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Genetic Interactions Among Late-Flowering Mutants of Arabidopsis

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ABSTRACT

Flowering time in Arabidopsis is controlled by a large number of genes, identified by induced mutations. Forty-two double mutants involving 10 of these loci were obtained and analyzed for their flowering behavior under long-day conditions, with and without vernalization, and under short-day conditions. The genetic interactions between the various mutants proved to be complex, although a major epistatic group (called group A) could be identified corresponding to the mutants, which are relatively insensitive to vernalization and daylength. In contrast, the genetic behavior of the mutants much more responsive to these environmental factors (group B) is more complex. The vernalization responsiveness of the group B mutants did not compensate for the lateness of the group A mutants. This indicated that these genes do not control vernalization sensitivity as such, but provide a factor that becomes limiting in short days. The classification of these mutants in different physiological groups is discussed in relation to the detected genetic interactions, and based on these interactions a more detailed model of their role in flowering initiation is proposed.

THE genetic control of the transition to flowering in Arabidopsis is complex. This is indicated by the large number of loci identified by the analysis of both mutants and natural variants (reviewed by Martinez-Zapater *et al.* 1994; Haughn *et al.* 1995; Weigel 1995; Coupland 1995; Amasino 1996; Peeters and Koornneef 1996; Koornneef and Peeters 1997). The onset of flowering is usually measured as the number of days to flower (flowering time) or the total number of leaves produced by a plant, both characteristics being very tightly correlated (Koornneef *et al.* 1991). No mutations have been described in Arabidopsis that abolish flowering completely, and therefore the effect of mutations and natural variants is mainly quantitative. The large number of flowering mutants available in Arabidopsis have been classified first of all according to their effects on flowering time: in early mutants, for example, those advancing flowering in comparison to the wild type, and late mutants that delay it. Furthermore, these mutants have been classified physiologically on the basis of their responsiveness to environmental factors such as daylength, light quality, and vernalization. The large number of loci, the exclusively quantitative effects of the mutations, and their different response to environmental factors suggest that these genes encode different factors that more or less independently modify flowering but are not decisive for the process to occur. Physiological arguments for a multifactorial control of flowering are given by Bernier (1988) and Bernier *et al.* (1993).

One of the best characterized groups of flowering mutants in Arabidopsis are the late mutants reviewed by Koornneef and Peeters (1997). These loci have been physiologically classified in two major groups: one group comprises the mutants *fca*, *fld*, *fpa*, *fve*, *fy*, and *ld*, which are responsive to daylength and vernalization treatment and are supposed to be mutated in the constitutive floral promotion pathway (Martinez-Zapater *et al.* 1994). The second group is represented by the mutants *co*, *gi*, *fd*, *fe*, *fha*, *ft*, and *fwa*, which are not or are much less responsive to environmental factors and which are assumed to represent mutations in the long-day (LD) floral promotion pathway. (Martinez-Zapater *et al.* 1994; Coupland 1995). This classification has been mainly based on the behavior of the monogenic mutants. However, different genetic relationships and epistatic groups are expected depending on whether the genes are working in the same or different developmental pathways. If one mutant masks the effect of a mutation in another gene, the first mutant is epistatic to the second one. It is then assumed that these genes control subsequent steps in a specific developmental pathway. When the genes control steps in two parallel pathways leading to the same end result, one expects that the phenotype of the double mutant is additive or synergistic. When mutants have large (qualitative) effects, the analysis of genetic relations is generally straightforward. However, in the case of mutants with strictly quantitative effects, the phenotype of the double mutant is also described quantitatively (Eshed

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and Zamir 1995), which implies that one should quantify the degree of interaction. The interpretation of such quantitative effects is not always obvious.

The analysis of genetic relationships among a large number of mutants with quantitative effects has not often been described. The availability of flowering-time mutants such as those of Arabidopsis provides an attractive system for this type of analysis. Examples of clear epistatic relationships among flowering-time genes in Arabidopsis are the interactions between *FLC* and, respectively, the *FRI* and *LD* loci, which could be interpreted in a model where the *LD* gene inhibits the *FLC* gene, which together with *FRI*, is required for the inhibition of flowering (Koornneef *et al.* 1994; Lee *et al.* 1994b). A strong interaction between *FLC* and other late mutants has been described by Sanda and Amasino (1996). The genetic relationships between some late-flowering mutants and early mutants affected in phytochrome genes were described by Koornneef *et al.* (1995).

In the present article, a systematic and quantitative comparison of double mutants of representative alleles at 10 of the late-flowering mutant loci has been made, and thus epistatic groups have been established. Preliminary data on the epistatic groups were given in Koornneef *et al.* (1991). The double and single mutants were tested under long-day (LD) and short-day (SD) light conditions, and with and without vernalization treatment. The classification of these mutants in different physiological groups is discussed in relation to the detected genetic interactions and, based on these interactions, a more detailed model of their role in flowering initiation is proposed.

