68 d) x I-A-5-2 (62 d) and I-B-20-4 (71 d) x I-A-5-3 (56 d). In each cross, the female parent was slightly later than the male parent. Parental, F₁, and F₂ populations from both crosses were seeded without replication 6 Mar. 1992. The same generations and F₃ families for the first cross only were seeded in two replications 2 Apr. 1993.

PI 236396 (*L. sativa*) (E) is a primitive oilseed type with elongated leaves, no spines, and anthocyanin on leaf margins (tinge). It flowers early during long days and later during short days (Table 1). PI 250020 (*L. sativa*) (E) is also an oilseed type, very similar in appearance and flowering time to PI 236396 (Table 1). PI 236396 and 250020 were each crossed with 'Salinas' and 'Vanguard 75', to produce F₁, F₂, and F₃ families.

Seed was sowed in a sand-soil mixture and transplanted at the three- to four-leaf stage to small plastic pots (~75 cm³), one plant per pot. All plantings were grown in the greenhouse. Flowering time was measured as the number of days from seeding to first open flower. Flowering times for winter and summer conditions

Fig. 1. Distribution of flowering times for F₂ populations of 'Vanguard 75' (L) x C-2-1-1 (VL) grown in 1988 and 1990 (L, late; VL, very late).

in the greenhouse are shown for all parents used in these studies (Table 1).

Flowering time varies over seasons within a year due primarily to changes related to photoperiod. From year to year, materials planted around the same planting date vary in flowering time due to temperature, greenhouse location, and other environmental differences (Ryder, 1996). Plants flower earliest when planted in the period from early March to early July; they flower latest when planted in the period late August to early December. Therefore, populations reported in this paper are identified by planting date. Genetic analysis was done by χ^2 for one or two gene segregation data.

Results

'Vanguard 75' x C-2-1-1

The parents and F₁ populations were seeded 2 Oct. 1987. Six plants of 'Vanguard 75' and five of C-2-1-1 averaged 169.5 and 200.8 d, respectively. There were only two F₁ plants, flowering in 176 and 197 d (mean 186.5 d), respectively. Therefore F₁ flowering time appeared to be intermediate between the parents. An F₂ population planted 24 June 1988 showed a bimodal distribution, with the preponderance of plants in the very late (VL) class (Fig. 1, Table 2). If the F₂ distribution is divided at the low point, the proportion of VL:late (L) is approximately 3:1. A second seeding was made 18 May 1990. The F₂ distribution was bimodal; flowering times approximated 3 VL:1 L (Fig. 1, Table 2).

The flowering times for the parents in the first experiment were outside the range of the F₂ population component means. The plants were located several feet away from the F₂, on another bench. In a small greenhouse, temperature and other conditions may vary from bench to bench, which may account for the difference. The relationships between the parents and the F₂ were closer to expectation in the second planting. A segregating F₃ family, in lieu of a third F₂ population, for which there was insufficient

seed, was grown in 1995, with random placement of the parents. This population segregated approximately: 3 VL:1 L, and the parental means coincided with the means of the VL and L classes, as expected (Table 2).

As there were only two F₁ plants grown in 1987, an estimate for an F₁ putatively grown along with the 1988 F₂ population was made by obtaining the mean for F₂ plants that produced segregating F₃ families and were therefore heterozygous. The mean obtained was 143.5 d, which is less than, but close to, the mean for the VL class of the F₂ (150.5 d) and therefore supports the hypothesis that VL is dominant.

Among F₃ families from VL F₂ plants of the 1988 planting, the ratio was approximately 1 VL:2 segregating. Within segregating F₃ families, the ratio VL:L was again approximately 3:1. The conclusions are that a single gene controls the difference in flowering time and that VL is dominant to L.

