FLOWERING IN *PISUM*: RECIPROCAL GRAFTS BETWEEN KNOWN GENOTYPES

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Abstract

The action of the flowering genes *Lf*, *E*, and *Sn* was studied under short days by reciprocal grafting between young seedlings of six pure lines of peas having genotypes *Lf E Sn*, *Lf e Sn*, *lf E Sn*, *lf e Sn*, *lf E sn*, and *lf e sn*.

Grafting caused changes in flowering node ranging from a 17-node promotion to an 8-node delay. The early and late classes were fully distinct and grafting caused a between-class change in seven of the 36 graft types. A significant within-class response occurred in a number of the remaining grafts.

It is proposed that *Sn* produces a flower inhibitor in the cotyledons and shoot (favoured by short days), *E* lowers the level of inhibitor in the cotyledons, and *Lf* increases apical sensitivity to inhibitor. There is also evidence of a cotyledonary flower promotor.

A scheme of flowering control is developed for peas, including the concept that flowering is determined by the balance between promotor and inhibitor.

I. INTRODUCTION

The physiology of flowering in *Pisum* was recently reviewed by Haupt (1969). The present experiment was designed to follow up work reported by Murfet (1971*a*, 1971*b*) where it was shown that several different flowering types may be recognized in peas under short days by observing both node of first initiated flower and time of open flower. Three distinct classes—ED (early developing), EI (early initiating), and L (late)—were subjected to genetic analysis. Under short days ED plants flowered early in node and time, EI plants early in node but late in time, and L plants late in both node and time. Between-class differences were determined by three dominant genes, S_1, *E*, and S_2. It was suggested (Murfet 1971*b*) that the historic symbols *Lf* and *Sn* be redefined to take on respectively the meaning attached to S_1 and S_2, and *Lf* and *Sn* are used here in the redefined sense. Genotype *lf e sn* is ED. Addition of *Sn* gives an L-type. *E* is epistatic to *Sn* in terms of flowering node and *lf E Sn* is EI. *Lf* is epistatic to *E* and *Lf E Sn* is L. *Lf e Sn* is also L. Genotypes *Lf E sn*, *Lf e sn*, and *lf E sn* are essentially ED although *Lf* may cause some delay in flowering node with a concomitant increase in flowering time.

Sn has several pleiotropic effects. It opposes flower initiation, floral development, and senescence thereby increasing height and yield. *Sn* also confers the ability to respond to photoperiod. *Lf* and *E* seem mainly concerned with the regulation of flowering node. Although *E* cancels the effect of *Sn* on flowering node in *lf E Sn* plants, *Sn* manifests its presence by suppressing development of the lower flower buds under short days and by causing a short period of vegetative reversion in a percentage of the plants.

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A speculative theory of gene action was developed from the information made available by the genetic program. It was proposed that *Sn* produces in the cotyledons and shoot a substance which opposes flower initiation, floral development, and senescence, that *E* lowers the level of flower inhibitor in the cotyledons, that *Lf* increases the sensitivity of the apex to inhibitor, that short days favour the production of inhibitor in the shoot, and that the level of *Sn* product falls inevitably with aging either through diminution of *Sn* activity or destruction of its product. The present experiment was designed to test this theory, in particular the site of gene action, by reciprocally grafting at an early age, stocks (with cotyledons) and scions of known genotype.

II. Materials and Methods

The plants were grown in a glasshouse in a 1:1 (by vol.) mixture of 6·4-mm dolerite chips and vermiculite. Nutrient in the form of a modified Hoagland's solution was supplied once per week. Night temperatures were not less than 14°C and day temperatures rarely exceeded 28°C. A photoperiod of 8 hr of natural light was used throughout the experiment.

Data were recorded from main shoots only and laterals were regularly removed. Flowering node was taken as the first node at which a flower bud was initiated irrespective of whether or not the bud developed into a mature flower. Nodes were counted from the cotyledons as zero. In some cases the first flowering was transient giving way to a second vegetative phase. Plants were grown on until it was clear that they had entered a stable flowering state and data were recorded on any vegetative reversion.

The six pure lines used for reciprocal grafting are as follows:

Line	*59*	*58*	*60*	*53*	*24*	*2*
Genotype	*lf E sn*	*lf e sn*	*lf E Sn*	*lf e Sn*	*Lf e Sn*	*Lf E Sn*
Phenotype	ED	ED	EI	L	L	L

Unfortunately pure lines of genotype *Lf e sn* and *Lf E sn* were not available for this experiment. Six lines give rise to 42 treatments—36 grafts and six ungrafted controls.

