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Studies of genes controlling early flowering in Lactuca sativa

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STUDIES OF GENES CONTROLLING EARLY FLOWERING IN *LACTUCA SATIVA*

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By

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Abstract

Studies of Genes Controlling Early Flowering in *Lactuca sativa*

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Abstract. Early flowering genes carried by *Lactuca sativa* cultivar Saffier were studied by crossing Saffier with three lines carrying none, one or two other known early flowering genes, *Ef1* and *Ef2*. This study reports the generation of resulting F2 populations, the analysis of the F2 segregation, and a characterization of Saffier photoperiod responses and early flowering behavior. Saffier is shown to be capable of flowering in forty days. Saffier crossed with cultivars both carrying and not carrying known early flowering genes generates progeny with days to first flowering decreased by as much as 8% or 10 flowering days in reference to the earliest flowering parent. Saffier may also carry a regulatory region capable of exerting a photoperiod inhibition effect on flowering similar to the previously characterized *Tt* gene.

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Introduction

Lactuca is a member of the Cichoreae tribe of the Compositae family. *L. sativa*, which has 2n = 18 chromosomes, is sexually completely compatible with *L. serriola* and and partially compatible with *L. virosa* and *L. saligna* (Ryder and Whitaker, 1985). These wild species provide a pool of variation useful for introducing beneficial traits such as disease resistance (Michelmore, 1986).

Lettuce is a cool season leafy vegetable which is grown around the world, with greatest production in Europe and the United States. The earliest evidence of the use of lettuce is found in tomb paintings in Egypt from about 4500 B.C. (Ryder, 1986).

Although lettuce is occasionally smoked or used as a cooked vegetable, it is used almost exclusively as a fresh raw product. For nutritional value, it is rated twenty-sixth on the list of common fruits and vegetables (10 vitamins and minerals). However, when weighted for actual consumption, lettuce places fourth, behind tomatoes, oranges and potatoes. Cos and leaf lettuces are most valuable nutritionally, with 18mg of ascorbic acid, 1900 IU of vitamin A, and 68 mg of calcium in 100g (Ryder, 1986).

For more than sixty years plant breeders have attempted to solve the mysteries involved in timing lettuce for the fresh market from a specific growing environment. Rapid, lush growth under short days, low light intensity in cold

temperatures would be ideal for greenhouse growers, but not for growers in the California desert. Since no one buys bolted lettuce, growers from all environments want bolting and flowering delayed in order to maximize the marketing window. The study of each individual gene involved in the timing mechanism is useful for understanding the growth of lettuce under different environmental conditions.

The purpose of this study is to evaluate the hypothesis that genes in the cultivar Saffier affect early flowering. Saffier was crossed with three other lines, known to carry either none, one, or two characterized early flowering genes, *Ef1* and *Ef2*, and the interaction of the known early flowering genes and Saffier genes was observed. Questions to be answered are as follows:

1. What is Saffier's days to flowering behavior under different photoperiod regimes?
2. Are there genes in Saffier that produce earlier flowering in the progeny when Saffier is crossed with other lettuce cultivars?
3. Do the F2 segregations demonstrate whether Saffier carries one gene with pleiotropic effects, or separate early flowering and photoperiod regulator genes?

Literature Review

Historically, lettuce investigations have focused on four main aspects of the timing question: cultivar - environment interaction, genes controlling heading, genes controlling bolting (stem elongation), and genes controlling flowering. Lettuce must bolt in order to flower. If lettuce is heading it cannot be bolting, and flowering is delayed.

The studies available on cultivar - environment interaction are numerous. However, most of these studies are cultivar specific, applicable only to certain unique environments, or production oriented. This literature review will focus primarily on gene action, which is more universally applicable, and specific information closely related to the parent lines used in this study.

Photoperiod and heading behavior. Durst and Lewis in 1930 reported that heading is a quantitatively controlled trait (Robinson et al. 1983). Other investigators have reported at least three major recessive genes controlling heading. Whether there are more than three genes controlling heading is difficult to establish because appropriate allelism tests have not yet been done and some gene stocks have been lost (Robinson et al. 1983). Single gene control for heading has been reported by Bremer and Grana (1935). They named this recessive allele Kopfbildung (German for head formation) and gave it the symbol *k*. Bremer (1931) also identified a single dominant gene responsible for early

bolting under long days. Bremer and Grana (1935) named the recessive allele of this gene Tagneutral (German for day neutral) and assigned the symbol *t*. The dominant allele carries the symbol *T*. Chodat and Gagnebin in 1948 confirmed Bremer and Grana's findings and showed that the expression of *k* is modified by *t* (Robinson et al. 1983).

Lindqvist (1960) studied the inheritance of photoperiodism by crossing cultivated lettuce with primitive *L. sativa* and *L. serriola* which were long day types. He determined from F1 and F2 generations that a single gene could account for long day or day neutral responses. He found that *L. serriola* and primitive forms of *L. sativa*, all have the dominant allele *T* (which he called *L*). The recessive gene, *t* (Lindqvist called it *l*), for insensitivity to photoperiod, is found only in cultivated lettuce. The great differences which exist between various lines carrying the *T* (*L*) allele show that the effect of the gene *T* (*L*) is influenced by other genes in different ways. Lindqvist (1960) also reported that two more genes named *h* for hearting and *ca* for capitate control heading and that *k*, *h*, and *ca* are all modified by a large number of other modifying genes. Gagnebin in 1972 and Gagnebin and Bonnet in 1974 reported dihybrid ratios for heading in segregating generations from a cross of headed and nonheaded types (Robinson et al. 1983). These findings led to the possibility of multiple alleles on two unlinked genes for

heading and the probability of single gene control of a strong photoperiod regulation of bolting.

Early flowering. Most of the work on genes controlling time of flowering has been done by Ryder at the USDA Agricultural Research Station in Salinas. In 1971 he gave the symbol name *pa* to an allele causing pale yellow flowers (Ryder, 1971). A pleiotropic effect of this allele is early flowering compared to normal. Ryder (1983) reported a single incompletely dominant gene, designated *Ef*, that controls flowering time. This gene, when crossed into a late flowering line (125 days to first flower (DTFF)), will halve the flowering time of the next generation (68 days DTFF). Ryder (1985) described the use of this gene to transfer lettuce mosaic resistance to a new line. A total of six back crosses to the recurrent parent usually takes 854 days. Utilizing the *Ef* gene, six back crosses can be accomplished in 551 days, a saving of eleven months. Ryder (1988) reported a second partially dominant early flowering gene which he named *Ef2*. The original *Ef* was renamed *Ef1*. Ryder (1989) reported that *Ef1* is linked to plump involucre.

Characterization of Salinas and Vanguard types. All of the data emerging from the following early flowering experiments in Salinas, Ca. are particularly pertinent to this study, because seed stocks of these two early flowering types, containing *Ef1* and *Ef2*, were used as the known parent lines in the crosses evaluated in this study.

Rousos and Ryder (1987) studied the effect of daylength treatments on two early flowering genotypes carrying the *Ef1* gene. They found that increasing the day length from eight hours to fourteen hours reduced the DTFF from 89.99 to 69.50. They also found that the short day plus an eight hour extension of dim light of $10uEm^{-2}s^{-1}$ had no effect on DTFF. Rousos (1988) exposed these two early flowering genotypes to photoperiods of 8, 12, 16 and 20 hours of mixed fluorescent and incandescent radiation at an intensity of $480uEm^{-2}s^{-1}$. Both genotypes responded as quantitative long-day plants. Keeping the plants 15 degrees above the temperature of the controls during day or night did not induce earlier bolting or flowering; controls were 20°C days and 17°C nights.

Characterization of butterhead types. Butterheads come in three types: winter, late spring and summer lettuces, based on their flowering response. Thompson and Knott in 1933 showed that White Boston, a butterhead, produced no heads and an early seedstalk when kept in a greenhouse at 21°C to 27°C (Ryder and Whitaker 1985). Length of day had no effect on seedstalk initiation but longer days did increase the rate of growth when the seed stalk was initiated. At 15.5°C to 21°C head formation occurred first and seedstalk initiation was delayed, indicating a regulating effect of temperature in contrast to the stocks carrying one *Ef1* gene. Rappaport and Wittwer (1956) also found that longer days and higher temperature accelerated flowering in general.

However, they also found that the cultivar Bibb, a butterhead, responded primarily to photoperiod and less to temperature while Grand Rapids, a leaf lettuce, responded primarily to temperature and less to photoperiod.

Background information on the Saffier parent, a bibb or butterhead type, is sparse. Wilbert D. Meijsing, a Dutch lettuce breeder, sent to Dr. Edward Ryder the Saffier seeds in response to the publication of his papers on early flowering. They were purported to be an early flowering genotype, but initial plantings by Ryder in Salinas did not support this claim. However a strong photoperiod inhibition was demonstrated.

Vernalization. Vernalization is frequently mentioned for decreasing DTFF and generation time in many crops. Typically vernalization consists of chilling seeds or seedlings at 4°C to 5°C degrees for various periods of time. Early studies on lettuce were inconclusive. Thompson and Kosar (1948) reported that vernalization of Slobolt, a slow bolting variety, and Cos 3288, a fast bolting strain, had no effect. This result might be a mistake. Prince (1978) reported some vernalization experiments which suggest a mechanism by which this mistake could occur. Seeds of *Lactuca serriola*, a wild lettuce, were vernalized at 2 to 4°C for 28 days and devernalized at 25°C for 24 hours after vernalization. Repeated cycles of vernalization and devernalization can be applied and the last treatment before

germination is controlling. Thompson and Kosar may have accidentally devernalized their lettuces.

Light intensity. Sanchez and Allen (1987) studied the effect of reduced solar radiation on growth and development of lettuce. They used shades of different shading effectiveness for different durations at different stages of crop development. They found that shading always reduced crop growth, approximately in direct proportion to reduction in radiation. Lettuce was more sensitive to shading during the latter period of crop development when the growth rate was more rapid. They concluded that any model used to predict days to harvest must include intensity of solar radiation in addition to temperature.

Summary of literature review. Lettuce has been shown to respond to both temperature and light variation by either slowing or hastening seedstalk elongation and flowering. Colder temperatures delay flowering in general though vernalization temperatures at critical stages can hasten seedstalk elongation and flowering. Warmer temperatures do not in themselves initiate flowering sooner than cooler temperatures, though warmer temperatures in general do support more rapid growth and maturation. Light affects lettuce flowering in two ways. Photoperiod requirements for initiation of flowering are specific to cultivars. Light intensity affects growth and maturation quantitatively. While information specifically on Saffier is not now

available, butterheads in general are reported to flower earlier, primarily in response to photoperiod and secondarily in response to temperature.