MATERIALS AND METHODS

The mutant alleles, all in the Landsberg *erecta* (L*er*) genetic background, that were used are the following: *fca-1*, *fve-1*, *fy-1*, *fpa-1*, *fe-1*, *ft-1*, *fha-1*, *fwa-1*, *co-3*, and *gi-3*. These mutant alleles have been described in Koornneef *et al.* (1991). Putative double mutants were identified as the latest plants in the F_2 generations derived from crosses between two mutants. Subsequently, the double mutant nature was confirmed by testcrossing with the parental mutants. Because *co* and *fwa* mutants are dominant, in the double mutants involving these mutations, allelism was confirmed by the absence of early wild-type segregants in the F_2 generations derived from test crosses.

Flowering time (FT) was recorded as the number of days from the date the seeds were imbibed at 25° to the opening of the first flower. Total leaf number (LN) was scored as the number of leaves in the rosette plus the number of cauline leaves on the main stem, which has been shown to correlate highly with flowering time (Koornneef *et al.* 1991).

The interaction between each pair of mutations was tested by two-way anova. Given the correlation between means of leaf number and the corresponding variances, logarithmic transformation was applied to the leaf number data. The latter implies that interaction is based on the absence of additivity in a multiplicative scale. Long-day (LD) experiments were performed in an air-conditioned greenhouse supplemented with additional light from the middle of September until the beginning of April, providing a daylength of at least 14 hr and a light intensity sufficient to allow growth. Day temperature was 22–25° and night temperature 16–19°. Per genotype, groups of six plants were grown in a row. These groups were randomized over four blocs. The vernalization treatment was tested in the same LD experiments. This treatment was given as described by Koornneef *et al.* (1994). For this, seeds were sown on Murashige-Skoog medium supplemented with 2% sucrose and incubated in a cold room for the periods indicated. Subsequently, the Petri dishes were kept for 48 hr at 24° before they were planted in the greenhouse. The nonvernalized control was sown in the same way but incubated directly at 24° where it stayed for 72 hr before planting in the greenhouse.

Short-day (SD) experiments were carried out in a single climate chamber with 8 hr of light as described by Koornneef *et al.* (1995). Per genotype, four groups of five plants (each in one pot) were available and each group was randomized in one of the four blocs.

RESULTS

The isolation of double mutants: In most cases double mutants could be readily identified by the procedure described in materials and methods. However, three double mutant combinations were not obtained. Because of close linkage between *co* and *fy* (Koornneef *et al.* 1994) and the dominance of *co*, this double mutant was not identified in the material tested. Probably also because of linkage we failed to identify a clear *fwa fca* double mutant. Linkage should not have provided problems in identifying the *fpa fy* double mutant. Although no extreme late plants could be identified in the corresponding F_2, several plants that were homozygous for either *fpa* or *fy* and heterozygous for the second mutant were identified. From selfed progeny of such lines the latest plants were used for allelism tests with the mutant parent for which the line was heterozygous. In none of the 63 crosses did the tested plant appear homozygous, although one-third was expected to be so. This suggests that the *fpa fy* double mutant might be inviable.

The genetic relationships among late-flowering mutants: The various confirmed double mutants were grown in the greenhouse under LD conditions in three different experiments. Because the most complete collection of double mutants was present in the last experiment and the correlation between the various experiments was high, the data of this experiment (Table 1) are presented and discussed. Table 1 shows the total leaf number of all single and double mutants tested.

The double mutants can be classified in a qualitative manner, according to their phenotype (total leaf number) in comparison to the parental mutants in the following three classes: (1) double mutants with a flowering time approximately similar to the addition of the delay produced by each mutant (and therefore both mutations do not interact but behave additively), and (2) double mutants with earlier flowering time than the addition of the effects of both single mutations. In

TABLE 1

Total leaf number with standard error of monogenic late-flowering mutants and their double mutants