Further information on the genetics and history of these lines may be found in Murfet (1971*a*, 1971*b*). However, some remarks on the history of lines *24* and *59* should be given here. These pure lines were derived by several generations of single plant selection from the commercial pea cultivars Greenfeast (late dwarf) and Massey (early dwarf) respectively. These commercial cultivars have been used extensively in Australia for research on flowering but unfortunately there is some doubt as to their genotypes. The batch of commercial Massey from which line *59* was originally selected proved to be heterogeneous for alleles *E* and *e*. In addition it is understood that wilt resistance was bred into Greenfeast around 1960 and line *24* is derived from stocks obtained prior to this time. Rowlands (1964) also suspected heterogeneity in several commercial varieties used in his crosses. There are therefore doubts as to the consistency and purity of commercial cultivars and this makes difficult the comparison of results from workers using apparently identical material. The different genotypes within cv. Massey are phenotypically indistinguishable but they may not react the same way to all experimental treatments. It would seem desirable that physiological experiments are carried out with genetically known pure varieties.

The grafting procedure was as follows. The seeds were set to germinate at room temperature in wet vermiculite. The grafts were made at 4 days when the plumules were still crooked and some 8–16 mm long. For the stock the shoot was decapitated just below the first scale leaf. A small rubber band made from bicycle-valve rubber was slipped over the cut top of the stock and the epicotyl slit down the middle by a sharp scalpel. For the scion the epicotyl was cut off just above the cotyledons, cut into a wedge shape with a sharp razor-blade, and wedged into the stock. A firm union occurred within 24 hr and most of the grafts were growing vigorously within a week. The cotyledonary axils were checked at regular intervals and any lateral shoots excised.

Some grafts took 2–4 weeks to show appreciable growth and then seldom attained a state of vigorous growth. Such grafts, referred to here as *slow* grafts, have been analysed separately as their flowering behaviour is sometimes substantially different from that of their *vigorous* counterparts.

A few grafts failed altogether. In particular the red-flowered varieties lines *2* and *60* made very poor stocks. Flowering in early varieties like line *59* seems to be determined within 7 days from the start of germination and the aim was to start and complete the grafts on the fourth day.

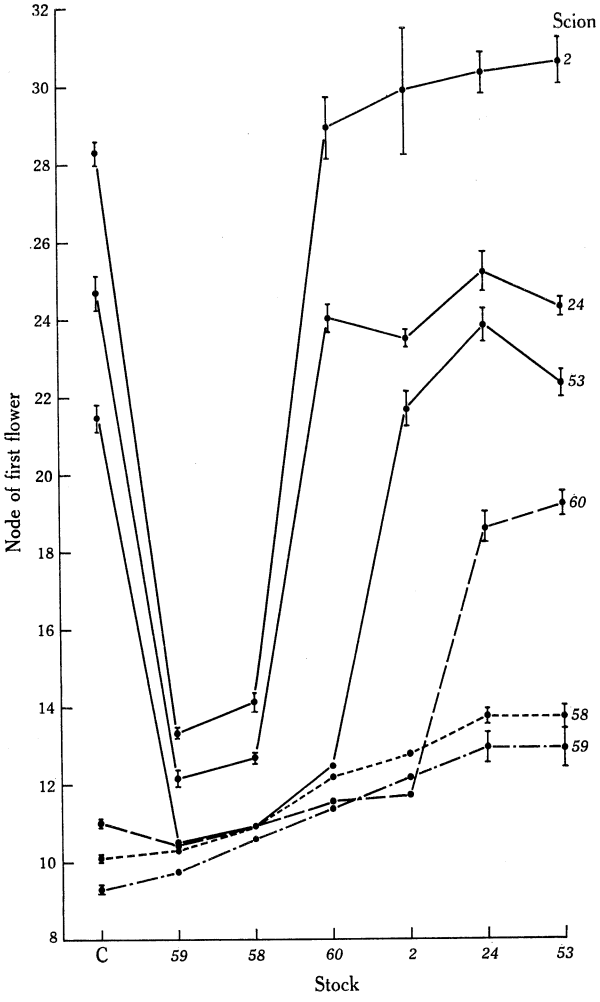

Fig. 1.—Node of first flower for reciprocal grafts between lines *59*, *58*, *60*, *2*, *24*, and *53*, with genotypes as given in the tabulation on the opposite page. C, control (ungrafted). Any standard errors not shown and sample sizes may be found in Table 1.

To meet this time requirement it was only possible to cope with six plants per treatment. The experiment was therefore carried out three times in order to get sufficient numbers, i.e. replicated in time and space. Results for ED scions from the first replicate are not included in the analysis as the grafts were made on the fifth day which was apparently too late in some cases for the node of first flower to be influenced. In addition the grafts of ED scions on line *2* stocks failed in the first replicate. Spare grafts were made to line *2* stocks in the second and third replicates in order to get workable numbers.

