PHYSIOLOGICAL GENETICS OF *PISUM* II.

THE GENETICS OF PHOTOPERIODISM AND VERNALISATION

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1. INTRODUCTION

THE genetics of photoperiodism or vernalisation has been investigated in several different species of plants (Lang, 1948). Rarely has it been possible to investigate the inheritance of both types of behaviour in the one series of crosses. This has meant that the relationship between the two patterns of behaviour has had to be worked out solely by the methods of experimental physiology. The physiologists' approach has led to the idea of a phasic development in those plants showing both types of behaviour. This idea was expressed in its crudest form by Lysenko (Whyte, 1948) and has been developed, particularly by Gregory (1948) working on cereals and Melchers and Lang (1948) using *Hyoscyamus*, into the concept that a period of growth at low temperatures (vernalisation) is necessary to provide the substrate ("vernalin") from which the flowering hormone ("florigen") is manufactured under the correct photoperiod.

If this idea of a chain of reactions each link of which is sensitive to a different environmental factor, and in which the whole chain leads to the synthesis of a positive flowering hormone, is of universal validity, it should be possible to isolate genes controlling the separate reactions. In different species allelic genes or polygenic systems are known controlling the following differences :—(a) vernalisation required : vernalisation not required ; (b) long-day : photoperiodically indeterminate ; (c) short-day : photoperiodically indeterminate. Segregation of genes controlling other possible differences in patterns of behaviour has not been realised nor has it been possible accurately to study genetic segregation for both vernalisation and photoperiodism in the one species. Attempts to isolate genes controlling both types of differences have been made in sugar beet (Owen *et al.*, 1940) and subterranean clover (Aitken, 1955, Davern *et al.*, 1957). The curious situation has arisen in both plants that segregation affecting differences in flowering behaviour affects both reactions simultaneously. Although analysis in both species is incomplete, the difficulty in separating the two reactions genetically might lead one to deduce that both types of difference in behaviour are functions of the one pair of alleles or of the one polygenic system. This situation is, perhaps, unexpected on the Gregory-Melchers scheme.

There are also indications at the physiological level that the idea of phasic development is not of universal application. Aitken, in particular, has described in subterranean clover a situation which is

best described as a competition between cold requirement and light, prior vernalisation reducing the photoperiodic response. This pattern of behaviour may be widespread although the experimental evidence is seldom sufficient to prove it (*e.g.* Owen's "photothermal" control in sugar beet, Thompson's (1953) horticultural studies on radishes, cabbages, celery, etc.).

There is another point at issue. It has become the fashion in some circles to discuss flowering almost entirely as though its control was by way of the formation of stimulating substances at definite periods during the development of the plant. Gregory mentions in passing that perhaps we should more often think in terms of removal of inhibitors. Hamner (1948) also discusses this general problem but decides there is little need to postulate inhibitors. Harder (1948) and von Denffer (1950) have advocated the view that inhibitors are active in the control of flowering. Von Denffer has suggested in this connection that we may have to add another function to the already numerous functional roles of auxin.

There is little conclusive evidence either way. There is good experimental evidence to show that a small region of the plant may be given a treatment which then makes the treated region capable of inducing flowers in remote parts of the plant. The induction of a single leaf by photoperiod or the grafting in of a vernalised apex are examples of this action-at-a-distance. It is natural to explain such a situation by postulating that the region inducing flowering is the source of a stimulator which is then transported to the apical regions which respond by the production of flowers. However, the experimental facts can often equally well be described in terms of the regions capable of inducing flowering acting as "sinks" for the destruction of hormonal substances inhibiting flowering. It seems time that some formal word to describe this functional class of substance be introduced. I therefore suggest that hormonal substances with the property of delaying or inhibiting the production of flowers be referred to as "*colysanthins*".*

It is impossible to determine how many of the presumptive "sources" of florigen or vernalin are really "sinks" for colysanthin. It is known that the interpolation of non-photo-induced tissue between an apex capable of flowering and the induced tissue, may nullify the effects of the induced tissue on the apex (Harder, 1948). This fact seems more easily interpretable on the idea of a "sink" removing a colysanthin than on the formation and subsequent destruction of a florigen. It is dangerous to rely, with Stout (1945) and Bonner and Galston (1952) on one theory of phloem transport to explain such effects.