	fca	*fve*	*fy*	*fpa*	*fe*	*ft*	*fha*	*fwa*	*co*	*gi*
fca	**22.5 ± 0.4**									
	11.4 ± 0.2									
fve	**31.1 ± 1.1**[b]	**18.0 ± 0.2**								
	10.4 ± 0.2	11.5 ± 0.3								
fy	**25.5 ± 0.9**[b]	**32.7 ± 0.5**[c]	**15.0 ± 0.2**							
	14.0 ± 0.3	13.3 ± 0.2	10.7 ± 0.2							
fpa	**44.5 ± 2.3**[c]	**17.5 ± 0.2**[b]		**14.8 ± 0.3**						
	20.4 ± 1.5	9.7 ± 0.2		11.3 ± 0.2						
fe	**35.7 ± 2.3**[b]	**39.6 ± 0.8**[a]	**28.6 ± 0.5**[a]	**52.1 ± 1.8**[c]	**19.4 ± 0.3**					
	12.7 ± 0.5	15.5 ± 0.4	18.5 ± 0.3	15.3 ± 0.3	15.0 ± 0.3					
ft	**40.6 ± 0.7**[a]	**39.6 ± 0.6**[c]	**28.8 ± 0.6**[a]	**56.8 ± 2.2**[c]	**32.2 ± 0.6**[b]	**18.0 ± 0.2**				
	17.8 ± 0.2	20.2 ± 0.4	20.3 ± 0.4	16.7 ± 0.4	28.7 ± 0.5	16.2 ± 0.3				
fha	**51.1 ± 1.6**[c]	**35.8 ± 1.3**[c]	**24.9 ± 0.5**[c]	**24.2 ± 0.9**[a]	**35.4 ± 1.2**[c]	**29.9 ± 1.0**[c]	**13.9 ± 0.2**			
	15.6 ± 0.3	15.2 ± 0.5	16.7 ± 0.5	15.5 ± 0.3	24.7 ± 0.6	25.2 ± 1.0	13.1 ± 0.2			
fwa		**37.0 ± 0.6**[a]	**31.3 ± 0.8**[a]	**28.1 ± 0.8**[a]	**36.5 ± 0.6**[b]	**21.3 ± 0.2**[b]	**27.1 ± 0.5**[a]	**20.0 ± 0.4**		
		21.0 ± 0.4	22.7 ± 0.4	19.7 ± 0.3	33.3 ± 0.5	20.4 ± 0.4	25.5 ± 0.4	17.8 ± 0.2		
co	**43.6 ± 0.8**[b]	**44.4 ± 0.5**[b]		**38.8 ± 1.7**[a]	**34.6 ± 0.4**[b]	**31.0 ± 0.5**[b]	**38.0 ± 0.9**[a]	**34.7 ± 0.7**[b]	**28.2 ± 0.7**	
	27.6 ± 0.6	34.9 ± 0.8		27.6 ± 0.5	32.8 ± 0.4	31.0 ± 0.5	32.2 ± 1.7	33.1 ± 0.5	24.0 ± 0.7	
gi	**47.7 ± 1.0**[b]	**48.8 ± 0.6**[b]	**42.5 ± 1.0**[b]	**35.3 ± 0.9**[b]	**35.0 ± 0.4**[b]	**35.5 ± 0.7**[b]	**36.0 ± 0.8**[b]	**37.7 ± 1.0**[b]	**36.3 ± 0.9**[b]	**33.3 ± 0.5**
	30.0 ± 0.6	31.0 ± 0.5	34.6 ± 0.8	27.5 ± 0.5	33.9 ± 0.5	34.1 ± 0.5	30.5 ± 0.5	34.8 ± 0.6	30.7 ± 0.7	25.7 ± 0.8

Ler (WT) **9.7 ± 0.2**
9.0 ± 0.2

Boldface, without a vernalization treatment; lightface, with a 3-wk vernalization treatment. All material was grown in the same experiment in the autumn of 1995.

[a] Indicates absence of a significant interaction ($P < 0.01$).

[b] A significant interaction with the double mutant having fewer leaves than expected on the basis of additivity.

[c] A significant interaction with the double mutant having more leaves than expected on the basis of additivity.

this case, the double mutants are considered as showing epistasis in the sense of less-than-additive interaction. The extreme situation of this type of interaction is when a double mutant shows similar flowering time to one of the parental mutants, usually the latest one. (3) Those double mutants showing a later flowering time than the simple addition of both mutations, for example, a more-than-additive effect, the mutations interacting synergistically. Mutants belonging to the same epistatic group are expected to have double mutants, which among them are epistatic and which will show a relatively similar behavior, either additive or synergistic, with the mutants outside the group. The significance of the interaction between two mutations was estimated from the interaction term of a two-way ANOVA for each pair of mutants (Table 1).

According to these criteria, taking into account not only the significance of the interaction but also the size of the interaction effect (Table 1), the most remarkable interactions detected among the 42 double mutants can be summarized as follows:

On one hand, *gi* behaves as epistatic with *co*, *fwa*, *fha*, *fe*, and *ft*; the *co* mutant behaves as epistatic with *fwa*, *ft*, and *fe*; and *fwa* shows epistasis with *ft*. In general, when considering the interactions among these mutants, the later mutants give rise to clear epistasis, whereas the earlier ones have a slightly additive effect. Therefore, these six mutants can be classified as one epistatic group, in agreement with the established physiological classification of these loci in a common group, which from here on will be referred to as group A.

On the other hand, the genetic relationships among *fca*, *fve*, *fy*, and *fpa*, which belong to the so-called responsive physiological group, seem more complex. This group will be referred to as group B. The only clear epistatic behavior was observed between *fca* and *fy* and between *fve* and *fpa*. The *fpa* mutant is the most deviating one as shown by the more-than-additive effect observed in the double mutant with *fca*.

When considering the interactions between mutants of group A and group B, in general an additive effect was observed, although strong synergistic interactions appeared in several double mutants. Particular complex interactions are shown by the double mutants involving *fpa*, which interacts synergistically with *ft* and *fe*, as shown by the extreme lateness of the double mutants. In contrast, the double mutants of *fpa* with *fha*, *fwa*, *co*, and *gi* are relatively early. The *gi* mutant is in fact epistatic with *fpa*. A last intriguing interaction is shown between *fha* and *fca*, which seem to interact synergistically as well. From this analysis it appears that genetic relations can be complex, and that this complexity depends on the mutant.