TABLE 1

DATA ON THE FLOWERING BEHAVIOUR OF RECIPROCAL GRAFTS

Plants were grown under a photoperiod of 8 hr. *59/58* indicates a scion of line *59* grafted to a stock of line *58*. Genotypes of the lines used are as follows: line *59*, *lf E sn*; line *58*, *lf e sn*; line *60*, *lf E Sn*; line *53*, *lf e Sn*; line *24*, *Lf e Sn*; line *2*, *Lf E Sn*. $\bar{x}$ is the mean value of the node of first flower or the mean value of the node at which vegetative reversion occurred or at which flowering resumed. S.E. is the standard error, and *n* the number of grafts

Type of Graft	Grafts showing Vigorous Growth			Grafts showing Vigorous Growth but Vegetative Reversion: Veg. Reversion		Grafts showing Vigorous Growth but Vegetative Reversion: Resume Flowering			Grafts showing Slow Growth			No. of Grafts which Failed
	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*	
59	9·33	0·14	12	10·0		11·0		1			0	0
59/59	9·75	0·25	8					0			0	4
59/58	10·57	0·30	7					0			0	5
59/60	11·36	0·32	8	12·0	0·0	13·0	0·0	3	11·0	0·0	2	2
59/2	12·17	0·17	12	13·0	0·0	14·0	0·0	2			0	6
59/24	12·90	0·38	10	15·0		16·0		1	14·0		1	1
59/53	12·88	0·48	8	11·0		13·0		1	11·0		1	3
58	10·08	0·08	12					0			0	0
58/59	10·33	0·21	6					0	11·5	0·5	4	2
58/58	10·90	0·18	10					0	11·0	0·0	2	0
58/60	12·17	0·17	6					0			0	6
58/2	12·75	0·25	12	12·0		13·0		1	12·5	0·5	2	4
58/24	13·71	0·18	7					0	13·0	1·0	2	3
58/53	13·70	0·30	10					0	14·0		1	1
60	11·00	0·11	18					0			0	0
60/59	10·42	0·21	12	11·7	0·3	13·7	0·3	3			0	6
60/58	10·94	0·14	16	15·0		16·0		1			0	2
60/60	11·56	0·29	9					0	18·0	1·0	2	7
60/2	11·67	0·17	9					0	18·6	0·6	11	4
60/24	18·57	0·36	14					0	18·5	0·5	2	2
60/53	19·17	0·30	12					0	18·0	0·7	4	2

TABLE 1 (*Contd.*)

Type of Graft	Grafts showing Vigorous Growth			Grafts showing Vigorous Growth but Vegetative Reversion: Veg. Reversion		Resume Flowering			Grafts showing Slow Growth			No. of Grafts which Failed
	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n	
2	28·28	0·60	18					0			0	0
2/59	13·33	0·14	12	17·6	0·7	24·8	0·6	9	25·0		1	5
2/58	14·09	0·25	11	16·3	0·5	23·0	1·7	4	26·5	1·5	2	3
2/60	28·91	0·78	11					0	27·5	1·5	2	5
2/2	29·83	1·62	6					0	28·4	0·8	5	7
2/24	30·29	0·53	14					0	29·5	0·5	2	2
2/53	30·58	0·57	12					0			0	6
24	24·67	0·46	18					0			0	0
24/59	12·15	0·22	13	14·7	0·4	21·3	0·3	13	21·0		1	2
24/58	12·64	0·15	11	15·0	0·6	21·4	0·7	10	14·0		1	1
24/60	24·00	0·36	11					0	23·2	0·4	5	2
24/2	23·46	0·22	13					0	24·0	0·7	4	1
24/24	25·18	0·50	11					0	23·5	0·9	4	3
24/53	24·23	0·28	13					0	24·0	1·0	2	3
53	21·44	0·35	18					0			0	0
53/59	10·50	0·15	12	15·5	0·5	18·0	0·0	2	19·0		1	4
53/58	10·88	0·13	16	15·0		16·0		1			0	2
53/60	12·45	0·28	11	15·3	0·4	18·2	0·8	6	19·6	0·4	5	1
53/2	21·64	0·45	11					0	21·5	1·5	2	4
53/24	23·80	0·42	10					0	21·4	0·9	5	3
53/53	23·30	0·34	10					0	20·5	1·5	2	6
Atypical vigorous grafts												
2/58	26·0	0·0	2									
24/59	21·0	0·0	2									
24/58	21·6	0·8	5									
53/60	19·0	0·0	1									
53/2	13·0	0·0	1									

III. Results and Discussion

(*a*) *Clear-cut Results*

A number of major points are apparent from the results given in Figure 1 and Table 1.