Materials and Methods

Materials. Seeds for three initial crosses were donated by Dr. Edward J. Ryder of the USDA Agricultural Research Station in Salinas Ca. Saffier is an early flowering, Dutch butterhead greenhouse variety. D-24, which carries both *Ef1* and *Ef2*, is a progeny of a cross of Salinas (commercial crisphead lettuce) and the original early flowering mutant described in Ryder (1983). C-2-1-33 which carries only the *Ef1* gene, is a progeny of Vanguard 75 (commercial crisphead) and the early flowering mutant. C-2-1-1 is a similar genotype with no known early flowering genes. Although this line has not yet been completely characterized, it is known to be homozygous recessive for *Ef1* and *Ef2* (Ryder 1990). C-2-1-1, C-2-1-33 and D-24 were all developed at the USDA in Salinas, Ca.

The hypothesis is that Saffier carries one or possibly two genes affecting early flowering. The first gene is named *Efs* for the purposes of this study.

Methods. Three sets of experiments were performed to characterize the genes related to early flowering carried by Saffier.

First. Saffier and other parent lines were planted monthly for one year outside in natural temperature, photoperiod and light intensity.

Second. A series of photoperiod experiments were performed in growth chambers.

Third. Saffier was crossed with three different well-characterized lines. Seed was collected from F2 progeny demonstrating a marker phenotype.

Experiment set 1. Photoperiod inhibition of Saffier

Plantings of the parent lines Saffier, C-2-1-1, C-2-1-33, and D-24 were made outside in Los Gatos, Ca. under natural light, photoperiod, and temperature conditions. Plantings were grown in one gallon black pots spaced on white concrete to ensure full sun. A minimum of six individuals of each genotype were planted at least once a month for a year. Nonsterile potting soils were used with Osmocote (by Sierra Chemical) 14-14-14 in the six month formulation added once at transplanting. Days to first flowering were recorded.

Experiment set 2. Effect of photoperiod on Saffier DTFF as compared to other early flowering parent lines

Early parent lines, C-2-1-33, D-24, and Saffier were grown under two sets of conditions; first, under four different light regimes, and second, under four different photoperiods. The average DTFF of a minimum of 6 individuals was calculated for each treatment.

Growth chambers were set at 22°C degree during the day and 20°C degree during the night. The relative humidity was set at 60%. Light intensities varied between $350uEm^{-2}s^{-1}$ and $500uEm^{-2}s^{-1}$ over the entire experiment but were consistent

within each subset. Fluorescent and incandescent lamps were used in all subsets. Both sterile and nonsterile potting soils were used over the entire experiment set but were consistent within each subset. Hoaglands nutrient solution was used for fertilization throughout.

The first regime was 16 hours lights on and 8 hours dark. A small subgroup of this population was removed after bolting initiated and placed into a short day growth chamber (8 hours of light).

The second regime was 8 hours lights on, 4 hours lights off, 8 hours lights on and 4 hours lights off (double day or DD).

The third regime was 15.5 hours lights on, 4 hours lights off, 0.5 hour lights on and 4 hours lights off (light break or LB).

The fourth regime was continuous light at high light intensity of $1500uEm^{-2}s^{-1}$. Temperatures were held at 23°C days and 20°C nights with R.H. between 50% and 60%. In addition to parent lines, a very early C-2-1-1 x Saffier F3 progeny was planted. After six days light intensity was reduced to $700uEm^{-2}s^{-1}$.

The four photoperiods examined were 14 hours, 16 hours, 18 hours, and continuous light. The 16 hour long day, the light break chamber and the double day chamber were able to be run at uniform illumination of $350uEm^{-2}s^{-1}$. The 14 and 16

hour chambers ran at a light intensity between 450 $uEm^{-2}s^{-1}$ and 500 $uEm^{-2}s^{-1}$.

Experiment set 3. Saffier cross with three parent lines

Saffier crosses. Lettuce, an obligate self-fertilizer, is a difficult plant to cross. The stamens are fused into a tube. The anthers shed pollen on the inside surface of the tube as the flower opens in the morning. The sticky elongating style with two stigmatic lobes picks up the pollen as it emerges from the top of the stamen tube, Figure 1 (Ryder, 1986). To make a cross, pollen is immediately and continually washed off with a fine stream of water and the foreign pollen applied by touching another flower to the two stigmatic lobes before they start to curl back, a signal of the end of receptiveness (personal communication Milligan and Robinson, 1987). Selfing is still common since some pollen grains will germinate before they are washed off. Lettuce flowers open once shortly after dawn for a few hours, so it was necessary to time the planting and flowering of the parent lines carefully.

The marker phenotype, a complex multigene phenotype characterized by anthocyannin expression and indented leaf margins, was carried in the C-2-1-1, C-2-1-33, and D-24 male parent lines. Saffier was used as the female so that only the successful crosses showed the marker phenotype.

The crosses were made, and F1 generations grown in greenhouses of Sungene Plant Technologies in Palo Alto, Ca. (C-2-1-1 x Saffier, C-2-1-33 x Saffier, D-24 x Saffier). Unsterile potting soils were used with Osmocote added at initial transplanting.

Preliminary experiments. Seeds were collected from the F1 of each cross and preliminary plantings were done to establish seed viability at 1,2,3,4,5,and 7 days after harvest. Prior to planting, seeds were dried and stored at room temperature in the dark, in unsealed plastic petri dishes.

A trial was run to assess the effects of imbibed seed vernalization. Seeds were soaked for thirty minutes in tap water, drained, and stored at 4°C on moist blotter paper in sealed plastic petri dishes for two weeks. A second sealed petri dish of dry seeds was chilled for two weeks at 4°C.

Nighttime temperature effects on DTFF variation were examined by moving two each of the three F1 types and two Saffier into a 60°F minimum temperature Brassica greenhouse five nights out of the week instead of the 75°F minimum temperature corn greenhouse from the fifth leaf stage until flowering.

F2 population plantings. Two F2 populations, each a full set of the three crosses, were planted at the USDA agricultural Research Breeding Station greenhouse in Salinas on 2-18-88 and on 6-10-88. Three F2 populations, each a full

set of the three crosses, were planted in growth chambers at San Jose State University in 3-13-88, 5-9-88, and 5-6-89. Seeds were germinated in sterile and unsterile potting soils, consistent within each population, and transplanted into more potting soils at San Jose State University and into natural soils in Salinas. Growth chamber populations were fertilized with Hoaglands. No fertilizer was used in Salinas populations.

Photoperiod for the Conviron growth chamber populations was 16 hours light at a light intensity of $500uEm^{-2}s^{-1}$ with temperatures 24°C days, 22°C degree nights and relative humidity at 70%. Lights were both fluorescent and incandescent. Light intensity measurements were made with a Licor model LI 185 with a quantum sensor. Salinas greenhouse populations were grown with natural photoperiod conditions, in an environment close to natural light intensities and temperature fluctuations. Days to first flowering were recorded for each individual of each population.

Some of the growth chamber experiments were consolidated after six weeks. The plants, in various stages of seed stalk elongation, were grouped according to their degree of seed stock maturity, and only one or two plants of each group were retained. When these individuals flowered, their whole groups were recorded as flowering on that day. The growth chamber experiments were moved when 75% of the population had flowered. The remaining plants were grown to

flowering at either Sungene Plant Technologies in Palo Alto or at the USDA Agricultural Research Breeding Station in Salinas.

Seeds were collected from all F2 plantings for future F3 plantings.

Analytical methods

Saffier photoperiod inhibition analysis. A method of compensating for the changing photoperiod was developed. The slope of the curve relating flowering time to hours of daylight for each month was used to generate a negative percentage value for the effect of photoperiod inhibition of bolting and flowering shown by Saffier in the Los Gatos plantings. Data from Salinas were used to fill in the missing months, since they would be even better predictors of progeny responses in Salinas (personal communication Ryder, 1990). A Saffier photoperiod inhibition factor, SPIF, is defined as the ratio of the difference in days to first flowering between Saffier and C-2-1-33 divided by days to first flowering of Saffier.

SPIF = (DTFF (Saffier) - DTFF (C-2-1-33))/(DTFF (Saffier))

Determination of number of active flowering genes. Flowering data were analyzed to determine if Saffier has one or more genes affecting early flowering. Five plantings of this F2 seed were analyzed for the number of days to first flowering (DTFF). The C-2-1-1 x Saffier distribution will

show a three to one segregation ratio in the F2 population if one *Efs* gene is active in Saffier and strongly or completely dominant. The C-2-1-33 x Saffier distribution shows a 15:1 segregation ratio in the F2 population if one *Efs* gene is active in Saffier. The D-24 x Saffier distribution will show a 63:1 segregation ratio in the F2 population if one *Efs* gene is active in Saffier. If two genes are active in Saffier, the ratios in the segregating F2 populations will be as follows, 15:1 in the C-2-1-1 cross, 63:1 in the C-2-1-33 cross, and 255:1 in the D-24 cross.

Data on the growth chamber planting of 5-9-88 and the Salinas greenhouse plantings of 2-18-88 and 6-10-89 were first plotted as a distribution of flowering days. Hypotheses of how many genes were active in affecting early flowering were formulated based on the preliminary experiments. The frequencies of all possible unique genotypes were calculated based on the hypothesized number of active genes. Phenotypes, expressed as DTFF, were predicted for each genotype in relation to DTFF of the controls.

Each segregating population was analyzed for the number of pertinent genes by examining phenotype classes with discrete visible groupings to determine if the appropriate segregation ratios could coincide with hypothesized number of genes and the groupings observed in the distribution.

Segregating classes were compared to expected 3:1, 15:1, 63:1, and 255:1 ratios under 1,2,3, and 4 gene hypotheses to determine the probable number of genes active in each cross. Chi-square tests were performed to determine goodness of fit.

Results

Experiment set 1. Photoperiod inhibition of Saffier

Saffier planted in the winter flowers in 235 days, whereas Saffier planted in the summer flowers in 71 days. C-2-1-1 varies from 207 in the winter to 99 days in the summer, a much flatter curve. C-2-1-33 DTFF is 185 days in winter to 44 days in summer. The DTFF differences between C-2-1-1 and C-2-1-33 are more pronounced in the summer, 55 days, than in the winter, 22 days. D-24 also had a flat curve. DTFF varied from 146 days in winter to 36 days in summer with a big drop between January and February of 54 days.

All genotypes planted in July and August either remained dormant until the weather cooled or germinated but were unable to survive the intense light and heat although they were kept moist. The only exception was a single Saffier out of 24 (Figure 2). Plantings in dappled shade germinated normally and after three weeks could be put out in full sun gradually. These data gave a rough approximation of the DTFF as if the climate were more moderate and are not included in Figure 2.

The results of the SPIF values plotted on a graph indicate that on the shortest days in January there is no difference in photoinhibition of seedstalk elongation between Saffier and C-2-1-33. Saffier shows a sharp increase in relative inhibition between January and February. An even

sharper increase is apparent in May. June demonstrates a lessening in the difference of DTFF. However, starting in late July the photoinhibition again dominates and flowering is delayed. Meanwhile C-2-1-33 is still decreasing its time to flowering. In October the difference in photoinhibitory response to daylength appears to decrease. By January the difference between the two lines is back to zero (Figure 3).

Experiment set 2. Effect of photoperiod on Saffier DTFF

Growth chamber data showed Saffier capable of flowering in 35 to 40 days in high illumination in the continuous light growth chamber.