Vernalization responsiveness in the double mutants: Because the mutants differ strongly in the effect of a 3-wk vernalization treatment it was tested for all single

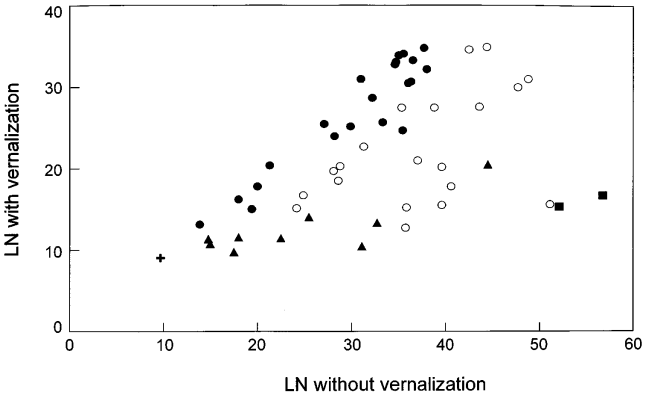

Figure 1.—The correlation between total leaf number of genotypes treated with or without a 3-wk vernalization treatment. +, wild type; ●, mutants and double mutants of group A; ▲, parents and mutants of group B; ○, double mutants combining group A and B mutants; ■, double mutants of *fpa* with *fe* and *ft*.

and double mutants (Table 1, Figure 1). The vernalization response of the single mutants was similar to previous reports (Martinez-Zapater and Somerville 1990; Koornneef *et al.* 1991), *fca*, *fve*, *fy*, and *fpa* being responsive, the rest of the mutants responding little or almost not at all to the treatment. In general, double mutants derived from either vernalization-responsive or -nonresponsive mutants behaved as the parents. Only the *fca fpa* double mutant shows less response than expected. In double mutants, between responsive and nonresponsive mutants, the sensitivity to vernalization is intermediate with the exception of double mutants of group B with *fe* and *ft*, which all are very responsive to this treatment. Although *fe* behaves similar to *ft* in many aspects, it seems slightly more responsive to vernalization than *ft*, and this is confirmed by the double mutants with *fe*, having slightly fewer leaves than similar *ft* double mutants.

A number of mutants from group A (*co*, *gi*, *fwa*, and *fe*) and from group B (*fca* and *fve*) were selected to test different periods of vernalization treatment (Figure 2). The comparisons of the double mutants with different periods of vernalization show a similar pattern, as with the 3-wk treatment. Double mutants within group A did not increase their response when increasing the vernalization treatment to 5 wk (Figure 2A). It appears that the vernalization responsiveness of *fca* and *fve* is additive. In combinations where one of the mutants is vernalization-responsive and the second one is not, the additional lateness due to the vernalization-responsive mutant (*fca* and *fve*) can be overcome by vernalization, but this treatment cannot compensate for the lateness of the second unresponsive mutant. In other words, vernalization cannot compensate for the lateness conferred by the nonresponsive mutants, as it can do for the responsive parent.

