FLOWERING IN *PISUM*: MULTIPLE ALLELES AT THE *lf* LOCUS

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SUMMARY

Two further alleles *lf^a* and *Lf^d* are proposed for the flowering gene *Lf*. The dominance order is *Lf^d* > *Lf* > *lf* > *lf^a*, with ability to delay flowering descending in that order. Homozygous *lf^a* plants can flower as early as node 5 or 6 but generalised statements regarding the flowering node for these alleles are avoided since the position depends on the status of the other major flowering loci, the background for various quantitative systems and the environmental conditions. In the present crosses heterozygotes of *lf^a* with the other alleles were always distinguishable from the *lf^a* homozygotes and segregation was therefore distinct even in long days. Parental lines 60 (*lf E Sn hr*) and 7 (*lf^a E Sn hr*) flowered less than four nodes apart but dominance of *lf* over *lf^a* was 0.69 and strong canalisation of development in this early region allowed classic Mendelian analysis of the data. A relatively strong polygenic system contributed to within-class variation of *lf^a* and *lf* plants. The degree of dominance of *Lf* over *lf^a* varied with the photoperiod and the background of internode-length genes. *Lf^d Lf* heterozygotes were not clearly distinguishable from either homozygote. The results of graft studies are in agreement with the proposal that the *Lf* alleles determine sensitivity of the apex to the flowering hormones. Type lines are appointed for the four alleles.

1. INTRODUCTION

UNDER field conditions flowering often shows continuous variation and many workers using these conditions have analysed their data by the methods of quantitative genetics (Clay, 1935; Rowlands, 1964; Watts *et al.*, 1970; Snoad and Arthur, 1973*a, b*). These workers generally conclude that flowering node and flowering time are under the control of simple, additive, polygenic systems with dominance of little importance but Rowlands also found evidence of a major gene or "effective factor" (possibly *Sn*) which delayed flowering and whose effect was increased in short days. Distinct segregations can occur under the daylength conditions operating in the field, and this paper contains examples, but the likelihood of obtaining a discrete segregation is generally improved by choosing environmental conditions which magnify the effect of a particular gene or gene combination. This technique was used by Barber (1959) and Marx (1968, 1969) and has been used in the present studies to attempt a Mendelian analysis of flowering.

The first paper in this series showed how six phenotypic classes could be recognised in a diverse group of pea varieties by the use of flowering node and flowering time data obtained under controlled photoperiod conditions (Murfet, 1971*a*). Each class was defined in relation to the behaviour of a standard variety. Four of these classes namely ED (early developing), EI (early initiating), L (late) and LHR (late high response) have now been investigated and four major genes—*Lf* (= *S₁*), *E*, *Sn* (= *S₂*) and *Hr*—have been found to determine between-class variation and to some

extent within-class variation, but there is also evidence of polygenic systems which in contrast seem to contribute mostly to within-class variation but occasionally to between-class variation as in the case of the penetrance modifiers of *Sn* (Murfet, 1971*b*, 1973*b*). Genotype *lf e sn hr* is ED; it is early (flowers about node 10) and day-neutral. Addition of *Sn* creates an L-type

TABLE 1

Distribution of node of first initiated flower for parents, F₁, F₂ and F₃ of cross 7 (lf^a E Sn hr) × 60 (lf E Sn hr). F₃ families of 12 plants were used to genotype the small F₂ and check the critical 8/9 region in the large F₂. Photoperiod 8 hours

Generation	Genotype	Node of first flower									
		6	7	8	9	10	11	12	13	14	15
Line 7	<i>lf^a lf^a</i>	1	10	1	—	—	—	—	—	—	—
Line 60	<i>lf lf</i>	—	—	—	—	10	9	3	—	—	—
F ₁	<i>lf lf^a</i>	—	—	—	—	5	9	—	—	—	—
small F ₂ *	— —	1	9	9	2	8	28	8	—	—	—
F ₂ †	<i>lf^a lf^a</i>	1	9	9	—	—	—	—	—	—	—
F ₂ †	<i>lf lf^a</i>	—	—	—	2	8	22	4	—	—	—
F ₂ †	<i>lf lf</i>	—	—	—	—	—	6	4	—	—	—
F ₃	<i>lf^a lf^a</i>	19	178	28	1	—	—	—	—	—	—
F ₃	— —	5	70	38	36	133	90	43	12	3	—
F ₃	<i>lf lf</i>	—	—	—	1	27	37	27	13	1	1
large F ₂ *	— —	—	27	24	13	72	69	16	3	—	—
F ₃	<i>lf^a lf^a</i>	1	74	21	—	—	—	—	—	—	—
F ₃	— —	2	30	5	24	62	6	—	—	—	—
F ₂ +F ₃ ‡	— —	8	136	76	75	275	193	67	15	3	—