The subpopulation removed from the 16 h photoperiod and placed in the 8 h photoperiod stopped elongating. Leaves lengthened and broadened. The edges of the leaves began to cup in and grow to the one uniform height from the soil line as if returning to a heading behavior.

The LBC slows DTFF in all lines compared to the 16 hour chamber. Saffier responds to the DD chamber by flowering 24% earlier. No counting effect was observed (Table 1).

The continuous light chamber with high light intensity of 1500uEm^{-2}s^{-1} showed inhibition of germination. Those that germinated had a frosted appearance, and leaves also showed bronzing. Saffier had white "pimples" on leaves. Leaves did not elongate but remained round small and sessile. As soon as the light was turned down rapid growth occurred and more germination commenced.

An early plant in the C-2-1-33 x Saffier progeny, named *CS13*, showed no delay in germination and flowered in 13 days. C-2-1-33 showed the most light inhibition of germination, followed by D-24, and then Saffier (Table 2).

Table 2 shows Saffier shortens DTFF by 35% when daylength is increased from 14 hours to 18 hours. C-2-1-33 and D-24 shorten DTFF by 18% and 7.5% respectively.

Experiment set 3. Saffier cross with three parent lines

The initial crosses of Saffier and C-2-1-1, C-2-1-33 and D-24 resulted in less than 15% of the putative F1's actually being selfs. The marker phenotype of anthocyanin expression and crinkly leaf margins was very distinct from the uniform round green leaves of Saffier. Allowing the plants to dehydrate slightly intensified the anthocyanin expression.

The F1's that were moved into the cooler Brassica house at night had an average of 76 days to first flower, and the F1's remaining in the warmer corn house at night flowered in an average of 69 days. This is a 10% difference. The Saffier plants moved into the cooler Brassica house at night flowered two days later than those remaining in the corn house, a 3% difference. The F1 generation flowered very uniformly under the 16 hour photoperiod in the Sungene greenhouses, but the F2 generation was extremely variable as expected.

Seed viability testing showed 100% germination when tested 1,2,3,4,5,& 7 days after harvest, indicating no primary dormancy. Seeds chilled at 4°C degrees, wet or dry, also showed 100% viability. There was a marked difference in DTFF between vernalized and unvernalized seed. At 64 days plants from vernalized seed had 10 inch stems, while plants from unvernalized seeds were still heading.

In July and August flats of germinating seeds in the greenhouse not only reversed their response to vernalization but entered a heat induced inhibition of germination. Some flats of moist seeds did not germinate for more than a month.

F2 plantings. The first F2 planting was planted in San Jose and transported to Salinas. Germination was irregular due to overheating problems during transport. When most plants had reached the 4 leaf stage each plant was scored corresponding to the number of days of delayed germination. When the plant flowered, this number was subtracted from its DTFF (Figures 4, 5, and 6). The second Salinas planting was planted in Salinas and germinated uniformly (Figures 7, 8, and 9).

Distributions of DTFF for the growth chamber F2 populations are shown in Figures 10, 11 and 12.

Results of segregation data analysis. The 6-10-88 F2 population planted in Salinas provides the best data for analysis. Figure 7 shows the DTFF distribution of the

C-2-1-1 x Saffier of 6-10-88. Clear early and late groups are visible. The data show a possible distribution with a visible break that occurs between days 104 and 110. Table 3 confirms this F2 population is a possible one-gene system that produces a 3:1 ratio with probability of 30%.

Figure 8 shows the DTFF distribution of the C-2-1-33 x Saffier F2 population of 6-10-88. A low point in the DTFF distribution occurs on day 100. Dividing the population here yields a 3:1 ratio with a probability of 70% to 80% (Table 5).

Figure 9 shows the DTFF distribution of the D-24 x Saffier F2 population of 6-10-88. This distribution did not yield clear results. (See discussion.)

Discussion

Experiment set 1 revisited

On Figure 3, the reason there is no difference in photoperiod inhibition of flowering in January between Saffier and C-2-1-33 is possibly because both are similarly inhibited long day plants at 9.8 hours of daylight. Another possibility is that during January cold may have been an overriding factor. During the longest days of the year, in mid June, a 14.75 hour day shows a lessening of the difference compared to surrounding months. This probably indicates a lessening in Saffier photoperiod inhibition. Extrapolating from the Conviron tests longer day lengths would presumably show even less difference. The greatest differences are shown in May and July with July peaking lower possibly because of an overriding effect higher temperatures. While C-2-1-33 demonstrates a growth response from that photoperiod, Saffier demonstrates an inhibition response. Still inhibited, Saffier is less able to utilize the high illumination and warm temperatures to support the same rapid growth and maturity demonstrated by C-2-1-33.

Experiment set 2 revisited

A further explanation of the purpose of the second set of experiments is appropriate. The purpose was primarily to evaluate the parent line responses to differences in photoperiod and light regimes. The high light intensity used

in the fourth regime was to determine responses to high light stress with no temperature stress, and to determine if there was a lower limit of DTFF when light intensity and heat stress were not limiting factors. Extreme high light intensity was shown to inhibit both germination and growth. More study is necessary to optimize light intensity for minimizing DTFF. Optimum light intensities would probably be cultivar specific and developmental stage specific with lower light intensities optimal for seedlings and higher light intensities usable in the later stages of development.

These experiments secondarily provided controls for the F2 populations. The 18 hour long day growth chamber was used as the no photoperiod effect control. The 14 and 16 hour growth chambers were used as controls for the 6-10-88 F2 population to check parent controls in the greenhouse plantings by comparison with the month of June, a 14.7 hours of daylight month. If the flowering times for the parent controls were not in the range of the 14 and 16 hour growth chambers it would indicate some other factor such as disease or light contamination was causing variation. The 16 hour growth chambers provided the same check on the F2 populations planted in the growth chambers at San Jose State University. DTFF of controls in the F2 populations in the growth chambers and in the Salinas 6-10-88 planting were within reasonable range allowing for variation in light intensities. The 2-18-88 planting could not be controlled in

this way because of its shorter photoperiod at planting time.

A third purpose of the second set of experiments was to determine if there was a counting mechanism at work and if so was there a critical amount of time the lights had to be on to be "counted." A counting mechanism might involve a phytochrome response related to the proportion of time the phytochrome far red irradiated structure of the molecule (*Pfr*) and phytochrome red irradiated structure of the molecule (*Pr*) were in equal amounts rather than *Pr* higher (*Pfr* is destroyed during dark time). There might be a requirement by the lettuce plant for a critical number of dark periods when *Pr* is higher while during the greater proportion of time *Pfr* and *Pr* are equal. Light affects phytochrome *Pr* and *Pfr* levels. Greater *Pfr* has been reported to stimulate *GA3* which stimulates seed stalk elongation (Barnes and Jones 1984). Long day plants like lettuce seem to need the higher level of *Pfr* for longer periods of time in order to flower.

The double day chamber would have a larger percentage of time with *Pfr* and *Pr* closer to equal than a straight 16 hour photoperiod. Saffier responded to this regime by a 24% decrease in DTFF, whereas the other early flowering lines showed little difference.

Experiment set 3 revisited

Some extremely speculative ideas can be examined by choosing other very arbitrary breaks in distribution data.

On figure 7, by using the visible grouping that peaks between days 61 and 63 a 15:1 ratio can be tested by Chi square. Table 4 suggests a possible 15:1 ratio with a probability of 20% to 30% indicating two genes.

If it were possible to select isolated phenotypes from the middle of a distribution, figure 8 could be examined for possible two gene activity. A low point occurs around day 89. Using the class peaking between day 89 and 100 a two-gene hypothesis could be supported with a probability of 80% to 90% (Table 6).

Figure 9 could be examined for two or three gene activity. The phenotype following the nine day break in the distribution between day 100 and day 109 fits a 15:1 ratio with a probability of 40% to 50% (Table 7). Another break in the distribution occurs between days 139 and 143. The phenotype following this break supports a 63:1 ratio with a probability of 70% to 80% (Table 8). The phenotype flowering from day 109 to 118 also supports a 63:1 ratio (Table 9).

Information gained from problems

One problem throughout this entire study was the difficulty in determining what was normal variation for DTFF for the Saffier controls. For both the Los Gatos and growth chamber populations Saffier controls usually varied from 16 to 20 in DTFF as compared to the other lines which varied approximately 10 days. In Salinas, although Saffier is thought to be very consistent with only a three or four day variation in greenhouse plantings, growth chamber data showed a 20 day variation within a population of six (personal communication, Dr. E.J. Ryder, 1990).

Saffier may be very sensitive to other variables not fully controlled or measured in the above experiments. Data from experiments in Salinas show Saffier flowers earlier in sterile soil (personal communication, Dr. E.J. Ryder). Personal observations in the growth chamber studies at San Jose State University, where complete rotation of plants within each chamber was not possible, showed greater seedstalk elongation and more rapid flowering of those individuals closest to the fans. Whether this response involved physical stimulation, CO_2 levels, O_2 levels, or other sensitivities is unknown. The growth chamber data mentioned above which showed a 20 day variation in Saffier DTFF was a fully rotated population.

Considering genes affecting early flowering only, in some of the F2 populations in this study there were probably

27 or 81 unique progeny distributed over a range of usually under 110 days. Just as the marker genes segregated independently in F2, giving a variety of phenotypes, other background genes affecting vigor and environmental responses were segregating independently also. With controls varying up to 20 days, overlap of phenotype classes was unavoidable and visual groupings are at best indicators of possibilities.

Another problem encountered was restricted growth chamber availability. Vernalization was investigated as a possible means of decreasing generation time. However, due to overheating problems, the possibility of devernalization occurring in only segments of the populations, and the possibility of response to vernalization varying according to genotype, it was decided not to vernalize. The consolidations of F2 growth chamber populations and moving them as soon as most of the experiment was accomplished was not ideal, but did allow the maximum number of experiments to be performed in the time allowed.

Other compromises from the ideal occurred due to facilities limitations. Los Gatos was not the best place to grow the parent lines for an understanding of F2 behavior in Salinas. Initial studies in Palo Alto had shown a strong temperature effect on the parental lines coming from Salinas and frosts were frequent in Los Gatos. Figure 2 is actually a graph of photoperiod and temperature effects on the parent

lines. However, by developing a percentage value from the difference in DTFF between C-2-1-33 and Saffier, the SPIF value, a useful chart was developed that aided in understanding the photoperiod distortion affecting the latest flowering individuals of Salinas 6-10-88 populations (Figures 7, 8, and 9). After the flowering of the Saffier controls there is a 2% per day increase in DTFF for all remaining individuals. Although the slow end of the distribution would normally show more variation than the faster end, the 2% slowing per day distorts the distribution even more. Data later made available to me by Dr. Ryder recording similar year round plantings of the early flowering lines produces a SPIF graph very similar to the SPIF graph based on Los Gatos data.