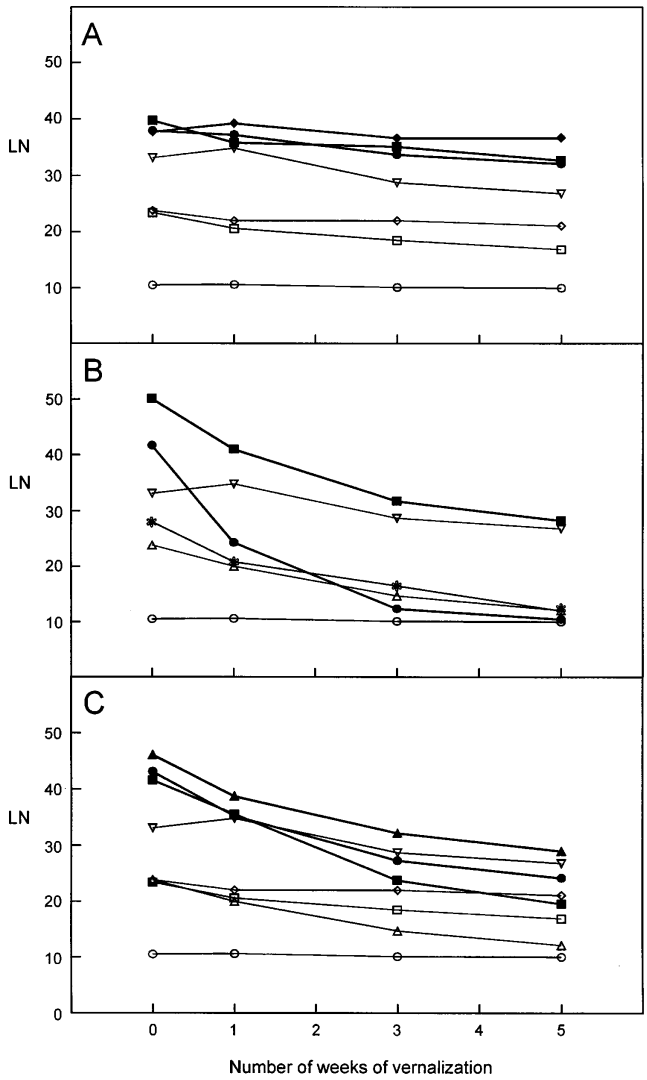

Figure 2.—The effect of different lengths of the vernalization period on total leaf number of double and monogenic mutants. (A) *fwa gi* (■), *fe gi* (●), *fwa fe* (◆), *gi* (▽), *fwa* (◇), *fe* (□), WT (○). (B) *gi fca* (■), *fve fca* (●), *fve* (△), *fca* (*), *gi* (▽), WT (○). (C) *gi fve* (▲), *fwa fve* (●), *fe fve* (■), *gi* (▽), *fwa* (◇), *fe* (□), *fve* (△), WT (○).

The effect of short days (SD): A number of mutants from group A (*co*, *gi*, *fwa*, and *fe*) and from group B (*fca* and *fve*) and all the corresponding double mutants were analyzed under SD light conditions (Figure 3). Two different greenhouse experiments (LD conditions) that are used for comparison show a considerable difference, where the plants in the summer experiment flowered earlier and with fewer leaves than in the autumn. This difference is probably mainly due to differences in light intensity, as has been reported before (King and Bagnall 1996). In SD conditions the monogenic mutants behaved as described previously (Koornneef *et al.* 1991). Flowering was strongly delayed in the *fca* and *fve* mutants, whereas the *co* and *gi* mutants were not delayed or only slightly delayed when compared to

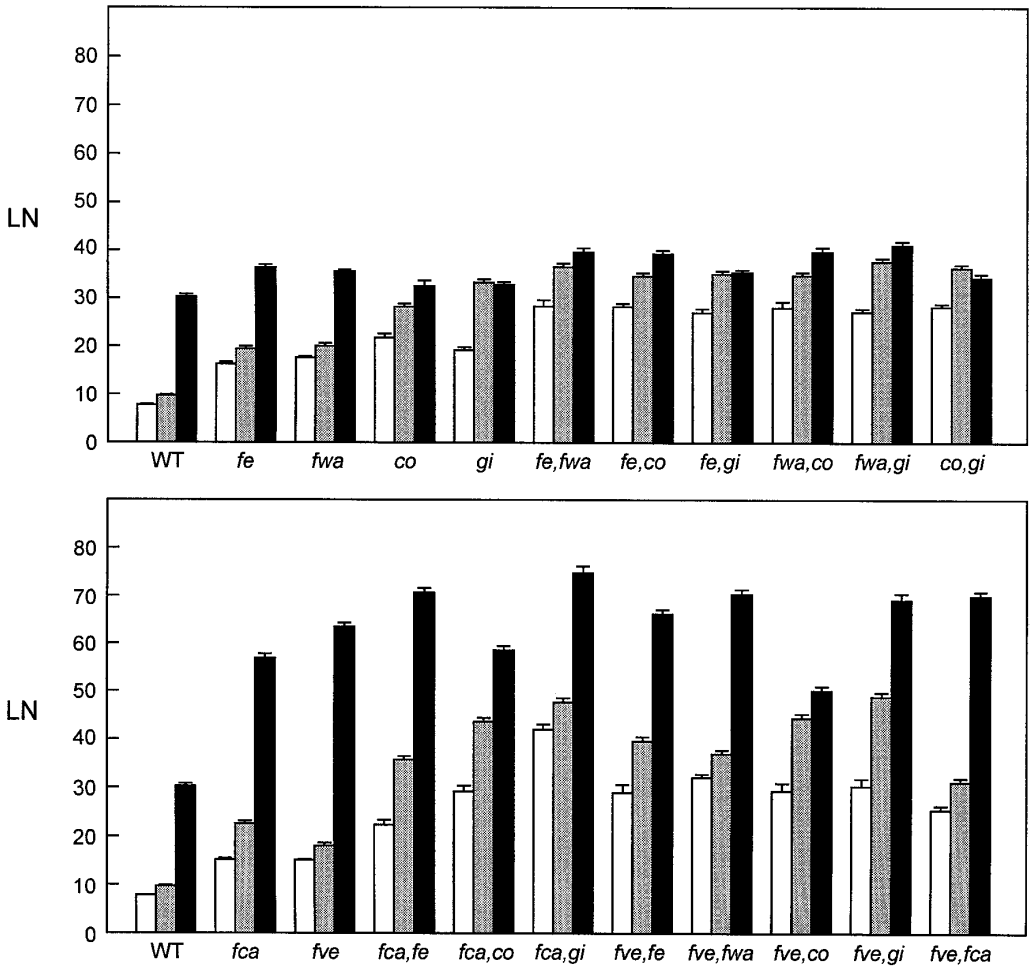

Figure 3.—Total leaf number of monogenic and double mutants grown in LD greenhouse conditions in summer (white bars), in autumn (grey bars), and SD climate chamber conditions (black bars).