It is, perhaps, pertinent to remark here that in their classical paper of 1938, Bonner and Hamner remark: "Two hundred and forty-six

* I am indebted to Professor J. R. Elliott for this suggestion. Colysanthin is derived from *Κωλινω* and *ἀνθος*—to delay flowers. I hesitate to suggest the abolition of the word "florigen"; but Professor Elliott feels that *anthophorin* or *anthogen* would be more comfortable words.

different kinds of extracts and extract fractions were made during the course of attempts to extract the floral initiation substance from leaves." They also tested between twenty and thirty pure compounds including vitamins, amino acids, plant acids, etc. They conclude : " There are indications, however, that if the vegetative cuttings are treated by slight modifications of the procedure here outlined, they may respond with the initiation of floral primordia to the application of a simple water extract of leaves from induced *Xanthium* plants."

An enormous effort has continued along these lines during the twenty years since publication of their paper. Florigen has still to be isolated and interest has naturally shifted to the study of ever more complex and labile fractions (*cf.* Finch and Carr, 1956, on nucleic acid fractions). Highkin (1956) has given a preliminary report suggesting that certain naturally occurring purine derivatives can accelerate flowering in peas. There have also been accounts in recent years suggesting that a variety of pure substances can accelerate flowering in the following ways :

(a) By removing the requirement for vernalisation (Lang, 1956 ; gibberellin on biennial *Hyoscyamus*, *Daucus*, etc.),

(b) By partially or wholly removing the photoperiodic requirement in long-day plants (Liverman and Lang (1956) on *Hyoscyamus* and *Silene* by indoleacetic acid ; Melchers and Lang (1942) on *Hyoscyamus* by sugars ; Langridge (1957) on *Arabidopsis*, Bunsow and Harder (1956) on *Lapsana*, Lang (1956) on *Samolus*, etc., by gibberellins),

(c) By some unspecified action on plants with a specific qualitative or quantitative vernalisation or photoperiodic requirement. (Highkin (1956) by guanosine on peas, Paton (unpublished) on peas and Wittwer *et al.* (1954) on celery by maleic hydrazide),

(d) By some unspecified action on plants with an indeterminate pattern of flowering (Clark and Kerns, 1942, van Overbeek, 1946, on pineapples, Shigeura, 1948, on the litchi, Howell and Wittwer, 1954, on the sweet potato, all of whom used indoleacetic acid).

Examples of chemical inhibition are fewer. Gibberellic acid delays flowering in early and late varieties of peas (Barber *et al.*, 1958). Harder and Bunsow (1957) have shown that gibberellic acid prevents flowering in the short-day plant *Kalanchoe* when it is applied to leaves receiving photo-inductive treatment. Indoleacetic acid will also destroy photo-induction of short-day plants under certain conditions (Bonner, 1948, on *Xanthium*). Haupt (1952, 1957) has demonstrated that yeast extract will delay flowering in excised pea embryos grown on a synthetic medium under short days. In darkness or continuous light the delay is much less.

Examples of non-chemically-defined inhibitions are more numerous, *e.g.* the non-induced leaves mentioned above ; the fact that removal of the leaves of vernalised *Hyoscyamus* will allow the plant to flower in short days (Lang and Melchers, 1943) ; Guttridge's (1956) postulation of a stimulus promoting vegetative as opposed to reproductive growth

in strawberries; Tashima's (1953) demonstration that the leaves of radish grown on a synthetic medium in short days delay flowering by 2-3 nodes as compared with total darkness or continuous light; Lockhart and Hamner's (1954) demonstration that a second dark period immediately following an inductive dark period in *Xanthium* reduces or inhibits flowering (*cf.* also Skok and Scully, 1954); Sprent and Barber's (1957) demonstration that soaking in water of young cuttings of late varieties of peas results in earlier flowering. There are few if any descriptions of experiments designed to test the inhibiting influence of grafted vegetative apices on the flowering of apices which are grown under conditions favourable to flowering.