In the 2-18-88 F2 planting in Salinas C-2-1-1, C-2-1-33, and D-24 controls showed earlier DTFF than were consistent with previously published data (Ryder, 1988) and the Los Gatos data for that time of the year. Since the daylight photoperiod was 10.7 hours at the time of planting, light contamination was indicated and had occurred (Ryder 1990). Saffier controls (at varying distances) on all benches showed only "normal" variation (within 20 days). Two possibilities may account for the normal variation. First, the contaminating light may have been uniform or second, the increasing photoperiod may have been the overriding factor to which to plants were responding. Variation in light

intensity within a minimum range may not be as significant in affecting DTFF in early stages of growth as in the later stages of rapid growth (Sanchez 1987).

The light contamination makes these data atypical for a February planting; however, the resulting DTFF distributions do reveal some important information. Unexpected flowering distributions, which do not show up in the other plantings, give us insight into gene interactions regardless of the exact photoperiod. The D-24 x Saffier of 2-18-88 shows the D-24 control blooming from day 47 to 54 right on schedule. There are no progeny blooming at the same time. For a population of 230, a minimum of three would be expected. With three active genes segregating, there should have been a minimum of 1 out of every 64. One possible explanation is that another overriding gene from Saffier is active in delaying the DTFF.

In the 2-18-88 planting of C-2-1-1 x Saffier, a large proportion of the progeny flowers before Saffier. A certain amount of transgressive segregation is to be expected because the Saffier early flowering gene(s) are now expressing in different background genotypes. However, in this F2 population of 231, 130 are flowering before the controls. This 56% could indicate that some portion of Saffier's early flowering capacity has been released by crossing it with C-2-1-1, a genotype with no known early flowering genes. This large early population could

substantiate the hypothesis that the early flowering effect and the photoperiod inhibition effect are carried on two separate genes, an *Efs* gene and a *Tt* gene. Possibly there is something in Saffier that works exclusively on *Ef* genes causing inhibition during short photoperiods. When that early flowering locus is not there it has no effect.

The fact that C-2-1-1 has not been fully characterized and may actually carry an early flowering factor of its own, could explain the unexpected segregations in the C-2-1-1 crosses with Saffier. This unidentified early flowering factor, *Ef0*, which when combined with Saffier's early flowering factor, produces extremely early flowering progeny. This model approaches the C-2-1-1 x Saffier as a two active gene system with one coming from Saffier and one coming from C-2-1-1. When a single Saffier gene combines with this factor from C-2-1-1 flowering occurs ahead of the Saffier control.

The Chi-square for either two gene assumption is 1.43 and the probability is 20% to 30% when the 6-10-88 greenhouse data are tested (Table 4). The Chi-square for the 2-18-88, or winter data, is 0.23 with a probability of 50% to 70% (Table 10).

This other possible active gene from C-2-1-1 could act either positively as an early flowering factor or negatively as a suppressor. C-2-1-1 may have a system of its own for

deactivating the Saffier inhibition effect without affecting the early flowering capacity.

Another possibility is that a mutation may have occurred in either the Saffier or the C-2-1-1 parent which generated this F2 population. It is possible that only a portion of the progeny carry this possible mutation. It is unclear whether other populations are responding in a similar manner and the changing photoperiod has masked the effect. Only 32% of the population flowered before the Saffier in the 6-10-88 population as compared to 56% in the 2-18-88 population. The counter argument can be made that this is a photoperiod effect. The C-2-1-1 controls are seven days earlier in the 6-10-88 planting than they are in the 2-18-88 planting. The Saffier controls are 47 days earlier in the 6-10-88 planting than they are in the 2-18-88 planting. Saffier is clearly responding more abruptly to the difference in photoperiod than is C-2-1-1. Heterozygotes or those individuals with only one Saffier gene responding to photoperiod may be less photoperiod inhibited in 2-18-88 planting and less responsive to the longer daylength in the 6-10-88 planting than Saffier controls.

Conclusions

The results of this study show Saffier has a strong photoperiod regulatory response, Saffier crosses with other early flowering lines produce both earlier and later flowering F2 progeny, and Saffier may carry a *Tt* factor either on a separate gene or pleiotropically as part of Saffier's early flowering gene, *Efs*. F3 populations are necessary to confirm a genetic hypothesis and further characterize the *Ef* genes in Saffier.

The Saffier photoperiod response produced the longest DTFF compared to other early flowering lines tested. When the limiting photoperiod was removed by placing the plants in continuous light, Saffier flowered in less than forty days, similar to D-24, an early flowering cultivar. All Fall and Winter plantings of Saffier have the characteristic of flowering together within the first two weeks of June.

All F2 populations planted in Salinas generated earlier flowering progeny than the parent controls. Crossing Saffier with a known early flowering line, C-2-1-33, produced individuals flowering up to 10 days earlier than the earliest parent control in the 6-10-88 Salinas planting. The same line planted on 2-18-88 produced individuals flowering up to 20 days earlier than the earliest parent control. On all 6-10-88 plantings at least 15% of the population flowered after the slowest parent control.

Saffier may have a regulatory region which operates in the same way as the *Tt* gene Bremer and Grana described in 1935. The F2 segregations are insufficient to determine the existence of a separate *Tt* in Saffier. A cross of F3 progeny, early cross late individuals in latest group and early cross late members in the early group would be required to determine if the *Efs* had separated from the *Tt* effect.

The future

Many factors, including examination of the transgressive segregation demonstrated by C-2-1-1 crosses, and possible dominance and linkage disturbances of the DTFF distributions, were beyond the scope of this paper and remain to be investigated.

The next important phase of investigation will be the growth of the F3 generation focusing on efforts to separate the early flowering effect from the photoinhibition effect, if possible.

F3 segregation analysis is preferred over backcrossing for lettuce because lettuce is difficult to cross. Also the inability to distinguish backcrosses from selfs makes the segregation of backcross data unreliable.

An unexpected result of this research was the generation of an early flowering F3 individual, *CS13*, from the C-2-1-33 x Saffier which flowered in 13 days under

growth chamber conditions. This is as fast as some of the rapid cycling Brassicas. This new item has potential uses for research, for rapid movement of new genes into commercial lines, and for educational purposes.

The photoperiod control in Saffier has potential uses in gene transfer. This strong regulatory region of the gene would be useful in extending crop ranges, possibly preventing early flowering in regions where certain crops are lost to late frosts.

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Table 1. Days to First Flowering under Different Photoperiod Regimes. Fall 1987 through Summer 1988.

Line	LD-16	LBC	DD
D-24	51 (11-29)	58 (12-12)	36 (Fall)
			52 (11-29)
C-2-1-33	70 (11-29)	78 (11-29)	63 (Fall)
		77 (12-12)	74 (11-29)
Saffier	60 (Fall)	79 (11-29)	60 (11-15)
	70 (11-29)		53 (11-29)

LD-16 = Long Day. 16 (16 h on, 8 h off).

LBC = Light Break Chamber. 15.5 h on, 4 h off, 0.5 h on, 4 h off.

DD = Double Day Chamber. 8 h on, 4 h off, 8 h on, 4 h off.

Fall - all new bulbs both incandescent and fluorescent
- light meter unavailable.

11-15 - attempt to repeat Fall run of Saffier (breakdown) but bulbs had dimmed.
- light meter unavailable.

11-29 - 350 $uEm^{-2}s^{-1}$ at start to no less than 300 $uEm^{-2}s^{-1}$ at end.

12-12 - repeat 11-29 (breakdowns).

Table 2. Days to First Flowering under Different Long-day Photoperiods. Fall 1987 through Summer 1988.

Line	LD-14	LD-18	C
D-24	40	37	Elongate flower ball at 30 days.
C-2-1-33	55	45	Not bolting. Germination inhibition.
Saffier	78	51	Bolting at 30 days.
C-2-1-33 x Saffier			Flowered in 13 days.

LD-14 = Long Day. 14 h on, 10 h off.

LD-18 = Long Day. 18 h on, 6 h off.

C = Continuous Light.
Light intensity in 14 h and 18 h chambers 450 $uEm^{-2}s^{-1}$ to 500 $uEm^{-2}s^{-1}$.

Table 3. Segregation of DTFF C-2-1-1 x Saffier, Greenhouse Planting. Salinas planting of 6-10-88. 3:1 Ratio.

Hypothesis: Genes segregate in 3:1 ratio for a system of 1 or more genes.

Phenotype Classes:

1: Flowers early: Flowers before C-2-1-1.
2: Flowers late: Flowers during and after C-2-1-1.

Data: Sample size = 231. A 5-day breakpoint prior to day 109 in the bipolar distribution is used to separate the early and late segregations.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Flowers early	3	172.2	179	0.27
2. Flowers late	1	58.8	52	0.79
Total	4	231	231	1.06
	Probability (1 df)			30 %

Summary: Hypothesis PASSES the Chi-square test, indicating possibly one active gene is segregating.

Table 4. Segregation of DTFF C-2-1-1 x Saffier, Greenhouse Planting. Salinas Planting of 6-10-88. 15:1 Ratio.

Hypothesis: Genes segregate in 15:1 ratio for a system of 2 or more genes.

Phenotype Classes:

1: Flowers early: Flowers in first visible grouping.
2: Flowers late: Flowers after first visible grouping.

Data: Sample size = 231. The first pronounced grouping occurs between days 61 and 63 with 10 samples.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Flowers early	15	216.6	221	0.09
2. Flowers late	1	14.4	10	1.34
Total	16	231	231	1.43
	Probability (1 df)			20% to 30%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly two active genes are segregating.

Table 5. Segregation of DTFF C-2-1-33 x Saffier, Greenhouse planting. Salinas planting of 6-10-88. 3:1 Ratio.

Hypothesis: Genes segregate in 3:1 ratio for system of 1 or more genes.

Phenotype Classes:

1: Group 101-Last. Phenotype flowering after day 101.

2: All others. Group prior to day 101.

Data: Sample size = 263. Class 1 is the grouping that follows the Group 89-100 segregation of Table 5.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Group 89-162	1	65.8	64	0.05
2. All others	3	197.2	199	0.02
Total	16	263	263	0.07
		Probability (1 df)		70% to 80%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly one or more active genes are segregating.

Table 6. Segregation of DTFF C-2-1-33 x Saffier, Greenhouse planting. Salinas planting of 6-10-88. 15:1 Ratio.

Hypothesis: Genes segregate in 15:1 ratio for system of 2 or more genes.

Phenotype Classes:

1: Group 89-100. First phenotype flowering after Saffier, DTFF 89 to 100.

2: All others. Group of all other flowering days.

Data: Sample size = 263. This is the first grouping following the large grouping ending at day 87.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Group 89-100	1	16.4	17	0.02
2. All others	15	246.6	246	0.01
Total	16	263	263	0.03
		Probability (1 df)		80% to 90%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly two active genes are segregating.

Table 7. Segregation of DTFF D-24 x Saffier, Greenhouse Planting. Salinas planting of 6-10-88. 15:1 Ratio.

Hypothesis: Genes segregate in 15:1 ratio for a system of 2 or more genes.

Phenotype Classes:

1: Group 109-End. Isolated late flowering phenotype, including all plants that did not flower before day 109.
2: All others. Group flowering before day 109.