their LN in the autumn greenhouse experiment. Flowering delay in the *fe* and *fwa* mutants was intermediate. All double mutants among *co*, *gi*, *fe*, and *fwa* behaved as daylength neutral and flowered under short-day conditions with only slightly more leaves than the wild type. In contrast, a large delay by SD conditions is observed in all double mutants that involve *fca* and *fve*, indicating epistasis of the latter mutations. Only in the combination of *co* and *fve* does it appear that the *co* mutation also renders the *fve* mutant less sensitive to the inhibiting effect of SD.

The relationship between rosette and cauline leaf number and flowering time: In earlier analyses it appeared that the late-flowering mutants differ in the relative numbers of rosette and cauline leaves. Analysis of the ratio of cauline/total leaf number (Figure 4) indicated that a high ratio is present among single and double mutants of the group A and relatively lower ratios within the group B mutants, as it was described previously for the single mutants (Koornneef *et al.* 1991). The double mutants derived from representatives of both groups have an intermediate ratio, although considerable variation is observed.

The ratio between LN and flowering time showed a relatively low variation among the genotypes, although the group A mutants flowered slightly later at the same number of leaves than the double mutants involving the B group, indicating a slightly faster rate of leaf initiation in the latter (data not shown).

DISCUSSION

The large number of flowering-time loci in Arabidopsis (Peeters and Koornneef 1996)—not one abolishing flowering completely—led to the assumption that these genes only modify flowering time and that they are not the crucial switches of the developmental change called the transition to flowering. The different flowering-time mutants, both the late and the early mutants, have been classified in different physiological groups on the basis of their responsiveness to environmental factors such as daylength, light quality, and vernalization (Martinez-Zapater and Somerville 1990; Koornneef *et al.* 1991; Hicks *et al.* 1996). The different physiological behavior of the single mutants indicates that the corresponding genes control flowering initiation in different ways. The various genes have been integrated in a model published by Martinez-Zapater *et al.* (1994) and Haughn *et al.* (1995) and called the Martinez-Zapater, Coupland, Dean, Kornneef (MCDK)

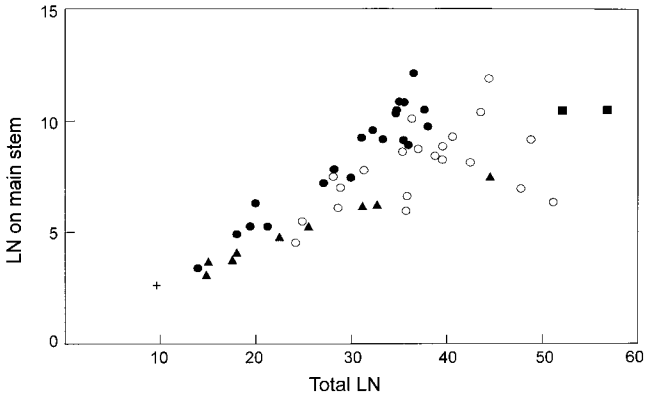

Figure 4.—The correlation between the number of cauline leaves and the total leaf number. Data derived from the experiment presented in Table 1. +, wild type; ●, mutants and double mutants of group A; ▲, parents and mutants of group B; ○, double mutants combining group A and B mutants; ■, double mutants of *fpa* with *fe* and *ft*.

model by Weigel (1995), in which these flowering genes either promote or inhibit a floral repressor. In the late-flowering mutants the physiological classification led to two major groups: one group comprising the genes *FCA*, *FLD*, *FPA*, *FVE*, *FY*, and *LD*, which are responsive to daylength and vernalization treatment and which are supposed to be involved in the constitutive floral promotion pathway, and a second group including *CO*, *GI*, *FD*, *FE*, *FHA*, *FT*, and *FWA*, thought to control the long-day floral promotion pathway. The aim of the research in the present article was to analyze the genetic relationships among mutants at 10 of these late-flowering loci, for example, to study the genetic interactions within and between these two major physiological groups of late-flowering mutants. Preliminary data on some of the genetic relationships are presented in Koornneef *et al.* (1991), but only a few double mutants were tested and the analysis was based on comparisons from different experiments. The results of the current analysis are in agreement with those preliminary data, except for the classification of *fwa* mutants, for which no reason is known.