It is still impossible to say whether any of the chemical treatments can be said to duplicate the normal action of any particular genotype in any particular environment. To answer this question it is necessary to demonstrate that the added chemical substance is a normal plant constituent which is produced by the genotype when, and only when, an environment appropriate for flowering is provided for that genotype. Proofs of this nature are possible by the ordinary methods used by the typical physiologist. They may, however, be facilitated by using as experimental material populations of plants differing by one or more genetically definable differences in pattern of flowering. Barber and Paton (1952 and 1955) by using such differences concluded that genetically late varieties of peas produced a colysanthin which could pass a graft union and delay the flowering of genetically early scions. The existence of a florigen was not excluded. The late varieties of peas are also vernalisable and photoperiodic whilst the earlies are non-vernalisable and non-photoperiodic. Further grafting experiments (Barber and Paton, unpublished) have shown that a vernalised rootstock of a late variety caused less delay in the flowering of an early scion than an unvernalsed stock and that a leafy late stock will delay flowering in an early scion under short days but not under long days.

This paper gives an account of the responses to photoperiod and to vernalisation of pure varieties, F_1 and F_2 hybrids. It has so far proved impossible to separate either genetically or physiologically the two reactions. Both reactions seem to be controlled by the one major gene, Sn , and a competitive relation exists between the two behaviour patterns. In addition to its effect on flowering the Sn gene has several other pleiotropic effects. An attempt has also been made to observe pleiotropic effects on flowering of other segregating genes, *e.g.* at the Le locus governing internode length.

2. MATERIALS AND METHODS

For the crossing programme the four varieties, Alaska (tall, early), Massey (dwarf, early), Telephone (tall, late) and Greenfeast (dwarf, late) were chosen. F_1 and F_2 seed was raised in Hobart and flown to the Earhart Laboratory, California Institute of Technology, where it was grown under controlled environments. A few tests were made using the varieties Zelka and Unica. Vernalisation treatments involved a period of three weeks at 4° C. immediately after soaking the seed.

The plumule was usually less than 1 cm. in length at the conclusion of the treatment. Photoperiodic treatment usually involved a period of 8 hours under natural illumination. This period was then supplemented by artificial fluorescent and incandescent light at 500-1000 foot-candles. All photoperiods were on a cycle of 24 hours. Temperature during growth was usually 17° C. In addition a few experiments were performed using artificial light entirely. However, at short photoperiods (8 or 12 hours) there was a tendency for loss of apical dominance making scoring impossible. Some experiments were also conducted at different growing temperatures (10° C., 17° C., 23° C.).

Cultural treatment was described in Went (1957), the plants being grown in a mixture of vermiculite and gravel and watered with a full culture solution. No nodulation occurred. In general a factorial experimental design was followed since this design allows the interactions of the different treatments to be most easily and accurately estimated. For the pure varieties twelve or sixteen replications were made. Mortality was low, the principal cause of loss being accidental decapitation. Where unequal numbers survived, plants were discarded at random to preserve the symmetry of the experiment and the analysis of variance.

Plants were scored for node at which the first flower was initiated, node of first leaf with more than two leaflets, length of stem between nodes 5 and 9, etc. The cotyledonary node was excluded. At short photoperiods and high temperatures abortion of the first flowers initiated is frequent. However, there is never any chance of confusion of an aborted flower with a vegetative axillary bud. Early abortion may result in the bracteoles of the flower hypertrophying into peculiar almost connate structures up to 0.5 cm. in diameter. Once a flower is initiated reversion to a vegetative axillary at higher nodes is very rare if the environmental cycle is maintained unchanged. Out of several thousand plants only two cases of reversion were noticed under these conditions. Photoperiodic induction is, however, reversible during the early period of development (see below).