87-41M-7 x D-3-22M

Flowering time was recorded for the F₁ population planted in 1999. There were two plants and both flowered in 48 d, which was equivalent to the mean for VVE plants in the F₂ population grown at the same time. This suggests that VVE flowering is dominant to VE flowering. All F₂ populations segregated approximately 3 VVE:1 VE (Table 3). Among F₃ families, segregation approximated 1 VVE:2 segregating:1 VE in both groups of families. Within segregating families, segregation approximated 3 VVE:1 VE in both groups, as in the F₂ (Table 3). The conclusion from these data is that a gene for flowering time has been identified and the earlier allele is dominant to the later allele.

796 Crosses

F₁ flowering times were estimated using the mean of F₂ heterozygous plants, seeded 14 July 2000. The parental and F₁ flowering times were 'Salinas' = 142.6 d; 'Vanguard 75' = 123.2 d; 796 = 39 d (one plant); 796 x 'Salinas' = 116.6 d; and 796 x 'Vanguard 75' = 111.7 d; these indicate a high level of dominance for lateness ('Salinas' is VL and 'Vanguard 75' is L).

The 796 x 'Salinas' F₂ population, seeded 14 July 2000, segregated 47 VL:26 E (Table 4), with clear separation between early and late values. However, 22 plants died before their flowering times were recorded; 15 of these were beginning to bolt and would have been late flowering plants. The others died too early to be identified. This would suggest segregation of 62 VL:26 E, not counting the seven unidentifiable plants ($\chi^2 = 0.97$; $P = 0.30-0.50$), giving a better 3:1 fit. Families of an F₃ population seeded 20 Apr. 2001, segregated approximately 1 all VL:2 segregating (Table 4). Within segregating families, segregation approximated 3 VL:1 E. In a second F₃ population, planted 22 May 2003, from VL F₂ plants only, segregation approximated 1 all VL:2 segregating among families, and 3 VL:1 E within segregating families (Table 4). The evidence suggests a single gene, with lateness (VL) dominant.

The 796 x 'Vanguard 75' F₂ population, seeded 14 July 2000, segregated 55 L:27 E (Table 5). Of 14 plants that died unrecorded for flowering time, nine would have been late flowering and the rest were unknown. This suggests a 64 L:27

Table 2. Segregation for flowering time in the cross 'Vanguard 75' x C-2-1-1. 'Vanguard 75' is late (L) and C-2-1-1 is very late (VL). Populations identified by date of planting.

Population	VL	Mean (d)	L	Mean (d)	χ^2	<i>P</i>
F ₂ (expect 3:1)						
24 June 1988	179	150.5	53	111.8	0.57	0.30-0.50
18 May 1990	90	141.9	29	104.7	0.04	0.80-0.90
5 May 1995						
(Seg. F ₃ family)	84	133.8	36	113.3	1.60	0.20-0.30
Pooled	353		118		0.006	0.90-0.95
F ₃ families						
(18 May 1990)	VL	Segregating	L			
Among (expect 1:2)	16	25			0.60	0.30-0.50
Within (expect 3:1)	228		74		0.04	0.80-0.90

Table 3. Segregation for flowering time in cross 87-41M-7 x D-3-22M. Populations identified by date of planting. 87-41M-7 is very, very early (VVE) and D-3-22M is very early (VE).

Population	VE	Mean (d)	VE	Mean (d)	χ^2	<i>P</i>
F ₂ (expect 3:1)						
21 Feb. 1997	73	42.3	23	50.0	0.05	0.80-0.90
5 Feb. 1999	68	48.7	26	56.7	0.36	0.50-0.70
10 Mar. 2000	71	40.3	21	46.0	0.23	0.50-0.70
Pooled	212		70		0.005	0.90-0.95
F ₃ families						
Among (expect 1:2:1)	VVE	Segregating	VE			
8 Aug. 1997	9	22	8		0.69	0.30-0.50
10 Mar. 2000	9	16	5		1.10	0.30-0.50
Pooled	18	38	13		1.44	0.30-0.50
Within segregating families (expect 3:1)						
8 Aug. 1997		202		73	0.35	0.50-0.70
10 Mar. 2000		130		50	0.75	0.30-0.50
Pooled		332		123	1.00	0.30-0.50

Table 4. Segregation for flowering time in cross 09-9607796-0 (796) x 'Salinas.' 796 is very early (VE) and 'Salinas' is very late (VL). Populations identified by date of planting.