The analysis of 42 double mutants involving 10 different loci show that genetic relationships among these genes are complex. This can be interpreted by assuming that these genes interact in a complex way and cannot be grouped into a few parallel pathways, each with a number of genes acting in a linear way. However, a complication in the interpretation of this type of genetic analysis is that some of the mutants might be "leaky." Leaky mutants in a linear pathway often show additive or even more-than-additive effects, which normally are interpreted as the genes controlling different pathways. Nevertheless, in most cases several alleles have been found, and mostly the more extreme alleles were included in the present analysis. The available sequence information for the alleles *co-3* (Putterill *et al.* 1995) and *fca-1* (Macknight *et al.* 1997) suggests that these mutations are true null alleles. Furthermore, it cannot be excluded that genes affecting essential metabolic functions may be lethal in the case of null mutations and that they may have an effect only on more subtle regulated processes such as flowering when they are "leaky." Genetic redundancy is an important cause to explain why even null mutants of essential genes show a leaky phenotype. Possibly, the unexpected interaction between *fpa* and *fy* can be explained in this way when these genes control in a duplicated way a similar essential function, but both genes cannot replace each other fully in the initiation of flowering. When both genes are not functioning, this may lead to lethality. That both genes function in a similar pathway is suggested by belonging to both the vernalization and daylength-responsive B group. However, in this group *fpa* deviates from most other mutants by its strong synergistic interaction with *fe* and *ft* mutants, suggesting that *FPA* shares some redundant functions in the constitutive promotion pathway, and also that *FPA* may play other different roles. Surprisingly, none of the other combinations of late-flowering mutants appears to lead to novel phenotypes such as lethality, reduced vigor, or other morphological changes. It is also important to note that in none of the double mutants is flowering absent. The latter indicates that the respective genes do not control in a duplicated manner floral initiation as such.

When small differences between double mutants, which might be explained by differences in leakiness, are neglected, the group A mutants (*co*, *gi*, *fe*, *fha*, *ft*, and *fwa*) behave as one epistatic group, which corresponds with their physiological properties. Their relatively limited responsiveness to environmental factors is also observed in the double mutants among the group A mutants. Moreover, these mutants have a relatively high proportion of cauline leaves and a slightly reduced leaf-initiation rate compared with the group B mutants. The *fe* and *ft* mutants can be considered a subgroup within group A, based on their similar genetic behavior with most late-flowering mutants, and particularly on their synergistic interaction in combination with the *fpa* mutant. This similarity between *ft* and *fe* may be surprising because the *fe* mutant has been described as a mutant somewhat intermediate in its response to environmental factors (Martinez-Zapater and Somerville 1990; Koornneef *et al.* 1991). Martinez-Zapater and Somerville (1990) suggested that this mutant might be a leaky allele of a locus of the unresponsive (group A) class. Nevertheless, *fe* and *ft* differ in their genetic relationship with *fwa*. In contrast to the *fwa fe* double mutant, the extreme epistatic phenotype of the *fwa ft* double mutant indicates a related function for the latter genes. This is also suggested by the specific synergistic effect of *ft* and *fwa* with *leafy* (*lfy*) mutants (Ruiz-Garcia *et al.* 1997) and by the observation that both are the only late mutants not rescued when

sucrose is provided to the shoot apex in continuous darkness (Madueño *et al.* 1996). This may suggest that *FT* and *FWA* play an (additional) role at the meristem level. This different genetic behavior of *ft* and *fe* in relation to *fwa* suggests their classification in two different subgroups.

The vernalization and daylength-responsive mutants (group B) behave more variably in their genetic relations with other mutants. Within this group the two mutants with the largest effect (*fca* and *fve*) show a clear additive effect even for their increased responsiveness to vernalization (Figure 2). On the other hand, *fpa* is epistatic with *fve*, and *fca* also shows less-than-additive interaction with *fy*. Thus, it is suggested that there might be two subgroups within group B. The more-than-additive effect observed between *fca* and *fpa* and the additivity between *fca* and *fve* suggests a redundant role for *FPA* and *FCA* and would locate *FPA* upstream to *FVE*. The possible lethality of the *fpa fy* double mutant would locate *FY* upstream to *FCA*, because otherwise the double *fpa fca* also would be expected to be lethal.

Several interactions between the two groups of late-flowering mutants are very interesting and might be interpreted in terms of interactions among the different pathways controlling flowering initiation (Figure 5). One of these is the extreme lateness of the double mutants *fpa ft* and *fpa fe*. To explain these synergistic interactions, we assume that *FPA* has a function in flowering similar to *FE* and *FT*, and in this way they may replace each other; only when both are absent is flowering strongly delayed. Taking into account that this extreme lateness is not observed in the double mutants between *fpa* and the rest of the group A mutants, then *CO*, *GI*, and probably *FHA* are likely to locate downstream of *FE* and *FT*. Nevertheless, some of these loci, although affecting specifically the same pathway, might be placed as branches from the LD-promotion pathway, as suggested for *FWA*. The interactions shown between *FPA* and genes from both groups *FCA*, *FE*, and *FT* suggest that the *fpa* mutation would affect flowering time through both the LD and the constitutive promotion pathways. This would explain the reduced responsiveness to vernalization observed in the *fpa fca* double mutants, because part of the lateness might come from an effect in the LD-promotion pathway through the interaction with *FE* and *FT*, and similarly would explain the large responsiveness to vernalization shown by the *fpa fe* and *fpa ft* double mutants. A last intriguing interaction between both groups of mutants is the very late phenotype of the *fha fca* double mutant. Because this extreme interaction is not observed between *fca* and the other group A mutants, this might locate *FHA* upstream in the LD promotion or, as discussed above, might determine a branching point of this pathway.