* All F₂ plants flowering at node 9 segregated in F₃.

† Degree of dominance of *lf* over *lf^a* = 0.69.

‡ Total *lf/lf^a* seg. 628/220 (χ^2_1 0.40), cutting between nodes 8 and 9.

which is quantitatively delayed (5-15) nodes) by short days but which is also a few nodes later in long days than the standard ED line if the seed is buried. Gene *E* is epistatic to *Sn* in terms of flowering node and genotype *lf E Sn hr* is EI; the node of first initiated flower is early and day-neutral but the flowering time is delayed in short days as a result of retarded development or abortion of the lower flower buds. *Lf* is epistatic to *E* and genotype *Lf E Sn hr* is again L-type. Genes *Hr* and *Sn* interact strongly and genotype *lf e Sn Hr* is LHR; it flowers with the L plants in long days but is very extensively delayed (25-50 nodes) in warm short day conditions. Genotypes *Lf e sn hr*, *lf E sn hr* and *lf e sn Hr* are basically ED but *Hr* confers

TABLE 2
Distribution of node of first initiated flower for the parents, F_1 , F_2 and F_3 of cross 7×2

Generation	Genotype	Photo- period	Node of first flower																											
			5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Line 7	$lf^a lf^a$	18	—	5	12	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Line 7	—	8	—	2	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Line 2	$Lf Lf$	18	—	—	—	—	—	—	—	—	—	—	—	4	9	4	3	—	—	—	—	—	—	—	—	—	—	—	—	—
Line 2	—	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	2	7	5	1	1	—	—	—	—
$7 \times 2 F_1$	$Lf lf^a$	18	—	—	—	—	—	—	—	—	1	1	12	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_1	—	8	—	—	—	—	—	—	—	—	—	1	0	0	2	1	1	8	1	1	—	—	—	—	—	—	—	—	—	—
F_2	—	18	1	1	9	7	—	—	—	1	13	15	20	10	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2	—	8	—	1	15	22	—	—	—	4	16	14	19	8	—	4	5	17	11	16	6	9	3	2	—	—	—	—	—	—
F_2^*	$lf^a lf^a$	8	—	—	6	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_2^*	$Lf lf^a$	8	—	—	—	—	—	—	—	2	5	6	4	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—
F_2^*	$Lf Lf$	8	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	2	2	2	2	1	1	—	—	—	—	—	—
F_3	$lf^a lf^a$	9	—	31	46	5	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
F_3	—	9	—	17	44	3	—	—	—	6	11	18	10	3	4	6	4	6	19	13	18	11	8	6	1	6	3	—	—	—
F_3	$Lf Lf$	9	—	—	—	—	—	—	—	—	—	—	1	0	1	—	—	—	1	9	16	19	25	17	10	8	12	10	5	2
slender $F_3^\dagger$	—	9	—	—	5	—	—	—	—	—	—	—	1	—	—	—	—	1	0	2	4	3	2	0	1	2	1	—	—	—
dwarf $F_3^\dagger$	—	9	—	7	12	2	—	—	—	2	4	3	6	—	—	2	0	1	5	7	6	6	3	1	0	1	—	—	—	—

* A sample of the short day F_2 was genotyped by growing 8 F_3 seeds from very-early segregates and 12 F_3 seeds from intermediate and late segregates.

† Grouped data from several F_3 families segregating for both Lf/lf^a and the slender/dwarf length difference.