Data: Sample size = 289. Group 109-End is preceded by 7 days of no flowering.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Group 109-End	1	18.1	21	0.47
2. All others	15	270.9	268	0.03
Total	64	289	289	0.50
		Probability (1 df)		40% to 50%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly two active genes are segregating.

Table 8. Segregation of DTFF D-24 x Saffier, Greenhouse Planting. Salinas planting of 6-10-88. 63:1 Ratio. (143-153)

Hypothesis: Genes segregate in 63:1 ratio for a system of 3 or more genes.

Phenotype Classes:

1: Group 143-153. Last phenotype flowering between days 143 and 153.
2: All others. Group of all other flowering days.

Data: Sample size = 289. Group 143-153 is preceded by 3 days of no flowering.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Group 143-153	1	4.5	5	0.06
2. All others	63	284.5	284	0.01
Total	64	289	289	0.07
		Probability (1 df)		70% to 80%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly three active genes are segregating.

Table 9. Segregation of DTFF D-24 x Saffier, Greenhouse Planting. Salinas planting of 6-10-88. 63:1 Ratio. (109-118)

Hypothesis: Genes segregate in 63:1 ratio for a system of 3 or more genes.

Phenotype Classes:

1: Group 109-118. Isolated phenotype flowering between days 109 and 118.
2: All others. Group of all other flowering days.

Data: Sample size = 289. Group 109-118 is preceded by 7 days of no flowering, and followed by 6 days of no flowering.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Group 109-118	1	4.5	6	0.50
2. All others	63	284.5	283	0.01
Total	64	289	289	0.51
		Probability (1 df)		40% to 50%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly three active genes are segregating.

Table 10. Segregation of DTFF C-2-1-1 x Saffier, Greenhouse Planting. Salinas Planting of 2-18-88. 15:1 Ratio.

Hypothesis: Genes segregate in 15:1 ratio for a system of 2 or more genes.

Phenotype Classes:

1: Flowers early: Flowers before last visible grouping.
2: Flowers late: Flowers in last visible grouping.

Data: Sample size = 236. The last pronounced grouping occurs between days 155 and 161.

Phenotype Classes	N	Expected	Observed	Chi-square $(E-O)^2/E$
1. Flowers early	15	221.2	218	0.01
2. Flowers late	1	14.8	13	0.22
Total	16	236	231	0.23
	Probability (1 df)			50% to 70%

Summary: Hypothesis PASSES the Chi-square test, indicating possibly two active genes are segregating.

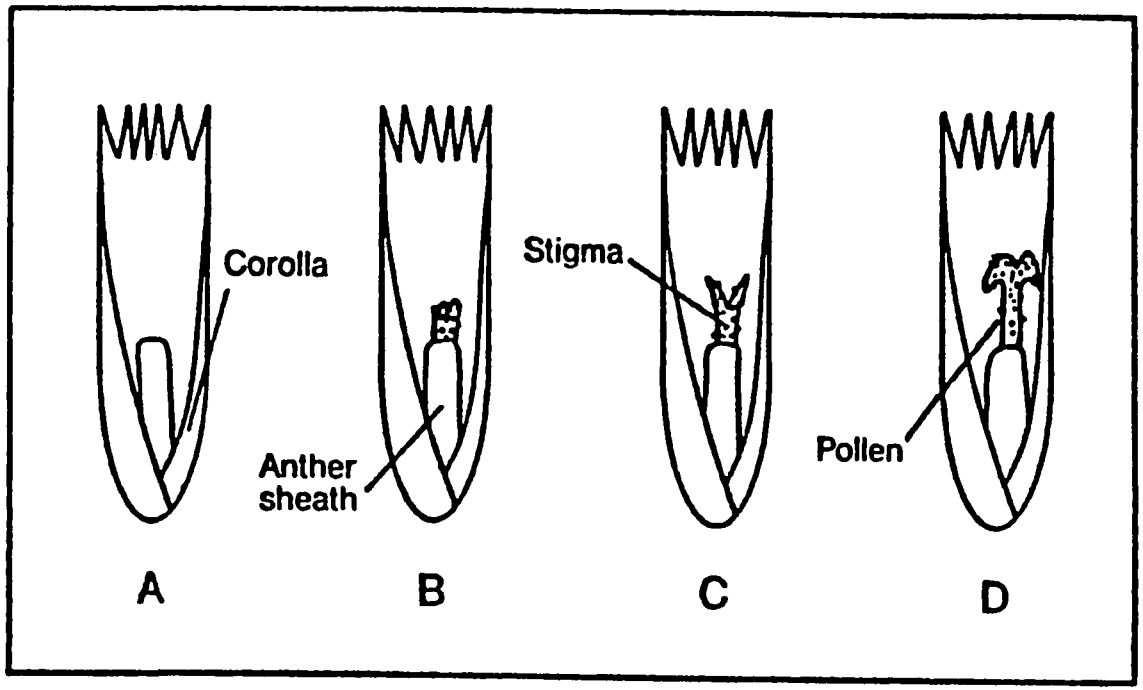

Figure 1. Lettuce Self-fertilization. Structure and stages of anthesis of a lettuce floret. A. Early stigma not yet emerged from anther sheath. B. Stigma emerging, covered with pollen. C. Ideal stage for pollen removal. D. Too late for crossing; selfing has occurred.

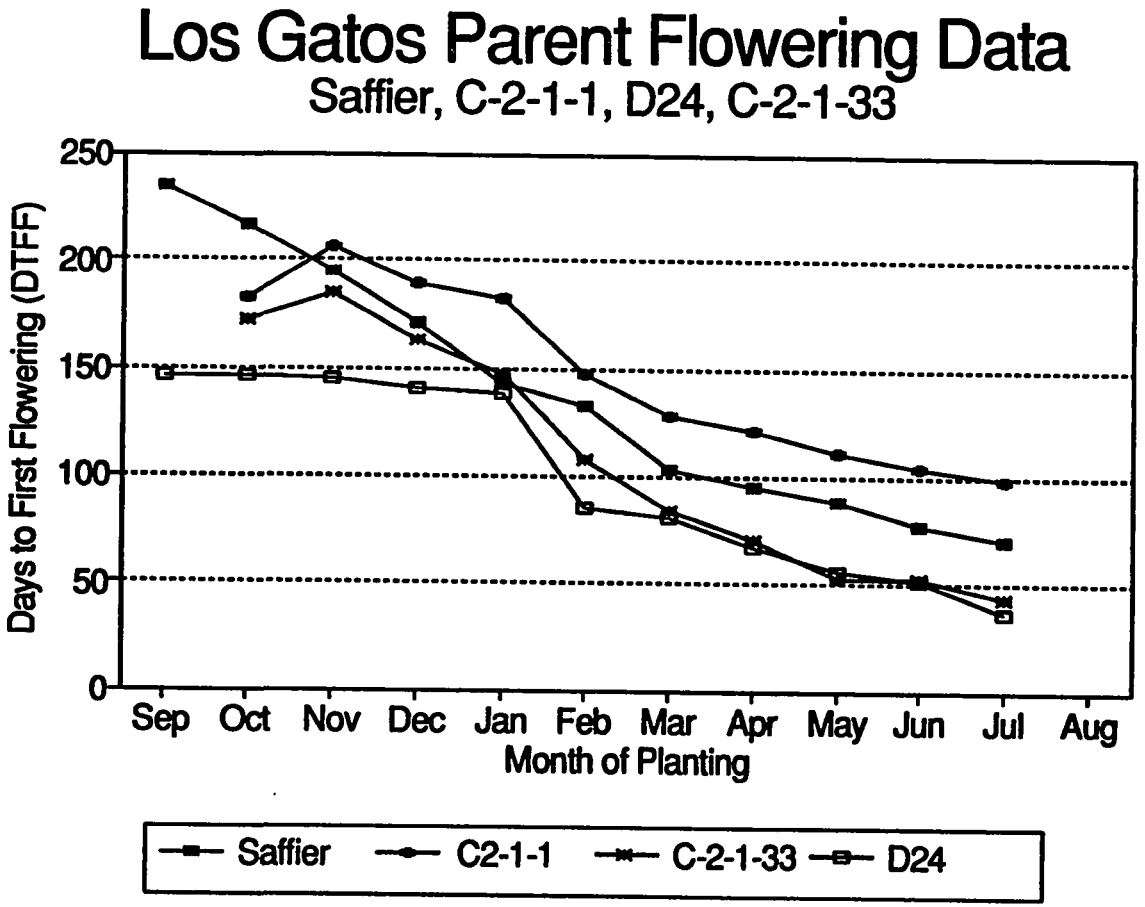

Figure 2. Days to First Flower Comparisons of Parent Lines. Los Gatos, California plantings from 1987 to 1988.

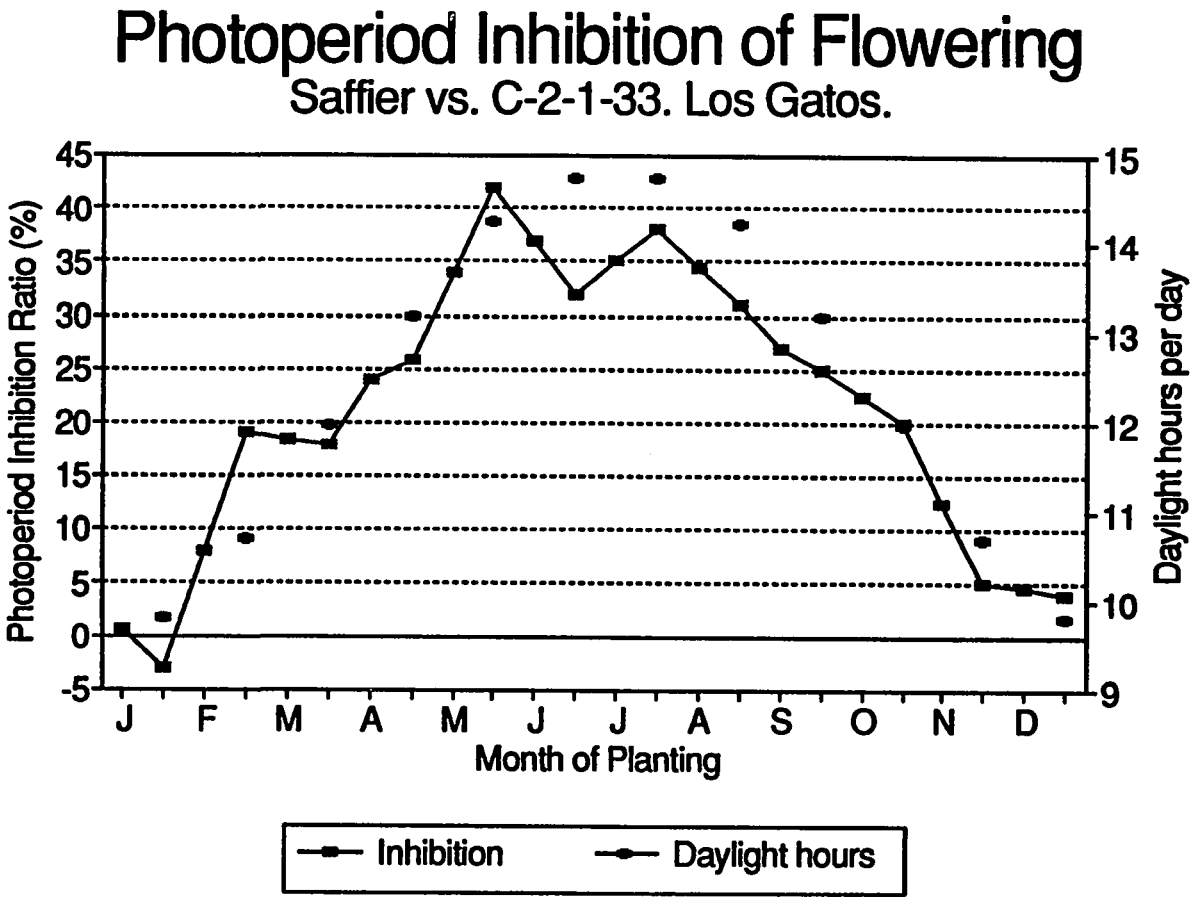

Figure 3. Photoperiod Inhibition of Saffier vs. C-2-1-33. Photoperiod Inhibition Factor, PIF = ratio of ((DTFF of Saffier) - (DTFF of C-2-1-33)) divided by (DTFF of Saffier).