3. RESPONSE OF PURE VARIETIES TO VERNALISATION AND PHOTOPERIOD

(a) *Node of initiation of first flower (F)*

The behaviour of the four varieties Alaska, Massey, Greenfeast and Telephone and the F_1 , Telephone $\times$ Massey, are illustrated in figs. 1 and 2 which are based on the data given in table 1. The graphs give the results of factorially designed experiments based on the combination of two temperature treatments (no vernalisation, UV, vernalisation, V) with three photoperiods (8 hours natural daylight, and 16 hours darkness, P_8 , 8 hours daylight and 4 hours artificial light and 12 hours darkness, P_{12} , and 8 hours daylight and 16 hours artificial, P_{24}). The temperature at which the plants were grown was 17° C. night and day. There were ten or twenty replications in each variety but only five for the F_1 . Error estimates are given in table 1.*

The varieties fall into two sharply separated groups. The early varieties flowering at node 9 or 10 show no response to photoperiod, Alaska also showing no response to vernalisation. Massey shows a slight but significant *negative* vernalisation, vernalised plants flowering

* Table 5 shows that there are very considerable changes in variance in the different environments, flowering becoming more variable in those environments unfavourable to it. In view of the size of environmental effects, it is clear that in most if not all cases the changes in variance would not alter the statistical significance of the effects of environmental variables and their interactions.

0.5-1 node later. The effect is highly significant statistically ($P < 0.001$). The apparent reduction in this negative vernalisation with long photoperiod is not statistically significant ($P > 0.2$). The negative

EFFECT OF PHOTOPERIOD AND VERNALISATION
ON FLOWERING NODE OF PEA VARIETIES

FIG. 1.—Graphs giving effect on node of first flower of factorially combined vernalisation and photoperiodic treatments for the varieties Massey (M) and Telephone (T) and their F_1 . Number of replications and standard errors of estimates of treatment means are given in table 1.

vernalisation of Massey is similar to the results of Leopold and Guernsey (1954) on the variety Alaska where they obtained evidence of an optimum temperature between 18 and 22° C. following short (5-day) temperature treatments. Haupt (1952) using the early variety "Kleine Rheinländerin" could obtain no response to either photoperiod or temperature.

The late varieties and the F_1 are both vernalisable and quantitative long-day plants, increasing photoperiod lowering the node of first flower apparently almost linearly in both vernalised and unvernalsed plants. For unvernalsed plants increasing the photoperiod from P_8 to P_{24} lowers the node of first flower 8-12 nodes in the different varieties. In vernalised plants this photoperiodic response is lower, being 5 to 7

INTERACTION OF PHOTOPERIOD AND VERNALISATION

FIG. 2.—Dependence of response to vernalisation on photoperiod for varieties Massey, Telephone and their F_1 and varieties Greenfeast (G) and Alaska (A).

nodes. Correspondingly the response to vernalisation is greatest at the shortest photoperiods, falling from 5 to 8 nodes at P_8 to 1 to 3 nodes at P_{24} (fig. 2). These interactions are all very highly significant ($P < 0.001$).

Similar experiments using the varieties Zelka and Unica were also performed. Zelka with factorially arranged treatments of V, UV, P_8 , P_{16} and P_{24} , is very similar in behaviour to the late varieties already discussed. Unica was tested under photoperiods of artificial light only. Growth was poor, most of the plants grown at P_8 dying before scoring was possible. Both V and P responses were smaller

than in the other varieties ($\Delta P = 0.36$ node in UV plants grown at P_{12} and P_{24} , $\Delta V = 1.54$ node at P_{12}) and there was no significant interaction of the P and V treatments.