Population	VL	Range (d)	VE	Range (d)	χ^2	<i>P</i>
796 x 'Salinas' F ₂ (expect 3:1)						
14 July 2000	47	112-149	26	44-54	4.39	0.02-0.05
F ₃ Families	VL	Segregating	VE			
Among (expect 1:2)						
20 Apr. 2001	7	12			0.11	0.70-0.80
22 May 2003	5	14			0.42	0.50-0.70
Pooled	12	26			0.06	0.80-0.90
Within (expect 3:1)						
20 Apr. 2001	98		37		0.41	0.50-0.70
22 May 2003	127		39		0.20	0.50-0.70
Pooled	225		76		0.012	0.90-0.95

Table 5. Segregation for flowering time in cross 09-960796-0 (796) x 'Vanguard 75.' 796 is very early (VE) and 'Vanguard 75' is late (L). Populations identified by date of planting.

Population	L	Range (d)	VE	Range (d)	χ^2	P
796 x 'Vanguard 75' F ₂ (expect 3:1)						
14 July 2000	55	90–140	27	43–57	2.75	0.05–0.10
F ₃ families (23 Mar. 2001)	L	Segregating	VE			
Among (expect 1:2)	6	14			0.10	0.70–0.80
Within (expect 3:1)	126		42		0	>0.99

Table 6. Segregation for flowering time in the cross PI 175735 x C-2-1-1. PI 175735 is early (E) and C-2-1-1 is very late (VL). Populations identified by date of planting.

Population	E	Mean (d)	VL	Mean (d)	χ^2	P
175735 x C-2-1-1 F ₂ (expect 3:1)						
24 June 1988	178	69.0	53	130.3	0.52	0.30–0.50
14 July 1989	85	76.2	33	143.3	0.56	0.30–0.50
Pooled	263		86		0.03	0.80–0.90
F ₃ families 14 July 1989	E	Segregating	VL			
Among (expect 1:2:1)	20	44	15		1.65	0.30–0.50
Within (expect 3:1)	371		142		1.96	0.10–0.20

E ratio ($\chi^2 = 1.05$; $P = 0.30$ – 0.50). An F₃ population, seeded 23 Mar. 2001, segregated approximately 1 all L:2 segregating among families and 3 L:1 E within segregating families (Table 5). The evidence suggests a single gene, with lateness dominant.

Crosses with PI accessions

PI 175735. The F₁ of 175735 x C-2-1-1 (VL) flowered in 151.8 d, compared to 148.5 d for the early parent in a 2 Oct. 1987 seeding. The mean of heterozygous F₂ plants grown in lieu of F₁ plants, seeded 24 June 1988, flowered in 69.7 d, compared to 67.3 d for the early parent. Strong dominance for earliness is suggested. Two F₂ populations were grown, seeded 24 June 1988 and 14 July 1989, respectively. Distributions were bimodal with a slight overlap. Divided at appropriate low points, they each segregated approximately 3 E:1 VL. Seventy-nine F₃ families were seeded 14 July 1989. They segregated approximately 1 all E:2 segregating:1 all VL. Within segregating families, segregation was approximately 3 E:1 VL, as expected. The evidence indicates a single gene, with earliness dominant (Table 6).

Variation within early and late classes in the distribution of F₂ plants suggested additional genetic variation for earliness. Crosses between earlier and later plants in the early class were analyzed for further variation. In the non-replicated test, planted 6 Mar. 1992, a continuous distribution skewed towards the early side occurred in the F₂ for both crosses and the F₁s were very close to the early parents, suggesting a quantitative basis, with earliness dominant. Similar distributions of the F₂ and among F₃ families were shown in the replicated experiment, seeded 2 April 1993. Regression of F₃ means on F₂ values was a straight line with slope $b = 0.48$, also suggesting a quantitative genetic basis for the variation (Fig. 2). The nature of the genetic basis was not investigated further.