EI tendencies (Murfet, 1973a) and *Lf* raises the flowering node particularly where assisted by a system of polygenic modifiers (Murfet, unpublished). In the present work the two remaining phenotypic classes, namely the extreme types VEI (very early initiating) and VL (very late) are brought into the genetic analysis. VEI plants are some three to four nodes earlier than the standard EI line and VL plants show the extensive flowering delay in short days characteristic of LHR plants but are also five to eight nodes later in long days than the standard L and LHR lines.

The findings from this series differ widely from those in the quantitative papers in that dominance and interaction are very marked and substantial transgressive segregation was reported in some crosses (*e.g.* Murfet, 1971b,

TABLE 3

Distribution of node of first initiated flower for the parents, F₁ and F₂ of cross 24 (a Lf e Sn hr m) × 16 (A Lf^d E Sn Hr M). Photoperiod 18 hours

Generation	Genotype	Node of first flower											$\bar{x}$	S.E.	n
		15	16	17	18	19	20	21	22	23	24	25			
Line 24	<i>Lf</i>	—	9	2	1	—	—	—	—	—	—	—	—	—	—
Line 16	<i>Lf^a</i>	—	—	—	—	—	—	—	1	1	8	1	—	—	—
F ₁	<i>Lf^a Lf</i>	—	—	—	—	1	2	6	6	4	—	—	—	—	—
F ₂	— —	2	12	12	22	34	21	19	10	5	9	3	—	—	—
F ₂	<i>A M</i>	—	1	3	4	19	17	15	8	5	9	2	20.6	0.2	83
F ₂	<i>A m</i>	1	1	1	11	13	3	2	—	—	—	1	18.8	0.3	33
F ₂	<i>a M</i>	—	6	4	5	2	—	2	1	—	—	—	17.8	0.4	20
F ₂	<i>a m</i>	1	4	4	2	—	1	—	1	—	—	—	17.3	0.5	13

The strong linkage of genes *Lf* and *Hr* with the pigment genes *A* and *M* respectively allows partial recognition of the effects of the flowering genes in this continuous F₂.

Differences: *a m*—*A m* 1.49, *t* = 2.50*; *a M*—*A M* 2.83, *t* = 6.11***

a m—*a M* 0.50, *t* = 0.75; *A m*—*A M* 1.84, *t* = 4.67***.

table 8). However, the different results are possibly reconcilable on the grounds that the relative contribution of each gene or gene system and properties such as dominance, interaction and transgression are all dependent on the genetic and environmental circumstances operating. Whether a gene shows clear-cut segregation or is obscured in a quantitative system is therefore a function of circumstance.

2. MATERIALS AND METHODS

Five of the varieties used here have already been investigated (Murfet, 1971b). They are lines 60, *lf E Sn hr*, EI; 22, *lf E/e sn hr*, ED; 2, *Lf E Sn hr*, L; 24, *Lf e Sn hr*, L; and 63, *lf e Sn Hr*, LHR. Lines 60, 24 and 63 are the standard varieties for their respective classes. Line 22 is a bulk selection from cultivar Massey with heterogeneity at the *E* locus. The two new varieties brought into the crossing programme are line 7 (Lamm L7, Nilsson L54, Vilmorin's "Acacia") phenotype VEI and line 16 (believed to be Wellensiek's "Dominant" line) phenotype VL.

The crosses reported are 7 × 60, 7 × 2, 7 × 16 and 24 × 16. Data on parents, F₁ and reference varieties in tables 1-4 come from plants grown simultaneously with the F₂. In addition the physiological action of the

TABLE 4

Distribution of node of first initiated flower for parents, F_{13} , F_2 and F_8 of the cross between lines 7 (11^a E Sn hr) and 16 (11^a E Sn Hr). The standard lines 24 (late—11 e Sn hr) and 63 (late high response—11 e Sn Hr) are included as controls