The distribution of node of first flower is discontinuous with a gap between 14·09 and 18·57. This gap separates the plants into two discrete classes, early and late. A between-class change (compare *24/24* with *24/59*) is therefore referred to as a qualitative response. A within-class change (compare *59/59* with *59/24*) is referred to as a quantitative response.

The response of slow grafts is often qualitatively different from that of vigorous grafts of the same type (e.g. *2/59* or *53/59*). Vigorous and slow grafts are therefore analysed separately.

Vigorous self-grafts in all cases flower slightly later than ungrafted controls. They never show a qualitative response and the quantitative differences are relatively small compared to those shown by some cross-grafts (compare *58*, *58/58*, and *58/53*). However, slow self-grafts may show a qualitative response (see *60/60*).

Scions with *Sn* have the potential to flower in either the late or the early class and in several instances show a qualitative response to grafting, changing from late to early and vice-versa (compare *24/24* with *24/58* or *60/60* with *60/53*). These cases provide striking examples of cotyledonary influence on flower initiation. With *Sn* scions as many as three types of behaviour may be observed with a single graft type. For example, with vigorous grafts of type *24/58* one plant commenced stable flowering at an early node, 10 plants flowered transiently at an early node then reverted to a second vegetative phase before entering, at a late node, a stable flowering state, and five plants showed no qualitative response to grafting because they commenced flowering at a late node. With this graft type the most common reaction was to flower at an early node, even if transiently in some cases, and data on these plants are given in the body of Table 1 and in Figure 1. Data for plants showing the less common reaction are given at the foot of Table 1 under the heading of "atypical" plants. Although the first and last types of response are qualitatively distinct they may be recognized as the extreme members of a series in which the penultimate members show vegetative reversion for a single node or for a substantial stretch (14 nodes in one plant).

The comparative behaviour of *Lf Sn* and *lf Sn* scions may be judged from the presence or absence of a between-class response (compare *24/60* and *53/60*), the degree of vegetative reversion apparent in those scions which flower early (compare *24/59* and *53/59*), and the relative within-class position of the scions (compare *24/59* and *53/59* or *2/53* and *60/53*). All criteria indicate that *Lf* scions have a stronger tendency to maintain the vegetative state. (They may be considered more sensitive to inhibitor or less sensitive to promotor than *lf* scions.)

Scions lacking *Sn* are always early but they may show a quantitative response to grafting. For example, grafts *58/60*, *58/2*, *58/24*, and *58/53* are all significantly later than the self-graft *58/58*. These results again illustrate the influence of the cotyledons on flowering and the importance of their genotype. The means for *58/59*, *58/60*, and *58/24* are all significantly different at the 0·1% level. *e Sn* and *E Sn* cotyledons both exert a significant delaying influence although the delaying influence

of *Sn* is significantly moderated by the presence of *E*. The flower-delaying ability of the stocks, either as measured by the quantitative response of *sn* scions or the qualitative response of *Sn* scions is given by the sequence $53 = 24 > 2 \approx 60 > 58 \approx 59$. In terms of promoting ability of course the sequence is reversed.

Only *Sn* scions show a between-class response and whether or not they respond depends on the genotype of the scion at *Lf* and the genotype of the stock at *Sn* and *E*. The actual flowering node of scions which flower in the early region, i.e. the within-class position, is not influenced by the scion genotype at *Sn* (e.g. *58/58*, *60/58*, and *53/58* all flower at the same node) but it is strongly influenced by the genotype of the cotyledons at *Sn* and *E* and the scion genotype at *Lf* (*Lf* scions are always later than *lf* scions). The consistently earlier position of scion *59* relative to scion *58* also reflects a genetic difference between the scions. This is probably polygenic in nature. (It may seem that the earlier position of line *59* both as a stock and as a scion is due to the presence of *E*. However, there is as yet no evidence (Murfet 1971*a*) that genotype *lf E sn* is generally earlier than *lf e sn*.) The actual flowering node of grafts flowering in the late region is in most cases hardly influenced by the stock but it is strongly influenced by the genotype of the scion. For example, compare grafts of scion *2* on stocks *60*, *2*, *24*, and *53* with grafts of scions *2*, *24*, *53*, and *60* on stock *53*. *Lf* scions are always later than *lf* scions and there are obviously other real differences. Genetic influence on within-class variation is discussed in Murfet (1971*b*). Significant influence of the stock on flowering in the late region is apparent in certain instances. For example, with *2/59*, *2/58*, *24/59*, and *24/58*, the first flowering in atypical grafts and the second flowering in reversibly induced scions is some 4–6 nodes earlier than the *2/2* and *24/24* controls.