Fig. 2. Joint distribution of flowering times for F₂ plants and F₃ family means of PI 175735 (E) x C-2-1-1 (VL). Regression equation: $Y = 34.17 + 0.48X$; $F = 119.81$; $P = <0.0001$ (E, early; VL, very late).

PI 236396. The flowering time range for 10 plantings over 1 year for PI 236396 in the greenhouse is 45–124 d. Parental, F₁, and F₂ populations of crosses with ‘Vanguard 75’ and ‘Salinas’ were planted 26 May 2000. Parental means were PI 236396 = 53.3 d, ‘Salinas’ = 140.2 d, and ‘Vanguard 75’ = 120.4 d. The F₁ with ‘Vanguard 75’ flowered in 62.3 d, and with ‘Salinas’ at 69.5 d. The F₂ distribution of 236396 x ‘Vanguard 75’ was divided into four distinct groups: a large early group and three later groups. The ratio of early to the sum of the later groups approximated 3:1 (Table 7). The F₂ of 236396 x ‘Salinas’ also was divided into four groups and the ratio of early to the sum of the late groups was also 3:1 (Table 7). If the numbers for the three late groups are summed for both crosses, the totals are 27:12:8. This approximates 9 early-late:4 mid-late:3 late-late ($\chi^2 = 0.09$; $P = 0.95$ –0.98), suggesting two additional genes, exhibiting recessive epistasis, segregating within the late class. In the early class, it is difficult to distinguish among the subclasses, suggesting a much stronger effect of the primary gene. F₃ families of 236396 x ‘Vanguard 75’ segregated approximately 1 all E:2 segregating:1 all L, and approximately 3 E:1 L within segregating families. F₃ families from early F₂ plants of 236396 x ‘Salinas’ segregated approximately 1 all E:2 segregating and approximately 3 E:1 L within segregating families (Table 7).

PI 250020. The flowering time range for 10 plantings over 1 year for PI 250020 was 47–116 d. Parental and F₂ populations of crosses with ‘Vanguard 75’ and ‘Salinas’ were seeded 2 June 2000. The parental means were PI 250020 = 55.5 d, ‘Vanguard 75’ = 111.0 d, and ‘Salinas’ = 130.4 d. Estimated F₁ means from heterozygous F₂ plants are 63.2 d for the cross with ‘Vanguard 75’ and 63.9 d for the cross with ‘Salinas.’ The F₂ distributions of both 250020 x ‘Vanguard 75’ and 250020 x ‘Salinas’ were each divided into one large early group and three late groups. Each segregated approximately 3:1, early to sum of late groups (Table 8). When the numbers for the late groups for both crosses are summed the ratio of 24 early-late:10 mid-late:4 late-late is approximately 9:4:3 ($\chi^2 = 1.72$; $P = 0.30$ –0.50). As with the crosses with 236396, the segregation was apparently masked in the early group. F₃ families of both 250020 x ‘Vanguard 75’ and 250020 x ‘Salinas’ segregated approximately 1 all E:2 segregating:1 L among families and 3 E:1 L within segregating families (Table 8).

Discussion

Genes for flowering time were identified in six sources: a commercial cultivar, a mutant line, a breeding line, and three PI accessions (Table 9).

Several pieces of evidence show that the gene identified in ‘Vanguard 75’ is *Ef-2Ef-2*. The original cross of 56679 x ‘Vanguard 75’ segregated for two genes (Ryder, 1988). It is most likely that a single mutation occurred in the 56679 parent, *ef-1* > *Ef-1*,

Table 7. Segregation for flowering time in crosses ‘Vanguard 75’ x PI 236396 and ‘Salinas’ x PI 236396. ‘Vanguard 75’ is late (L), ‘Salinas’ is very late (VL), and PI 236396 is early (E). Populations identified by date of planting.