C-2-1-1 x Saffier Salinas 2-18-88

236 Plants

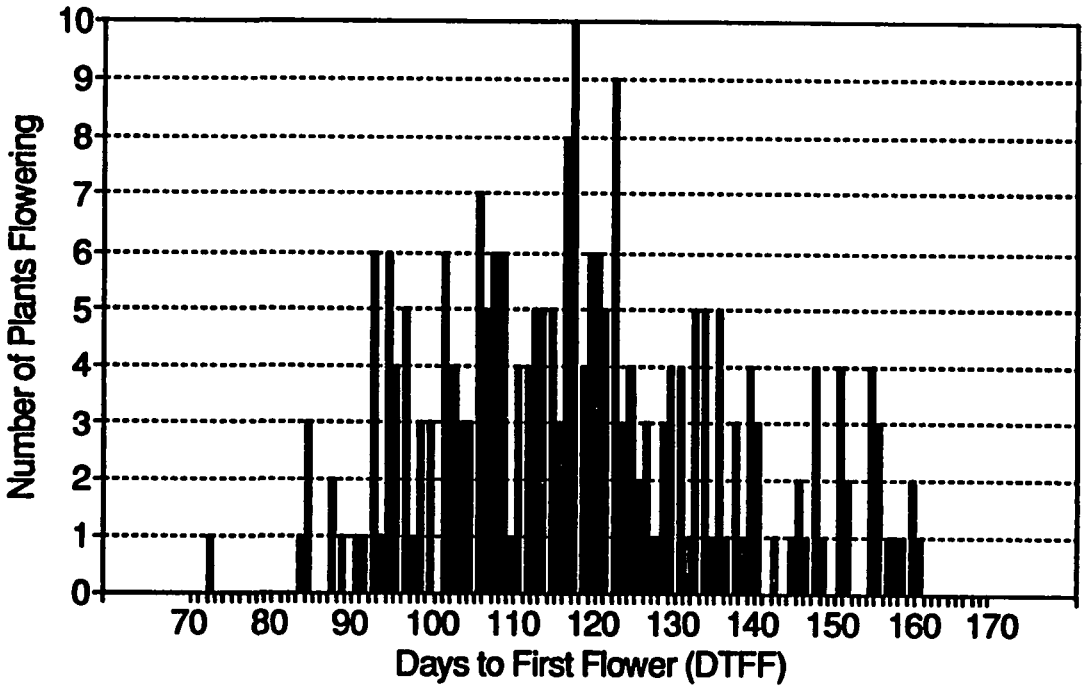

Figure 4. Salinas 2-18-88 Planting of C-2-1-1 x Saffier. Total population 236. C-2-1-1 control flowered 1(day 121), 1(123), 1(124), 1(125), 2(126), 1(127), 3(128), 1(131), 1(132), 1(134), 1(139), 1(140), 1(142), 1(142). C-2-1-1 mean DTFF 130.4. Saffier control flowered 1(122), 1(123), 1(124), 1(125), 1(128). Saffier mean DTFF 123.5.

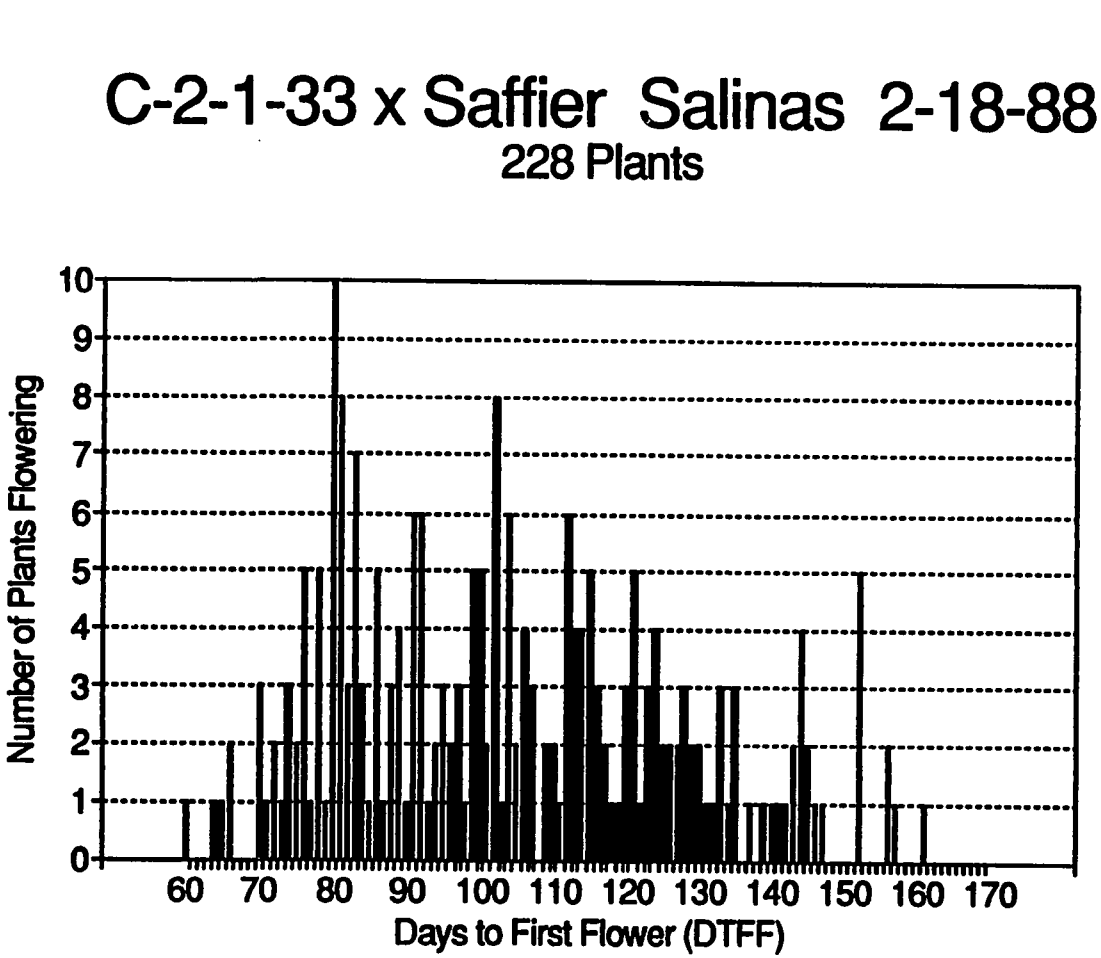

Figure 5. Salinas 2-18-88 Planting of C-2-1-33 x Saffier. Total population 228. Saffier control flowered 5 (day 108), 1(110), 1(111), 1(112), 3(113), 2(115), 1(117), 3(120), 1(121), 1(128). Saffier mean DTFF 114.1. C-2-1-33 control flowered 2(73), 4(74), 3(75), 1(76), 2(77), 3(78), 1(79), 3(80), 1(81). C-2-1-1 mean DTFF 76.6.

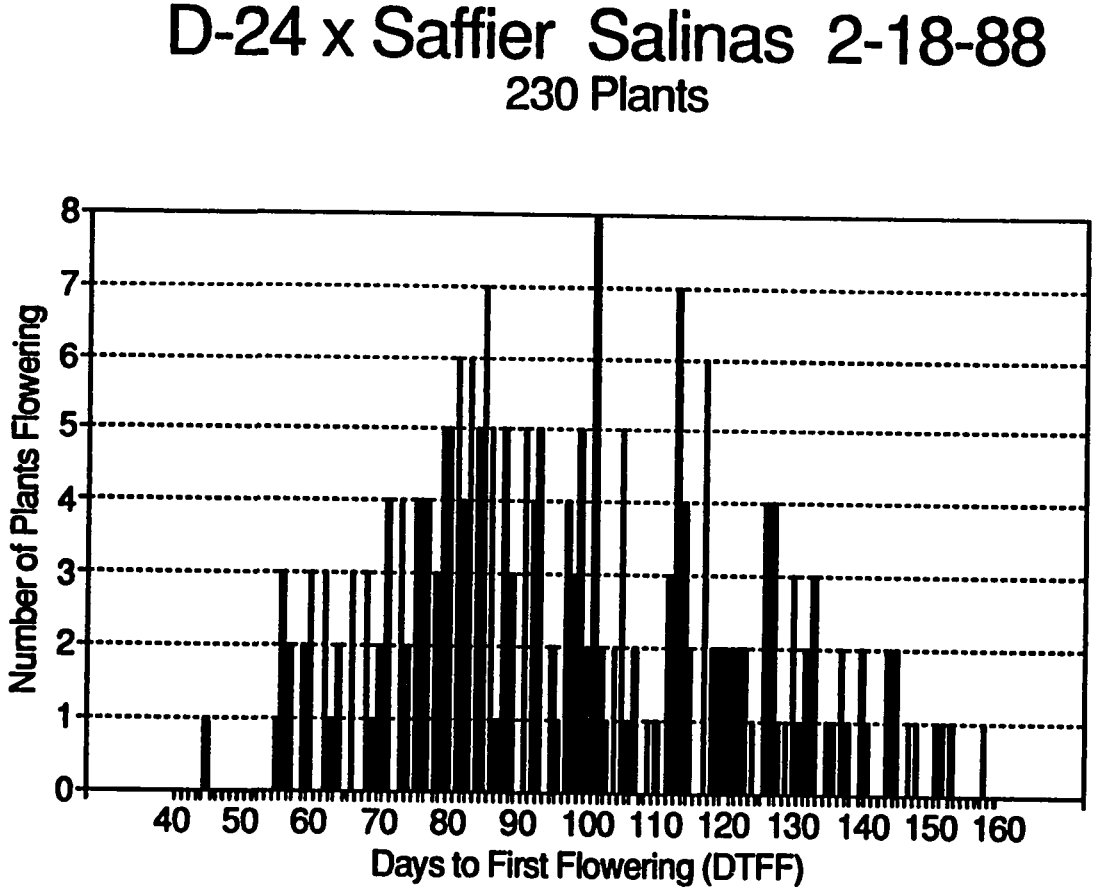

Figure 6. Salinas 2-18-88 Planting of D-24 x Saffier. Total population 230. D-24 control flowered 1(day 47), 2(48), 5(49), 3(50), 3(51), 2(52), 2(53). D-24 mean DTFF 50.1. Saffier control flowered 1(105), 1(111), 1(112), 1(122), 1(123), 1(124) and 1(128). Saffier mean DTFF 117.9.