In the present study we observed that the vernalization responsiveness is not abolished in double mutants involving responsive and nonresponsive mutants, which indicates that the group A genes are not required for the vernalization responsiveness of the group B mutants. However, vernalization does not compensate for the lateness due to the group A mutants, in agreement with the observation that a saturating vernalization treatment of wild type in SD greatly reduced flowering time but did not compensate fully for the SD delay (Lee and Amasino 1995). Taken together, this suggests that vernalization promotes flowering in the same pathway in which the group B genes act. The group A genes apparently control a flowering factor that becomes limiting when the vernalization requirement has been completely satisfied. This is in agreement with the MCDK model, which explains the increased vernalization responsiveness of group B mutants, because such genes control a pathway with an effect similar to that of a vernalization treatment. In this model the common target of both vernalization and the group B genes has been suggested to be gibberellin metabolism or sensitivity.

The model that in group A mutants the LD promotive pathway is blocked leads to the expectation that in SD the effect of the group A mutants will not be observed as was found (Figure 3). However, the observation that the *co fve* double mutant in SD is somewhat earlier than the monogenic *fve* mutant suggests that *CO*

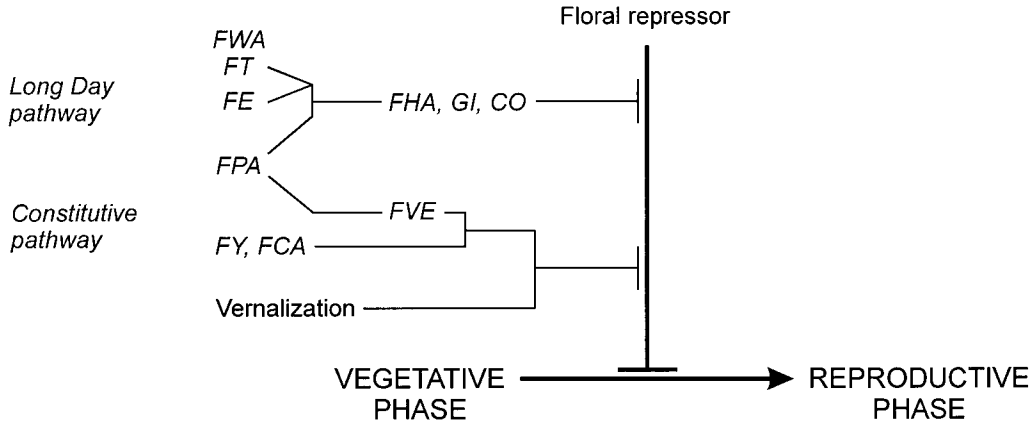

Figure 5.—A model describing the pathway of flowering affected by the flowering-time genes *CO*, *GI*, *FCA*, *FE*, *FHA*, *FPA*, *FT*, *FY*, *FWA*, and *FVE*.

not only promotes flowering under LD but somehow also promotes the inhibition by SD. A scheme summarizing the relations among the various late-flowering genes is shown in Figure 5, which can be considered a refinement of the MCDK model.

One important gene, *FLC*, was not included in the present analysis because all mutants were isolated in the L*er* genetic background which probably carries a loss-of-function allele at this locus (Sanda and Amasino 1996). The *FLC* gene has been considered an inhibitor of flowering that is normally repressed by genes such as *LD* and probably all members of the group B genes, because wild-type *FLC* makes *fca*, *fpa*, and *fve* much later but does not do so with *gi*, *fe*, *ft*, and *fwa* (Sanda and Amasino 1996). This different genetic relationship between *FLC* and the late-flowering mutants supports the classification into two groups. *FLC* has been shown to interact with the *FRI* gene, the combination of both producing extreme lateness, which is fully compensated by vernalization. The *FRI* allele behaves very similarly to the group B mutants in its response to vernalization, photoperiod, and light quality (Lee and Amasino 1995), and this has led to the placement of *FRI* in the same "general response" group of the constitutive pathway. However, because the delay due to the *FRI* allele is dominant, it is thought to control a repressor of flowering. The vernalization promotion would act downstream of *FRI* and *FLC*. However, it is also possible that vernalization regulates the expression of these genes as daylength regulates *CO* expression (Putterill *et al.* 1995).

The present genetic analysis has shown that the physiological pathways thought to control flowering promotion in Arabidopsis also interact. In the near future more genes affecting flowering time and further genetic interactions probably will show a larger complexity in the flowering gene network. Some of the genes already have been cloned (Lee *et al.* 1994a; Putterill *et al.* 1995; Macknight *et al.* 1997) and the knowledge of the flowering process will increase rapidly in the near future. It will be very interesting to integrate the genetic and molecular analyses in order to understand the genetic classifications and interactions in terms of gene function and thereby to translate a genetic model into a functional model.

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