Population	E	Range (d)	L or VL	Range (d)	χ^2	P
F ₂ (expect 3:1)						
26 May 2000						
‘Vanguard 75’ x 236396	65	51–72	27	91–149	0.93	0.30–0.50
‘Salinas’ x 236396	65	50–78	20	86–151	0.09	0.70–0.80
F ₃ families						
‘Vanguard 75’ x 236396 (29 June 2001)						
Among (expect 1:2:1)	6	9	5		0.30	0.80–0.90
Within (expect 3:1)	83		24		0.37	0.50–0.70
‘Salinas’ x 236396 (9 Feb. 2001)						
Among (expect 1:2)	5	11			0.03	0.80–0.90
Within (expect 3:1)	102		30		0.36	0.50–0.70

Table 8. Segregation for flowering time in crosses ‘Vanguard 75’ x PI 250020 and ‘Salinas’ x PI 250020. ‘Vanguard 75’ is late (L), ‘Salinas’ is very late (VL), and PI 250020 is early (E). Populations identified by date of planting.

Population	E	Range (d)	L or VL	Range (d)	χ^2	P
F ₂ (expect 3:1)						
2 June 2000						
‘Vanguard 75’ x 250020	75	53–74	21	91–153	0.51	0.30–0.50
‘Salinas’ x 250020	73	51–100	17	106–155	1.79	0.10–0.20
F ₃ families						
‘Vanguard 75’ x 250020 (3 Sept. 2001)						
Among (expect 1:2:1)	8	11	5		1.02	0.50–0.70
Within (expect 3:1)	95		37		0.64	0.50–0.70
‘Salinas’ x 250020 (6 July 2001)						
Among (expect 1:2:1)	5	12	6		0.13	0.90–0.95
Within (expect 3:1)	95		26		0.80	0.30–0.50

Table 9. Characteristics of lettuce flowering time genes.

Symbol	Source	Dominance	Effect in days ^z
<i>Ef-2ef-2</i>	‘Vanguard 75’	Lateness ^y	34
<i>Ef-3ef-3</i>	87-41M-7	Earliness	7
<i>Ef-4ef-4</i>	796*	Lateness	78
<i>Ef-5ef-5</i>	PI 175735	Earliness	70
<i>Ef-6ef-6</i>	PI 236396	Earliness	50
<i>Ef-6ef-6</i>	PI 250020	Earliness	64

^zReduction in time to first flower compared to late group in F₂ population. 87-41M-7 was crossed to D-3-22M, which is *Ef-1Ef-1Ef-2Ef-2*, very early, limiting further reduction by *Ef-3*.

^yIn absence of *Ef-1* (Ryder 1988). Previous work showed that the effect of *Ef-1* is 75–80 d (Ryder 1983, 1988)

*Cos-like line 09-960796-0, received from A. van der Arend, Leen de Mos Seed Co., Gravenzande, The Netherlands.

which gives the more drastic earliness effect, and that *Ef-2*, which gives the more mild effect, already existed in 'Vanguard 75.' Lines developed from the cross, identified as *ef-1ef-1Ef-2Ef-2*, flower in the same time range as 'Vanguard 75.'

87-41M-7 is homozygous for the trait *endive-like* leaf, which provided the opportunity for a linkage study with the newly identified *early flowering-3* (Table 9). The F₂ populations, seeded 27 Feb. 1997 and 10 Mar. 2000, segregated for both traits, fitting the ratio 9 VVE, normal leaf:3 VVE, endive:3 VE, normal:1 VE, endive ($\chi^2 = 1.10$; $P = 0.70-0.80$), and indicating independent inheritance.