C-2-1-1 x Saffier

6-10-88 Salinas Planting. 231 Plants.

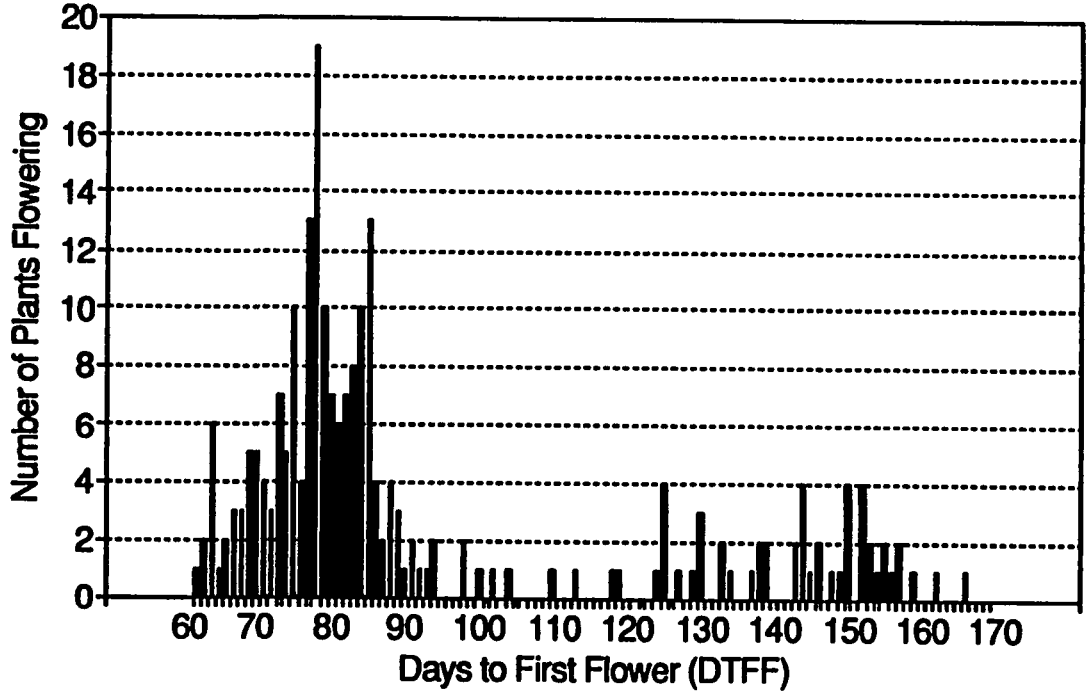

Figure 7. Salinas 6-10-88 Planting of C-2-1-1 x Saffier. Total population 231. C-2-1-1 flowering 1(day 116), 1(119), 2(120), 1(122), 1(123), 1(124), 1(125), 1(127), 2(133). C-2-1-1 mean DTFF 123.8. Saffier flowering 4(day 72), 4(73), 3(75), 1(76), 5(77), 1(78), 3(80), 1(81), 1(85), 1(90). Saffier mean DTFF 76.7.

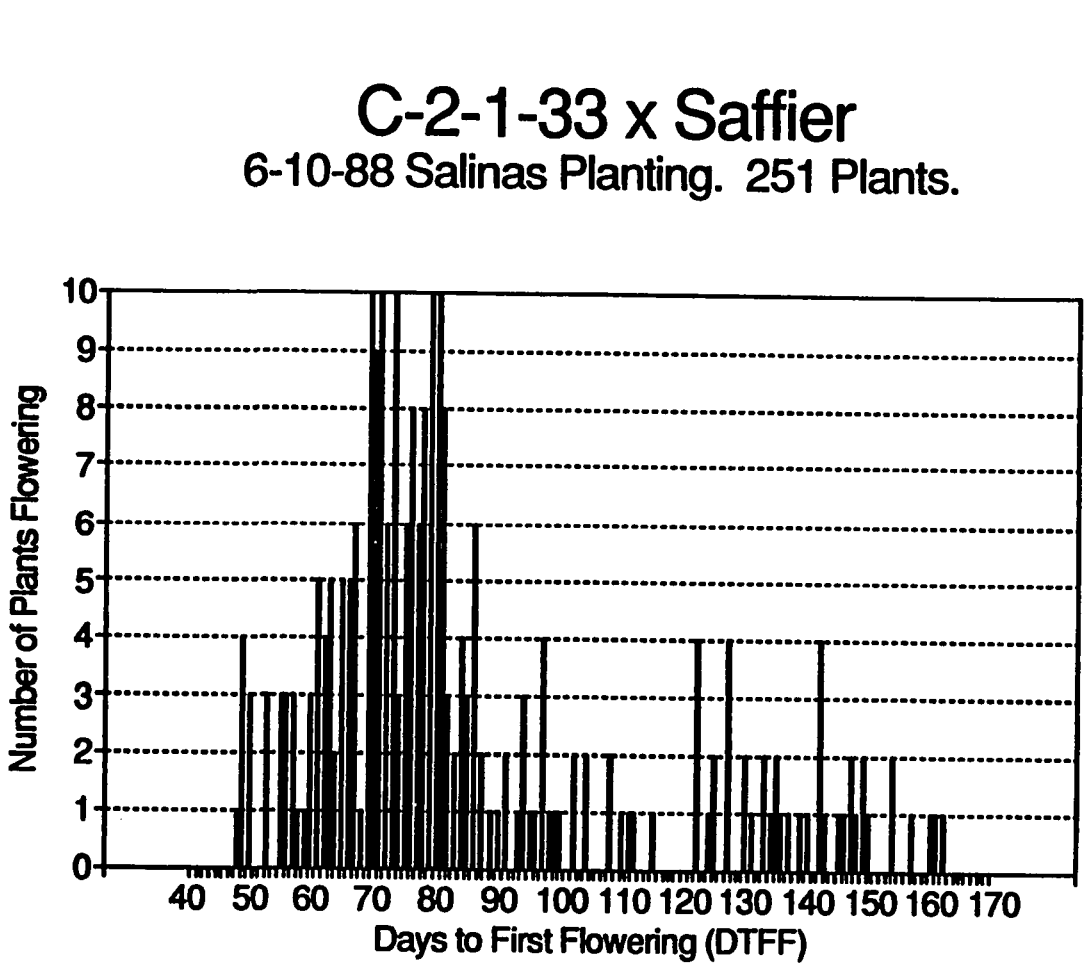

Figure 8. Salinas 6-10-88 Planting of C-2-1-33 x Saffier. Total population 263. Twelve had not yet flowered by day 171. C-2-1-33 flowered 2(day 59), 2(60), 3(63), 1(64), 3(66), 2(68), 1(71), 1(73), 1(75). C-2-1-33 mean DTFF 65.25. Saffier flowering 4(72), 4(73), 3(75), 1(76), 5(77), 1(78), 3(80), 1 (81), 1(85), 1(90). Saffier mean DTFF 76.7.

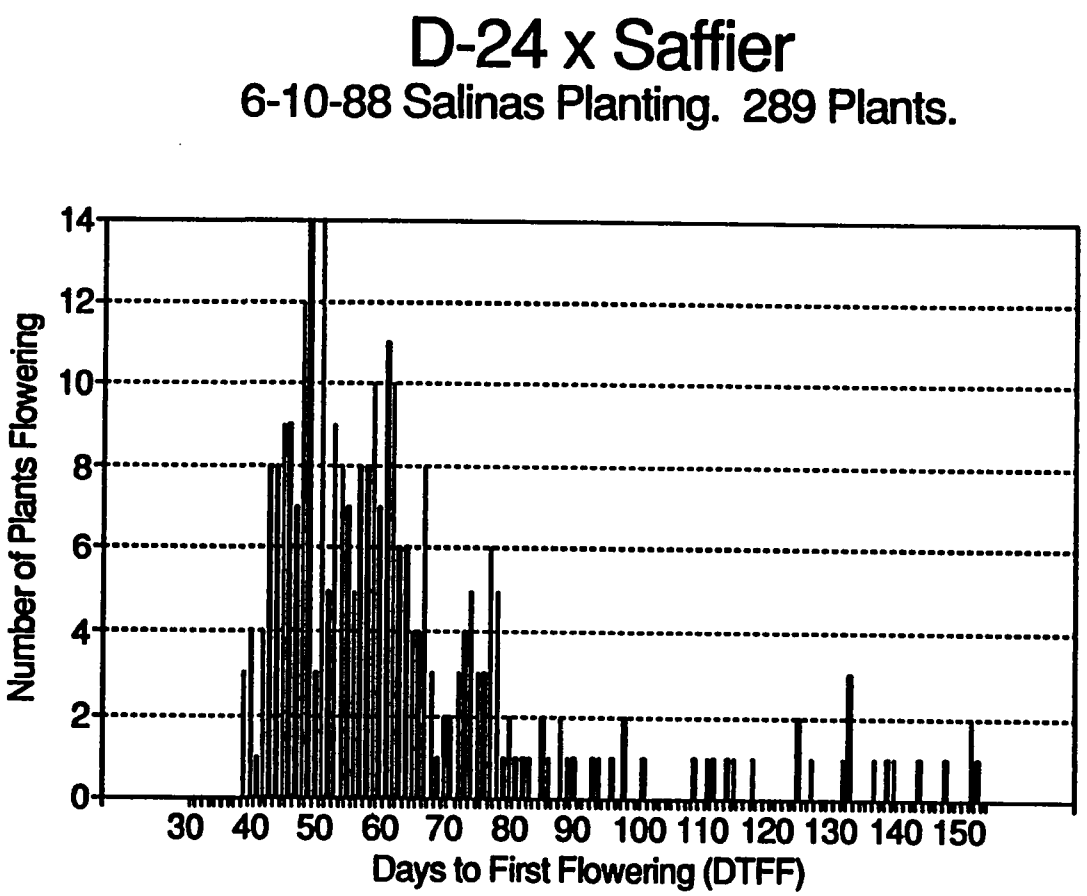

Figure 9. Salinas 6-10-88 Planting of D-24 x Saffier Total population 289. D-24 flowering 1 (day 41), 8(43), 12(44), 3(95). D-24 mean DTFF 43.7. Saffier flowered 4(72), 4(73), 3(75), 1(76), 5(77), 1(78), 3(80), 1 (81), 1(85), 1(90). Saffier mean DTFF 76.7.