The regions of gene activity are perhaps best seen by an overall comparison of the behavioural patterns of the scions and stocks. Comparing the scions they are seen to form three pairs: *59* (*lf E sn*) and *58* (*lf e sn*), *60* (*lf E Sn*) and *53* (*lf e Sn*), and *2* (*Lf E Sn*) and *24* (*Lf e Sn*). The flowering behaviour of the scion is therefore independent of the genotype at *E* but dependent on the genotype at *Lf* and *Sn*. Comparing the stocks they also are seen to form three pairs: *59* (*lf E sn*) and *58* (*lf e sn*), *60* (*lf E Sn*) and *2* (*Lf E Sn*), and *24* (*Lf e Sn*) and *53* (*lf e Sn*). The behaviour of the stock is independent of the genotype at *Lf* and dependent on the genotype at *Sn* and also at the *E* locus when dominant *Sn* is present. *Sn* is therefore active in the scion and stock, *E* in the stock only, and *Lf* in the scion only. (Graft *53/2* is out of step with the general pattern as the majority of grafts are late not early. It will be argued later [Section III(*c*)(iii)] that the results for *53/2* are not as far out of step as a first impression of Figure 1 might suggest.)

These results show support for the model of gene action proposed in the Introduction but there is also evidence of a flower promotor. Further analysis of the results requires a degree of speculation and is therefore deferred at this point.

(*b*) *Development of a Model*

Kohler (1965) working with the late cultivar Alderman and the early cultivar Kleine Rheinlanderin reported a qualitative response for the Alderman/Kleine Rheinlanderin graft similar to that observed with *24/59* but he did not find any quantitative influence of the stock on flowering in the late region as observed with

24/59, *24/58*, etc. In contrast Paton and Barber (1955) and Amos and Crowden (1969) found no qualitative response but report a quantitative lowering of flowering node in the late cultivars Telephone and Greenfeast when grafted to stocks of the early cultivar Massey. The latter workers, along with other Australian workers (Barber and Paton 1952; Sprent and Barber 1957; Barber 1959; Johnston and Crowden 1967; Paton 1969), have claimed evidence for a flower inhibitor in peas. On the other hand, Kohler (1965) and Haupt (1952, 1954, 1957, 1958, 1969) have evidence of a flower promotor and favour the view that postulation of a flower inhibitor is unnecessary. Indeed most of the *Pisum* data may be interpreted solely in terms of either promotor or inhibitor and therefore permit no unequivocal conclusion.

Evidence pointing towards a flower inhibitor is as follows. Cotyledon excision caused a maximum delay with cv. Massey when performed at 4 days (Johnston and Crowden 1967). They observed in these circumstances a flowering node of 11. In contrast scion *59* (line *59* is a direct selection from cv. Massey) flowered at node 13 when grafted to stock *53* at 4 days and Paton (1969) reports a flowering node of 13 for cv. Massey scions grafted to unvernalized cv. Greenfeast stocks (probably *Lf e Sn*) under short days. These results certainly suggest that *e Sn* stocks actively delay flowering although the suggestion requires support from comparable data obtained in a single experiment. Again, the results of Sprent and Barber (1957) for cuttings of cv. Greenfeast seem best interpreted as leaching of a flower inhibitor. It may be noted that these effects are quantitative and fairly small in size. The further case for an inhibitor in peas is more a question of convenience than argument. It is convenient to assign a positive role to a dominant late gene as did Barber (1959) but as suggested by Haupt (1969) the dominant gene may be suppressing a promotor. Again, although it is convenient to consider a positive cause for the forward step vegetative to flowering, with the frequent occurrence of vegetative reversion in the grafts it is no less convenient to reverse the model and attribute the vegetative state to a high level of inhibitor. The hormonal substances controlling flowering are undoubtedly of adaptive significance to plants in their natural state and, as selection selects only for an effect, there seems no paramount reason why selection should have chosen only genes which achieve their effect through regulation of a positive stimulus.