Variety or cross	Photo- period	Node of first flower																					
		6	7	8	11	14	17	20	23	26	29	32	35	38	41	44	47	50	53	56	59	62	63
Line 7	16	10																					
Line 7	8	12																					
Line 16	16									4	8												
Line 16	8																			4	6	1	
7 × 16 F ₁	16									4	1												
F ₁	8																1	2					
F ₂	16	22								37	11												
F ₂	8	26								1	4	2	1	1	1		3	5	12	24	13	4	
F ₃	8									2													
Line 24	16									10	6	2											
Line 24	8																						
Line 63	16																						
Line 63	8									2	6	4											
F ₃	8	157																	1	0	1	4	2
F ₃ ^m	8	9								1	4	1	1	1				1	0	2	1		
F ₃ M	8	17															3	4	12	22	12	4	

flowering genes was investigated by making reciprocal grafts between lines 7, 22, 24 and 16 using the technique described by Murfet (1971*c*). Data are taken from main shoots only and laterals were regularly excised. Nodes are counted from the first scale leaf as node 1. Flowering node refers to the first node at which a flower bud was initiated irrespective of subsequent development. Since all early segregates in these crosses were of an EI or VEI nature, separation into phenotypic classes can be made without reference to flowering time. The seed marbling gene *M* is sometimes obscured in *a* plants but it was recognisable in crosses 7×16 and 24×16 by a distinct "ghost" marbling. The gene for long internodes (*Le*) contributed by line 16 leads to some handling difficulties in the controlled

TABLE 5
Segregation and linkage data for the flowering loci Lf and Hr and marker genes A and M.
All crosses are in coupling.

Cross	Photo-period	Phenotype Observed numbers				Total	Chi-squared			CrO%	S.E.
							Seg. 1	Seg. 2	Joint		
7×60	8	<i>A lf</i> 42	<i>A lf^a</i> 4	<i>a lf</i> 4	<i>a lf^a</i> 15	65	0.6	0.6	40.0***	12.4	4.4
7×2	18	<i>A Lf</i> 59	<i>A lf^a</i> 4	<i>a Lf</i> 2	<i>a lf^a</i> 14	79	1.0	0.2	39.2***	8.0	3.2
7×2	8	129	10	5	28	172	3.1	0.8	72.9***	9.4	2.4
7×16	16	<i>A Lf^d</i> 70	<i>A lf^a</i> 5	<i>a Lf^d</i> 9	<i>a lf^a</i> 17	101	0.1	0.6	36.0***	14.8	3.9
7×16	8	71	7	4	19	101	0.3	0.1	48.1***	11.3	3.4
$16 \times 24^\dagger$	18	<i>A Lf^d</i> 94	<i>A Lf</i> 22	<i>a Lf^d</i> 7	<i>a Lf</i> 26	149	0.7	4.1*	42.1***	18.4	3.6
7×16	8	<i>M Hr</i> 57	<i>M hr</i> 0	<i>m Hr</i> 4	<i>m hr</i> 14	75	0.1	1.6	43.3***	5.8	2.8

† Continuous F_2 arbitrarily cut at nodes 18/19; genotypes unsubstantiated.

photoperiod facility, particularly in short days where the delayed flowering may result in plants over 6 metres tall. Height difficulties were avoided in cross 7×16 by growing only *le* segregates and switching the short day plants to long days after the elapse of three months. Single, double and triple asterisks against χ^2 and other statistics indicate probabilities less than 0.05, 0.01 and 0.001 respectively. Other experimental details are given in previous papers.

3. RESULTS

(i) *The allele lf^a*

Flowering node data for cross 7×60 are given in table 1. The parental varieties flower on average little more than three nodes apart and the F_2 is apparently continuous. Nevertheless the flowering node distribution is

strongly bimodal and the full F_3 analysis shows conclusively that this is a distinct monohybrid segregation determined by alleles of the major flowering gene *Lf*. Referring to the region 6-8 as very early and 9-15 as early the F_1 classifies early and the F_2 segregates into 19 very early and 46 early plants which fits closely a 1 : 3 ratio ($\chi^2_1 = 0.62$). The 19 very early F_2 plants all bred true in F_3 . (Note one of the 226 offspring flowered at 9.) Of the early F_2 plants 10 bred true and the remaining 36 gave rise to both very early and early segregates in the proportion of 113/317, numbers which are in good accord with a 1 : 3 ratio ($\chi^2_1 = 0.37$). The F_2 could therefore be partitioned into 19 recessive, 36 heterozygous and 10 dominant plants which fits a 1 : 2 : 1 ratio ($\chi^2_2 = 3.25$). As a further check on the sharpness of the discontinuity between nodes 8 and 9 a larger F_2 was grown. The F_2 plants flowering at node 8 always bred true (17 progenies checked), whereas F_2 plants flowering at node 9 invariably segregated both very early and early plants (13 progenies checked). Many hundreds of plants of the parental varieties have also been grown; line 7 never flowered later than node 8 and line 60 never earlier than node 9. It is apparent that development is strongly canalised in the region of nodes 8 and 9 and that the transition across this zone requires a change in major gene state, at least with the type of genetic background present in cross 7×60 .