Mean flowering times for the VVE and VE portions of the three F₂ distributions of the cross 87-41M-7 x D-3-22M differed by 6, 9, and 6 d, respectively. The approximate reduction of flowering time for each gene, sequentially from *Ef-1* alone, to *Ef-1* plus *Ef-2*, to *Ef-1* plus *Ef-2* plus *Ef-3* is: *Ef-1* = 140 to 65 d; *Ef-2* = 65 to 45 d; and *Ef-3* = 45 to 38 d, when grown in the greenhouse during the summer. The effect of *Ef-3* in the absence of *Ef-1* and *Ef-2* is not known.

A group of 13 F₄ lines from the cross, selected from plants of relatively short stature were seeded 29 Mar. 2002, and were read for flowering time and height. They ranged from 42.3–43.9 d and from 8.5–19.6 cm. This compares to 51.2 d/37.8 cm for 81-41M-7 and 55.8 d/38.0 cm for D-3-22M, suggesting that selecting for shorter plants also selects for increased earliness.

The F₂ distributions of the crosses 796 x 'Vanguard 75' and 796 x 'Salinas' differ in the late classes. In the latter F₂, the late class ranges from 112–149 d, while in the former, the late class ranges from 76–140 d. The difference may be the effect of *Ef-2* from 'Vanguard 75'.

For the 796 x 'Salinas' cross, the mean of seven F₂ plants homozygous for lateness was 133.3 d and for 26 early plants was 48.6 d, a difference of about 85 d. The mean of homozygous late F₃ families of the 796 x 'Salinas' cross is 124.8 d, while the mean of early plants in the segregating families is 49.1 d, a difference of 76 d. For the 796 x 'Vanguard 75' cross, flowering dates for late F₃ plants were not recorded. Flowering dates for F₂ plants only were used to compare. The mean of six plants homozygous for lateness was 121.8 d, and for 27 early plants was 49.7 d, a difference of 72 d. Therefore, the average reduction in flowering time controlled by the gene is about 78 d.

PI accessions 236396 and 250020 are very similar in appearance as well as flowering time. PI 250020 was received from Egypt and is a typical oilseed type from that area. PI 236396 was received from Japan, but may have been originally collected from Egypt. The similarity in the F₂ distributions of the crosses with 'Salinas' and 'Vanguard 75' strongly suggest that the primary flowering time gene is the same for each.

The apparent 9 early-late:4 mid-late:3 late-late ratios in all four F₂ populations suggests another pair of genes in both PI lines, whose effects are noticeable only in the late group. It also suggests that the effect of *Ef-2ef-2*, which should show in the 'Vanguard 75' crosses, is effectively masked.

Only one allelism test was performed to establish relationships among the genes discussed in this paper or with *Ef-1ef-1* and *Ef-2ef-2*. *Ef-3* co-segregated with *Ef-1* and *Ef-2* and therefore must be different from either. Evidence strongly suggests they are all different genes, except for the indication that *Ef-6* is in both P236396 and PI 250020 (Table 9). The recessive earliness allele *ef-4* reduces flowering time by more than twice as many days as *ef-2*, and is therefore most likely different. Among the dominant early alleles, *Ef-5* has a similar effect to *Ef-1* and a slightly lesser effect than *Ef-6*, but the pattern of segregation of *Ef-5ef-5* is less discrete, showing an overlapping bimodal distribution in F₂, while the other two separate discretely. It is also substantially later than either *Ef-1* or *Ef-6* under short days (Table 1) (Ryder, 1983)). *Ef-6* and *Ef-1* have similar effects, but their origins are different: *Ef-6* occurs in a primitive oilseed lettuce, while *Ef-1* mutated recently in a crisphead breeding line (Ryder, 1988).

The full effect of *Ef-3* is not known in the absence of *Ef-1* and *Ef-2*. It is possible that *Ef-3* and *Ef-6* have the same magnitude of effect. However, the sources of the two genes are different, suggesting that they are not the same.

No linkage or marker tests were performed, so the location of the genes on the chromosomes is not known. Further work with these genes might therefore include mapping and allelism studies. The earliest alleles may be useful in educational applications and plant development studies.

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