Haupt (1958, 1969) and Kohler (1965) have advanced evidence of a transmissible flower promotor in the cotyledons of the early cultivar Kleine Rheinlanderin on the basis of cotyledon removal and graft experiments with this cultivar and the late cultivar Alderman. Their arguments are further supported by the present work. For example, the qualitative response reported by Kohler is shown in an even more striking manner by *2* scions grafted to *59* stocks which flower some 16 nodes earlier than the *2/2* controls. This response could perhaps be ascribed to the transfer of the *2* scion from an inhibitor-rich to an inhibitor-free stock. However, the single *2/59* slow graft flowers at a late node. The same phenomenon may be observed with *60/60*, *60/2*, *2/58*, *24/59*, *53/59*, and *53/60*; the great majority of the vigorous grafts are early and all of the slow grafts are late. If it is assumed that the stock cotyledons have made little contribution to the scion in slow grafts (and the growth of slow grafts is rather similar to that of decotyledonized plants) then early flowering in the vigorous grafts seems to be induced by a floral stimulus donated by the stock cotyledons. This conclusion is supported by the fact that plants of the late cv. Greenfeast

decotyledonized at an early age flower at a late node (Johnston and Crowden 1967; Amos and Crowden 1969; appropriate comparison—short day, unvernalized).

These findings lead to consideration of a model involving an interaction between promotor and inhibitor and such a model seems in line with recent findings on hormonal interaction (Galston and Davies 1969). Barber (1959) had earlier suggested a model in which promotor (ϕ = florigen) and inhibitor ($\varkappa$ = colysanthin) were interconvertible, the *Sn* gene converting ϕ to $\varkappa$ under short days but the early flowering of grafts such as *Lf Sn*/*e sn* opposes this proposal. There are two basic models for promotor–inhibitor interaction. Under the first, which may be called the "independent threshold model", flowering may take place when ϕ is present above a certain critical threshold level and the $\varkappa$ level is below another threshold. Under the second, which may be called the "balance model", flowering follows when the proportion of ϕ:$\varkappa$ exceeds a certain critical ratio. The balance model is convenient and offers at once flexibility in that there is an opportunity for genetic differences in the control of both ϕ and $\varkappa$ and yet there is also an inbuilt stability. For example, a treatment which causes a concomitant change in the level of both ϕ and $\varkappa$ may cause a change of state under the independent threshold model yet have no effect under the balance model. The usefulness of the balance model may be judged from the remaining discussion.

Finally, with any model employing a threshold it is quite conceivable that the substances concerned are never totally absent from a plant. Indeed this might almost be expected under the balance model although it is certainly not a necessary condition. Marushige and Marushige (1962) and Nitsan (1962) have results which imply that only slight changes in the balance of enzymes already operating in the bud determine the difference between the vegetative and flowering states in *Pharbitis* and that new proteins are not essential. Again the cotyledon removal experiments of Johnston and Crowden (1967), with the early cultivar Massey, suggest that some inhibitor is present in the cotyledons of this variety even though it lacks both the latening genes *Lf* and *Sn*.

In view of the preceeding remarks I propose to explain the graft results under the balance model using the scheme of gene action proposed in the Introduction (last paragraph) with the addition of the suppositions that all the pea varieties used are able to produce a flower promotor in their cotyledons and shoots, that they each have fairly similar capacities in this respect, and that a low level of inhibitor is available in recessive *sn* plants (*sn* could be a leaky mutant or inhibitor could be produced by another pathway). Although *Lf* is treated as giving increased sensitivity to inhibitor, it is assumed that under the balance model this is equivalent to considering *Lf* as giving reduced sensitivity to promotor. It may be noted that the proposed fall in *Sn* product with aging has something in common with Kohler's suggestion of autonomous initiation.

(c) *Application of the Model to the Present Results*

(i) *Grafts with Scions of Genotype* lf sn

Scions *59* and *58* have genotype *lf sn* and are expected to have a low sensitivity to inhibitor and a low capacity to produce inhibitor. Any delay which they show is therefore a measure of the amount of inhibitor contributed by the stock cotyledons.

The low capacity for inhibitor production in these scions is illustrated by the fact that they are never late in any graft combination. Even the inhibitor-rich stocks of *24* or *53* fail to cause more than a 3-node delay. (Statistically this delay is significant at the 0·1% level.) Presumably inhibitor donated by the *e Sn* cotyledons fails to maintain a balance unfavourable to flowering beyond about node 14. This supports the proposal (Murfet 1971*b*) that the vegetative condition above node 14 in intact *lf e Sn* plants is caused by inhibitor supplied through the activity of *Sn* in the shoot itself. The significantly lower level of inhibitor in *E Sn* cotyledons as compared to *e Sn* cotyledons is evident from comparisons such as *59*/*60* and *59*/*24* ($t_{16} = 3 \cdot 10$, $P < 0 \cdot 01$). However, *Sn* activity in the cotyledons is not completely counteracted by *E* because the level of inhibitor in *E Sn* cotyledons is still significantly higher than that in *E sn* or *e sn* cotyledons as seen by the comparison of *59*/*59* and *59*/*60* ($t_{14} = 3 \cdot 97$, $P < 0 \cdot 01$). Vegetative reversion in *lf sn* scions *58* and *59* is uncommon, of short duration, and occurs at a low node. It probably arises because the scion has commenced flowering before inhibitor passing through the graft union from the donor cotyledons has reached a significant level. In contrast, vegetative reversion in *Sn* scions is common, often extensive, and starts at a somewhat higher node than in *sn* scions. In this case reversion is caused by inhibitor produced in the scion itself.