The flowering genotype of line 7 may be deduced as follows, bearing in mind that L and LHR segregates are likely to flower above nodes 17 and 40-45 respectively under the short day conditions used for crosses 7×60 and 7×2 (the standard lines 24 [L] and 63 [LHR] occur in table 4). The absence of L plants in cross 7×60 (*lf E Sn hr*) at once shows that line 7 carries *E* (genotype *lf e Sn hr* is L) and a recessive form of *Lf* (genotype *Lf E Sn hr* is L). In addition, line 7 must carry *Sn* since line 53 (*lf e Sn hr*) was obtained from cross 7×22 (*sn*) and *hr* since no LHR plants (genotype *Lf E Sn Hr* is LHR) appeared in cross 2 (*Lf E Sn hr*) $\times$ 7 (table 2). Assumption of a multiple allele at the *Lf* locus recessive to allele *lf* will explain both the monohybrid segregation for flowering node in cross 7×60 and the lack of independence between this segregation and segregation for the anthocyanin gene *A*. The cross-over value of 12 per cent (table 5) is characteristic of the linkage between the *A* and *Lf* loci. Using symbol *lf^a* to represent the allele for very early flowering, the genotype of line 7 may be written as *lf^a E Sn hr*. Additional supporting evidence comes from cross 7×2 (*Lf E Sn hr*) where a discrete monohybrid segregation occurs for flowering node (*e.g.* the long day data in table 2) and a strong linkage is again apparent between the flowering locus and the *A* locus (table 5).

(ii) The allele *Lf^a*

Lines 24 and 16 are the standard lines for the L and VL classes respectively. The lines themselves flower quite separately in long days but their F_1 is somewhat intermediate in flowering node and the F_2 is continuous and essentially unimodal (table 3). The cross data therefore require rejection of class VL since the flowering difference in long days is not a suitable basis for distinguishing a discrete class. The cardinal property of line 16 is a very high response to photoperiod (table 4) which places it in class LHR. The continuous F_2 in cross 24 (*Lf e Sn hr a m*) $\times$ 16 (*A M*) shows little indication of major gene segregation. However, if the flowering node

distribution is partitioned according to the pigmentation classes $A M$, $A m$, $a M$ and $a m$, it is apparent that most of the white flowered F_2 plants (a) correspond to line 24 whereas most of the purple flowered plants (A) correspond with the F_1 or line 16. If the F_2 is arbitrarily cut between nodes 18 and 19, a 2×2 contingency analysis shows that the flowering node distribution is not independent of segregation at the A locus (table 5, $\chi^2_1 = 42.1***$). This effect is revealed in another way by the significant difference between the mean flowering node of the a and A segregates (see foot of table 3). These results can be explained by line 16 possessing a very late allele at the Lf locus, for which symbol Lf^d is proposed. Further supporting evidence is given in subsequent sections.

A full genotype of $Lf^d E Sn Hr$ is tentatively suggested for line 16 on the basis of the evidence below. With Lf^d epistatic to E , the combination $Sn Hr$ will account for the very large response to photoperiod in this line. The short day F_2 of cross 7×16 segregates clearly into 26 VEI, 14 L and 61 LHR plants (table 4). These numbers are in excellent agreement with the expected $4 : 3 : 9$ ratio ($\chi^2_2 = 1.62$) if the two recombinant genotypes $lf^a E Sn Hr$ and $Lf^d E Sn hr$ classify VEI and L respectively. The segregation VEI/L + LHR represents the lf^a/Lf^d pair of alleles and the characteristic linkage is again observed with the A locus (table 5). The segregation L/LHR represents the hr/Hr pair of alleles and the tight linkage with the M locus (tables 4 and 5) is consistent with the reported linkage of these loci (Murfet, 1973a). The $M Hr$ linkage is also visible in cross 24×16 (table 3), where the $A M$ segregates are significantly later than the $A m$ segregates ($t_{114} = 4.67***$). This difference indicates a significant interaction between the Lf^d allele and the combination $Sn Hr$ under long days which in turn supports the view that lines 16 and 24 carry distinctly different Lf alleles since no such interaction is visible in the white flowered segregates (compare classes $a M$ and $a m$ in table 3). Finally, the presence of E in line 16 is indicated since preliminary studies (Murfet, unpublished) suggest that plants of genotype $lf^a e Sn hr$ are likely to flower in the gap between the VEI and L segregates of cross 7×16 (table 4).