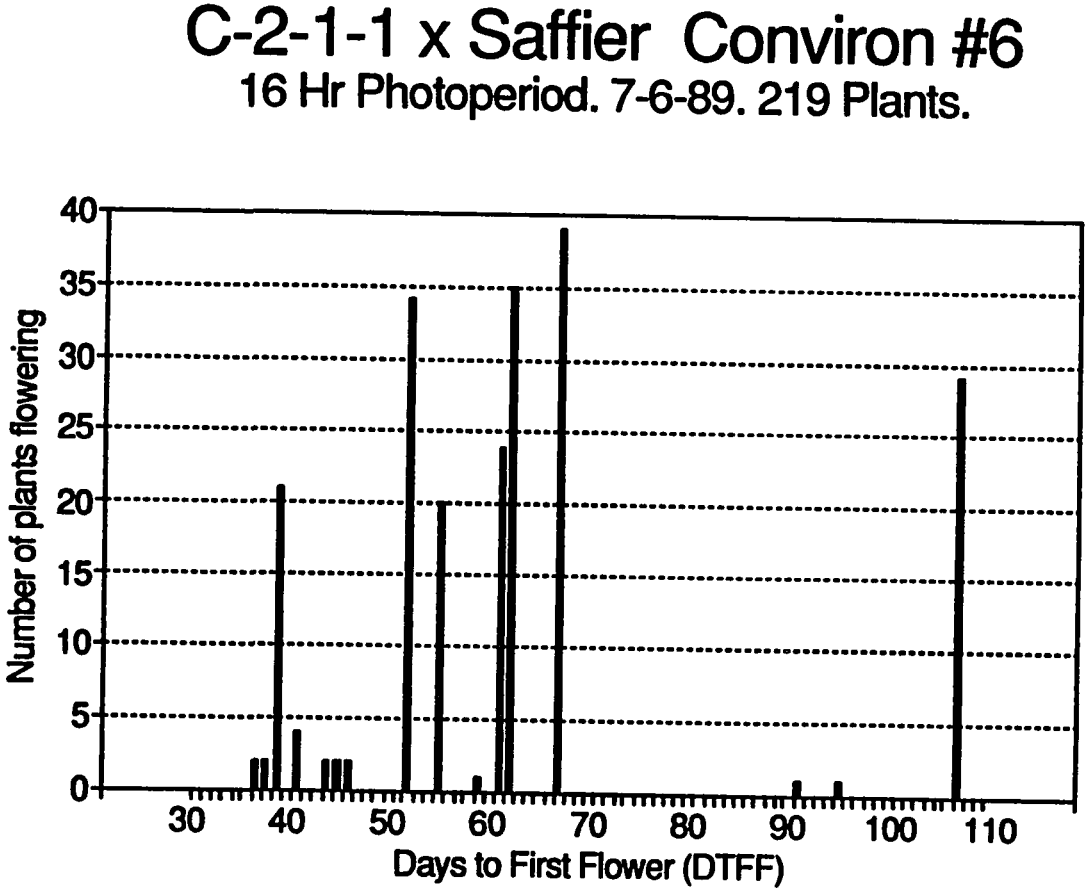

Figure 10. Growth Chamber Planting of C-2-1-1 x Saffier. Total population 223. Date 7-6-89. 16 hour photoperiod. Saffier control flowered 2(day 49), 2(50), 1(51). Saffier mean DTFF 49.8. C-2-1-1 control flowered 3(91), 3(92). C-2-1-1 mean DTFF 91.5.

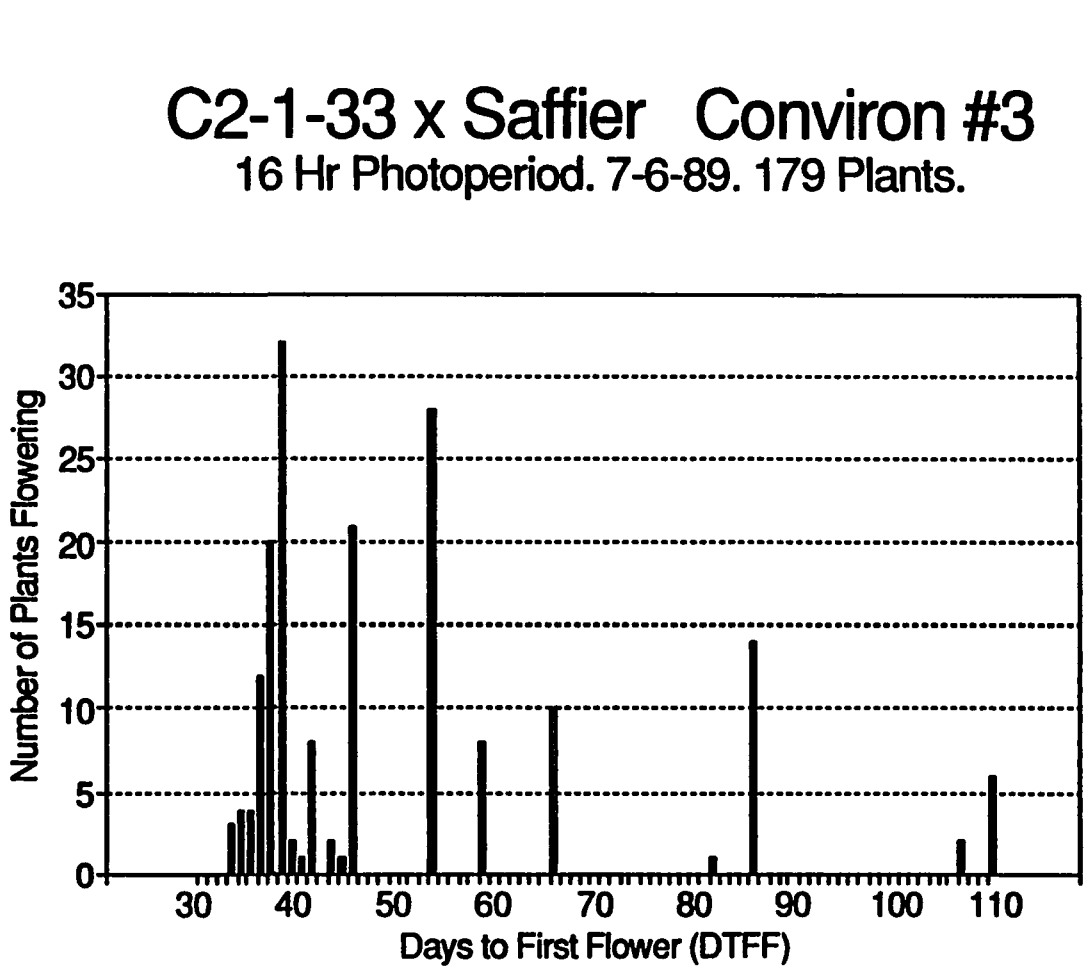

Figure 11. Growth Chamber Planting of C-2-1-33 x Saffier. Total population 179. Date 7-6-89. 16 hour photoperiod. Saffier control flowered 1(day 59), 1(62), 1(63), 3(66). Saffier mean DTFF 63.6. C-2-1-33 control flowered 1(52), 1(61), 1(63), 1(66). C-2-1-33 mean DTFF 60.5.

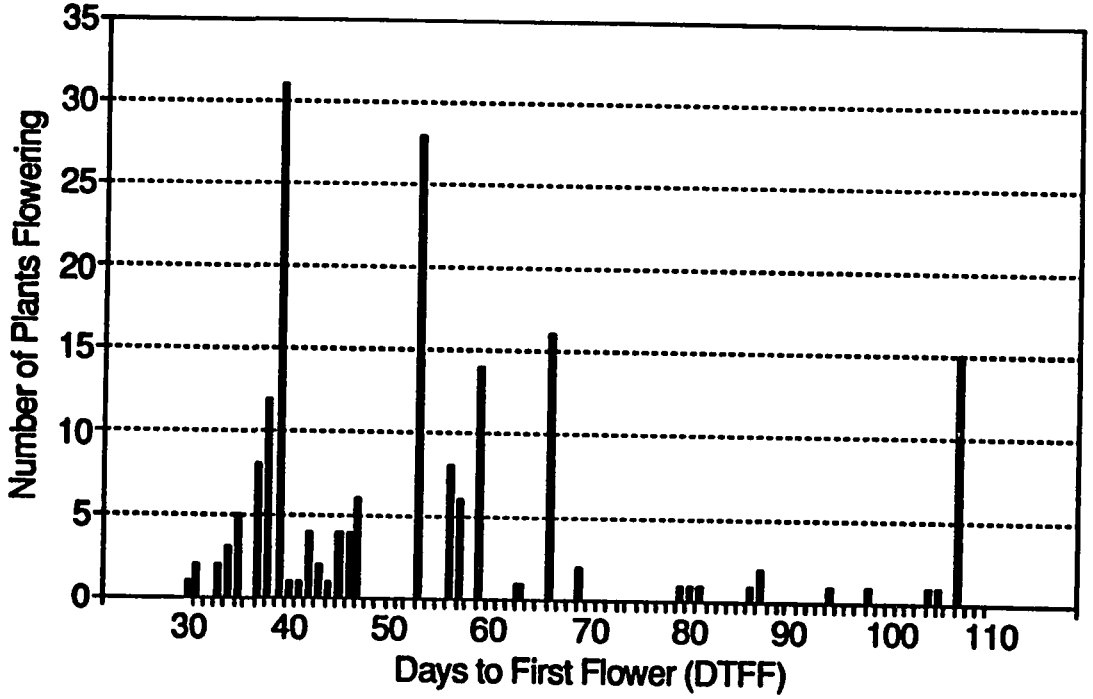

Figure 12. Growth Chamber Planting of D-24 x Saffier. Total population 187. Date 7-6-89. 16 hour photoperiod. D-24 control flowered 4(day 30). D-24 mean DTFF 30. Saffier control flowered 2(day 58), 1(59), 1(67). Saffier mean DTFF 60.5.

Appendix A. Hoagland's recipe.

Hoagland's nutrient solution. Combine the following in 1 liter of H_2O.

1. 10 ml. of 1M. $Ca(NO_3)_2$

2. 10 ml. of 1M. KNO_3

3. 4 ml. of 1M. $MgSO_4$

4. 2 ml. of 1M. KH_2PO_4

5. 2 ml. of EDTA stock solution. Iron complex of ethylenediaminetetra-acetic acid, each Ml. of solution contains 5 mg. of Fe.

6. 2 ml. of micronutrient stock solution. Combine following in 1 liter of H_2O.

2.86 g of H_3BO_3 (boric acid)
1.81 g of $MnCl_2$-$4H_2O$ (manganese chloride)
.11 g of $ZnCl_2$ (zinc chloride)
0.05 g of $CuCl_2$-$2H_2O$ (copper chloride)
0.025 g of Na_2MoO_4-$2H_2O$ (sodium molybdate)

Appendix B. Average Hours of Daylight, San Francisco Bay Region.

Month	Hours of Daylight
January	9.8
February	10.7
March	11.95
April	13.2
May	14.25
June	14.75
July	14.75
August	14.25
September	13.2
October	11.95
November	10.7
December	9.8