Although the present results give no indication as to why *sn* plants are vegetative for the first 8 or 9 nodes the results of Johnston and Crowden (1967) suggest under the balance scheme that the vegetative state is caused by the small quantity of inhibitor being mobilized more rapidly than the promotor.

(ii) *Grafts of* Sn *Scions on* e sn *Stocks*

These grafts concern scions *60*, *53*, *24*, and *2* on stocks *59* and *58*. The *Sn* scion tissue has the capacity to produce inhibitor and *e sn* stocks have abundant promotor and very little inhibitor. At the time of grafting (4 days) the balance in the scion is against flowering. Whilst the scion is small the balance at the apex is largely determined by the contributions of the stock. Abundant supplies of promotor fairly soon turn the balance in favour of flowering and flower initiation follows at a low node. However, as the scion grows, influence of the shoot tissue increases whilst influence of the cotyledons diminishes. Sufficient inhibitor may be produced in the shoot, which is under short days, to switch the balance against flowering and vegetative reversion takes place, often at node 15 or 16. As the shoot continues to grow and age, the level of inhibitor inevitably falls, the balance again turns in favour of flowering, and a stable flowering state is entered. Using *2*/*59* as an example, the second flowering phase starts a few nodes lower than the first flowering of both vigorous and slow *2*/*2* grafts. The explanation may be that in *2*/*59* a smaller total quantity of inhibitor has entered the system and in slow *2*/*2* a lower quantity of both inhibitor and promotor. A higher apical sensitivity to inhibitor in *Lf* scions will explain the difference in behaviour between *Lf* and *lf* scions. All vigorous *lf Sn*/*e sn* grafts flowered early whilst 16% of *Lf Sn*/*e sn* grafts were late. Again *Lf* scions initiate slightly later than *lf* scions and vegetative reversion is both more common and extensive. Grafts *53*/*58* and *60*/*58* flowered at exactly the same node as *58*/*58*. Presumably the presence of *Sn* in the scion has had no influence on the position of the first flower in *lf* scions

because they have initiated flowers whilst still very small. The presence of *Sn* in the scion is of course later betrayed by vegetative reversion in many cases and by the retarded development of the lower flower buds under short days. The effect of *Sn* on the node of the first flower in the *Lf Sn* scions cannot be gauged as grafts of type *Lf sn/e sn* are not available.

On the surface the few vigorous grafts of type *Sn/e sn* which failed to flower at a low node differ qualitatively from the majority behaviour for this type of graft. However, the internal situation in these plants may have been fairly close to that obtaining in those scions which flowered transiently at a low node. In the second instance the critical balance or threshold was transiently exceeded and in the first case it was never quite attained. Presumably the levels of the substances underlying the flowering reaction have varied in a continuous manner and the threshold nature of the phenomenon has permitted a small difference in level to cause a qualitative change in bud morphogenesis. By contrast, although *Sn/e sn* slow grafts are qualitatively similar to those vigorous grafts which failed to flower at a low node, the internal situations may have been substantially different at the time nodes 12–16 were being laid down. With slow grafts the stock cotyledons are assumed to make little contribution to the scion so that the apical balance is determined largely by the activity of genes in the scion itself. The presence of *Sn* in the scion has therefore led to a balance strongly against flowering. With *Sn* scions only one slow graft out of 63 flowered in the early region.

(iii) *Grafts of* Sn *Scions on* E Sn *Stocks*

The *Lf Sn* scions in grafts *2/60*, *2/2*, *24/60*, and *24/2* have a high sensitivity to inhibitor and the balance is held against flowering in the early stages by inhibitor donated in moderate levels by the *E Sn* stocks and later by inhibitor produced in the *Sn* scions themselves. In the case of the *lf Sn* scions *60* and *53* with their low sensitivity to inhibitor, the moderate supplies of inhibitor from *E Sn* stocks are insufficient to maintain the balance against flowering but inhibitor production in the *Sn* scion sometimes causes vegetative reversion.