(iii) Dominance relationships of lf^a , lf , Lf and Lf^d

A dominance order of $Lf^d > Lf > lf > lf^a$ is shown and some evidence obtained on the degree of dominance, which is seen to vary with the environment and genetic background in some cases. The degree of dominance of lf over lf^a is 0.69 calculated from the genotyped F_2 of cross 7×60 (table 1). The environment is probably of little consequence here since the flowering node of these genotypes ($lf/lf^a E Sn hr$) is particularly stable. On the other hand, the degree of dominance of Lf over lf^a on background $E Sn hr$ is markedly influenced by the environment in cross 7×2 (table 2). The long day F_2 was not genotyped but Lf is obviously showing a high degree of dominance. In contrast dominance is a low 0.06 in short days as estimated from the sample of F_2 plants genotyped by growing F_3 . The flowering node distribution has broken up in short days to form three separate groups; very early, intermediate and late. F_3 samples show that the very early group consists exclusively of homozygous $lf^a lf^a$ plants whereas the intermediate group is largely made up of heterozygotes and the late group largely of homozygous $Lf Lf$ plants. The heterozygotes have in general

failed to respond to photoperiod. These results for cross 7×2 and alleles Lf/lf^a contrast sharply with those for cross 7×16 (table 4), where the Lf^d/lf^a heterozygotes responded to photoperiod, never flowered earlier than node 19 and where dominance of Lf^d over lf^a is obviously high in either long or short days and with or without *Hr*. Lack of data on F_2 genotypes in cross 24×16 (table 3) again prevents precise calculation but dominance of Lf^d over Lf appears to be fairly low under these circumstances.

(iv) *Quantitative systems*

Fig. 1 contains some very definite evidence of polygenic influence in cross 7×60 . The heterozygous F_2 plants ($lf/lf^a E Sn hr$) have been taken and the mean flowering node of the VEI and EI segregates in each F_3

FIG. 1.—Regression of mean flowering node for early and very-early segregates in F_3 families from heterozygous F_2 plants in cross 7×60 plotted against the flowering node of the F_2 parent.

progeny plotted separately against the flowering node of the F_2 parent. It is clear that the node at which flowering occurs within the EI region is highly heritable and that the quantitative genes are capable of influencing flowering by as much as 1.5 nodes, which is approximately half as large as the effect of the major gene *lf* itself. The same genes are also observed to be having an effect in the VEI region but the size of the effect is diminished.

(v) *Pleiotropy*

Cross 7×2 is of the form *le La cry*^s $\times$ *le la Cry* with regard to genes controlling internode length. With an *le* background the presence of either *La* or *Cry* results in a dwarf phenotype whereas the recessive genotype *le la cry*^s results in very long internodes and a phenotype known as "slender". Approximately one-sixteenth of the F_2 were slender and a significant flower-delaying action by this combination of length genes was already apparent (table 6). Larger numbers of slenders appeared in some F_3 families and this pleiotropic action was confirmed. Perhaps the most striking feature was the near absence of slender plants in the intermediate region (nodes 12-16; see the lower portion of table 2). This suggests that slender has acted as a dominance modifier increasing the degree of dominance of *Lf* over *lf*^a in short days from near zero to a high value. This delaying action is consistent with a previous report (Murfet, 1971*b*) that slender increases the frequency of vegetative reversion in EI plants.

(vi) *The physiological action of the lf alleles*

The results of reciprocal grafts between lines 7, 22, 24 and 16 are given in table 7. The node of first flower in line 7 scions is little influenced by grafting which is not surprising since these *lf*^a apices initiate flower primordia soon after, if not before, germination. (Approximately 6 nodes are already present in pea embryos.) The flowering of line 7 shoots was also very stable and only one case of vegetative reversion occurred in 50 grafts. Johnston (unpublished) likewise found that flowering of line 7 is not delayed to any extent by cotyledon removal. The behaviour of *lf*^a *E Sn hr* shoots is

TABLE 6

Pleiotropic effect of the length genes on node of first flower in cross 7×2 in an 8-hour photoperiod

Generation	Background	Flowering node		Significance
		Dwarfs	Slenders	
F_2	<i>Lf- E Sn hr</i>	17.9	22.0	$P < 0.01$
F_3	<i>Lf- E Sn hr</i>	19.9	23.8	$P < 0.01$
F_3	<i>LfLf E Sn hr</i>	25.2	26.7	$P < 0.05$

therefore distinctly different from that of *lf E Sn hr* shoots where cotyledon removal or grafting to line 24 can cause a delay of seven to eight nodes (Murfet, 1971*c*, 1973*b*). However, these two genotypes perform in a similar manner when used as stocks.

Line 22 scions were unfortunately on the point of flowering at the time of grafting. However, the delaying action of the *Sn* stocks often caused a short period of vegetative reversion above the first flowering node. The node at which stable flowering commenced is therefore used as a statistic for these scions. The problem was compounded in the case of line 24 stocks as these were the slowest to germinate, which may explain why graft 22/24 does not show the three-node delay reported for comparable grafts Massey/Greenfeast, 59/24 and 58/24 (Paton, 1969; Murfet, 1971*c*). Stocks 7 and 16 have exerted a significant and approximately equal delaying action on scion 22 which contrasts with the wide difference in flowering node of these stocks grown as intact plants. Their behaviour as stocks for line 24 scions

(*Lf*) is again fairly similar and line 7, in spite of its very early flowering, has failed to elicit the striking promotion of flowering achieved by the recessive *sn* stock line 22. However, even line 22 proved incapable of inducing early flowering in line 16 scions. This resistance to induction appears to be conferred by *Lf^d*, since Reid (unpublished) has shown that *lf e Sn Hr* scions will flower at an early node in short days if grafted to *sn* stocks, but data on scion *Lf^d e Sn hr* would be useful here.

TABLE 7

Node of first initiated flower for reciprocal grafts between lines 7, 22, 24 and 16 performed on days 5 and 6. Photoperiod 9 hours

Stock	Scion											
	7			22*			24			16†		
	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n
7	7.1	0.1	14	11.5	0.3	13	22.9	0.3	12	47.7	1.6	7
22	6.5	0.1	15	9.9	0.2	11	12.8	0.3	15	47.3	1.3	12
24	7.3	0.2	12	10.6	0.4	14	24.9	0.2	13	49.3	1.6	9
16	7.0	0.2	9	11.6	0.8	10	25.4	0.5	8	48.0	2.4	6
Ungrafted	6.7	0.1	15	9.4	0.1	16	23.8	0.3	16	55.4	0.6	12
Genotype	<i>lf^a E Sn hr</i>			<i>lf E/e sn hr</i>			<i>Lf e Sn hr</i>			<i>Lf^d E Sn Hr</i>		

* The node at which stable flowering commenced is used since the first flower primordium was already determined in some scions at the time of grafting but vegetative reversion often followed.

† None of the line 16 shoots had initiated flowers at the time of transfer to long days (day 98).

In conclusion, these results with alleles *lf^a*, *lf*, *Lf* and *Lf^d* are consistent with the previous proposal (Murfet, 1971c), based on alleles *lf* and *Lf* only, that the *lf* locus operates in the shoot and not the cotyledons and is possibly concerned with sensitivity of the apex to the balance of flowering hormones. They also support the proposal that *Sn* controls the formation of a graft-transmissible inhibitor in the cotyledons and shoot. The earlier flowering of graft 22/24 relative to 22/7 and 22/16 does not support the proposal that *E* reduces inhibitor production in *Sn* cotyledons but this may be an aberrant result due to the maturity of the line 22 scions at the time of grafting to line 24.