The majority of *53/2* grafts are out of step with the other *lf Sn/E Sn* grafts. This situation is perhaps not as conflicting as it may first appear from a glance at Figure 1. One graft flowered early and that by itself is an event worthy of note as both graft partners are late varieties. In addition it was argued from a consideration of vegetative reversion that the balance is only just on the flowering side of the threshold in intact *lf E Sn* plants (Murfet 1971*b*) and the three factors discussed below would each tend to tip the balance against flowering. Firstly, the stock cotyledons are providing most of the floral stimulus. Any inefficiency in the graft will permit the scion tissue to exert a larger influence on the course of events and *53* scions having *Sn* will favour the vegetative state. Line *2* undoubtedly made the worst stock and vigorous *53/2* grafts were on the average less vigorous than *53/60* grafts. Secondly, *53* scions are somewhat later than *60* scions (compare *53/24* with *60/24*). Lastly, *2* cotyledons may possess slightly more inhibitor than *60* cotyledons (the difference is just significant at the 5% level in the case of *59/60* versus *59/2*). It seems likely that with 100% graft efficiency *53/2* would flower early as there is no

evidence so far that minor gene variation can cause genotype *lf E Sn* to flower in the late class but such plants would have been extremely difficult to recognize in cross 57 (Murfet 1971*b*).

(iv) *Grafts of* Sn *Scions on* e Sn *Stocks*

Finally, with grafts of the type *Lf Sn*/*e Sn* or *lf Sn*/*e Sn* sufficient inhibitor is donated by the inhibitor-rich stock to keep even the low sensitivity *lf* scions vegetative until this role is taken over by inhibitor produced in the *Sn* scions themselves.

(*d*) *General Discussion*

The balance model and scheme of gene action proposed here will permit a new interpretation of some previous results and a reconciliation between opposing points of view in some areas of controversy. For example, Johnston and Crowden (1967) have concluded that colysanthin (flower inhibitor) and photoperiod have an independent effect. This conclusion follows if the cotyledons are assumed to be the sole source of inhibitor but their data on decotyledonized Greenfeast may also be interpreted as supporting the present suggestion that *Sn* is active in the shoot as well as the cotyledons, the level of inhibitor being influenced by the photoperiod. Again, Amos and Crowden (1969) have proposed that vernalization has two separate effects but with the present scheme it is possible to explain their data in terms of a single vernalization effect, namely a reduction of *Sn* activity in the cotyledons and the shoot. Paton (1969) supports the view that inhibitor synthesis in the cotyledons is repressed by vernalization but he also proposes from the cv. Greenfeast (probably *Lf e Sn*) transfer experiment that vernalization conditions the apex in some way, which leads to the following speculation. If gene *Lf* increases apical sensitivity to inhibitor are there two vernalization effects: one achieved by a general reduction in *Sn* activity and the second achieved by a reduction in the effect of *Lf*.

Previous results in general support the present proposals. For example, the results of Barber (1959) on the reversal of photoperiodic induction in the late cultivar Zelka support the view that inhibitor production may occur in the shoot as well as the cotyledons and the results of Sprent (1966) for defoliation in peas support the view that it is the balance rather than the absolute level of substances which determines flowering. The present scheme will also explain the data of Haupt (1958, 1969) and Kohler (1965) and it removes the need to postulate the two types of flower initiation which they propose ("induced" and "autonomous"). Their results with the cultivars Alderman and Kleine Rheinlanderin certainly support their proposal for a promotor in early cotyledons which is absent in late cultivars. On the other hand, the results of the Australian workers with cultivars Greenfeast, Telephone, and Massey favour the idea of an inhibitor in late cultivars which is absent or almost absent in early varieties. These conflicting views are reconciled by the present proposals and the basic cause of the different results revealed as a matter of genotype. Clearly the early cultivars Massey and Kleine Rheinlanderin behave differently and are almost certainly genetically different. Embryos of the latter cultivar are sensitive to photoperiod and may flower as late as node 17 under short days (Kohler 1965, p. 443). Massey embryos are insensitive to photoperiod and do not flower later than node 11 (Johnston and Crowden 1967). Kleine Rheinlanderin seems more analogous to line

60 than lines *58* or *59* but a genotype for this cultivar cannot be assumed. The studies reported in Murfet (1971*a*, 1971*b*) do not cover all the genetic variation in *Pisum* and, in addition, although two cultivars are physiologically similar under certain conditions they are not necessarily genetically identical. For example, lines *58* and *59* show similar behaviour under a wide range of conditions and give similar graft results but their genotypic difference at the *E* locus is strikingly apparent if they are crossed to line *53* and the hybrids grown under short days. Clearly consideration of both genetical and physiological information is of mutual advantage in a study of the flowering problem.

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