4. DISCUSSION

Plants carrying genes *E*, *Sn* and *Hr* may have anything from five to over 100 vegetative nodes depending on the environment, the polygenic background and the genotype at the *Lf* locus. However, no circumstances are presently known to me, either genetic or environmental, which will cause *Lf* plants to flower before node 11 or *lf* plants before node 8 whereas *lf^a* plants flower as early as node 5. The evidence therefore points to the *Lf* series having a basic role in determining the minimum length of the vegetative period.

Two multiple allelic systems concerned with flowering have previously been proposed in *Pisum*. Wellensiek (1961, 1964, 1969, 1972a, b) has

reported three alleles No^h , No^m and no conditioning flowering at a high, medium and low node respectively. No^h shows no linkage with A but weak linkage with Fa which would place it in group IV. The no series therefore appears unrelated to the present case. Pellew (1940) provisionally represented as alleles for the first time genes L , l_1 and l_2 , renamed Li by Blixt (1969), determining flowering at the 18th, 15th and 9th nodes respectively. From Pellew's brief remarks it seems quite likely that she was working with the lf locus and possibly alleles Lf^d , Lf and lf . The symbol lf (White, 1917) has priority.

Very early flowering peas have been investigated by a number of workers. Lamprecht (1956) proposed two genes ib and iba determining *inflorentia basalis* with the earliest plants having as little as two sterile nodes. However, the flowering node of these forms may not be any earlier than the earliest lf^a segregates (four sterile nodes) if the present scoring techniques are employed. For example, Lamprecht reported difficulty recognising the lowest nodes, which may not have been counted, and he certainly scored laterals, which often have a flowering node lower than the main shoot. No linkage data are available for ib and iba but it is of interest that the Acacia line was an ancestor of Lamprecht's cross and the lf^a allele may possibly have contributed to the very early flowering of some segregates. Recessive mutants 46 and pra with a flowering node similar to lf^a plants were obtained by Gottschalk (1960) and Monti and Scarascia Mugnozza (1967, 1970) respectively. Mutant pra also shows linkage with A but the figure of 33 per cent is somewhat higher than the values reported here for lf^a .

The number of major loci concerned with flowering may not be as large as the multiplicity of gene symbols suggests. It would be desirable to draw together the body of genetic information on flowering and attempt resolution of the question of gene relationships by crossing. Such a programme would be facilitated if we had as reference points pure lines designated as types for proposed genes. Lines 65E, 59, 53 and 64 are therefore named as type lines for the dominants Lf , E , Sn and Hr respectively; line 58 covers the recessives lf , e , sn and hr ; line 7 lf^a and line 16 Lf^d . Line 60 ($lf E Sn hr$) is also useful as a test line.

Testing of the relationships between the flowering genes is rendered difficult by the non-availability of some reference material, the frequent need for controlled environments and by the complex gene interactions. For example, the phenotypic similarity of two parents and their offspring is not sufficient proof that the parents are genetically identical; e.g., $lf E sn hr \times lf e sn hr$ or $Lf e Sn hr \times lf e Sn hr$. It is best to attempt positive identification of the genetic complement by crossing to several key lines. Another problem concerns the relationship between the results of Mendelian analyses and biometrical analyses like those of Snoad and Arthur (1973a, b). These workers have assumed that genes Lf , E , Sn and Hr are not of consequence in long days but there is evidence to the contrary. The present results show that the lf locus can determine a qualitative difference under long days (tables 2 and 4) and the effect of Hr is quantitative in cross 24×16 (table 3) but detectable with the aid of a tightly linked marker, M . The Sn gene is certainly active and effective in buried cotyledons even if the shoot is in continuous light (Murfet and Reid, 1974), and cross 64×53 provides an example of a very obvious Sn segregation under a 24-hour photoperiod (Murfet, 1973a, table 1, row 25). By this result the modifier

E also becomes of significance and cross 60 × 53 provides an example of an obvious *E* segregation in long days (Murfet, 1975). Although the major genes may not be identifiable *per se* in the field they would be expected in many circumstances to exert at least a quantitative influence.

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