A few experiments on the effect of temperature at which the plants were grown were also carried out using the varieties Massey, Telephone and Unica. Temperatures chosen were 10°C ., 17°C . and

TABLE 1

Flowering response of pure varieties to vernalisation and photoperiod

The table gives mean node of first flower, F, for 6 treatments, combining 2 vernalisations. (UV and V) and 3 photoperiods.

Variety	Vernalisation	Photoperiod				No. of replications	S.E. of treatment mean	Significance of PV interaction
		P_{24}	P_{16}	P_{12}	P_8			
<i>Early</i>								
Alaska .	UV	10.0	...	10.2	10.2	10	0.120	N.S.
Massey .	UV	9.1	...	9.1	9.1	10	0.111	N.S.
Alaska .	V	10.3	...	10.2	10.1	10	0.120	...
Massey .	V	9.6	...	9.8	10.0	10	0.111	...
<i>Late</i>								
Greenfeast .	UV	16.5	...	25.7	(29.4)*	10	0.321	0.001
Telephone .	UV	18.0	...	23.95	29.0	20	0.330	0.001
F ₁ T × M .	UV	17.6	...	24.0	26.0	5	0.270	0.001
Unica .	UV	18.91	19.36	19.27	died†	11	0.275	N.S.
Zelka .	UV	15.8	17.4	...	26.0	10	0.359	0.05-0.01
Greenfeast .	V	14.9	...	19.8	(22.0)*	10	0.321	...
Telephone .	V	15.8	...	20.1	21.35	20	0.330	...
F ₁ T × M .	V	15.0	...	19.8	20.8	5	0.270	...
Unica .	V	16.82	17.09	17.73	died†	11	0.275	...
Zelka .	V	13.6	14.1	...	21.7	10	0.359	...

* Only 7 and 6 plants survived to scoring date under P_8 for Greenfeast. S.E. of estimates of means are UV, P_8 , 0.719 and V, P_8 , 0.516 node.

† Unica was grown under artificial light only. Sixteen replicates planned reduced to 11 by deaths most of which were due to poor growth.

23°C . Illumination was wholly artificial and particularly at the higher temperatures and under short photoperiods growth was poor. Results for Telephone and Massey are given graphically in fig. 3. As might be expected, Massey shows only a small response. In unvernalsed plants there is a delay in flowering of about one node at 10°C . as compared with higher temperatures. The effect is statistically significant ($P < 0.01$). Prior vernalisation appears to remove the effect of growth-temperature.

Telephone and Unica show a greater response to growth-temperature. The size of the effect depends on both photoperiod and vernalisation. In both varieties prior vernalisation and long photoperiod reduce the effect of growth-temperature. In other words,

the lower the temperature of growth, the less the effect of vernalisation and of photoperiod.

The interaction of vernalisation and growth-temperature is to be

FIG. 3.—Effects of growth-temperature on node of first flower in the varieties Massey (lower curves) and Telephone (upper curves). In Massey photoperiod (P₁₂ and P₂₄) has no effect on node of first flower. The data have therefore been grouped. In Telephone growth-temperature has no significant effect in continuous light (P₂₄). In photoperiods of 12 hours, the effect of growth-temperature is reduced by prior vernalisation.

expected. It presumably means that vernalisation in peas is a continuous process not restricted to the early stages of growth although as Highkin (1956) has shown the effect of temperature is most easily detected during the early stages of development. Both reactions seem

easily interpretable along the lines suggested by Melchers and Lang (1948), one of the biochemical reactions governing flowering having a lower temperature coefficient (Q_{10}) than the reactions promoting vegetative growth. Highkin has shown that vernalisation also reduces the growth in length of the stem and that this effect is not reversible by high temperatures applied immediately after vernalisation. The effect on node of first flower is reversible under these conditions.

To test whether the photoperiod induction of flowers is reversible, plants of the varieties Telephone and Zelka were grown under a photoperiod of 8 hours at a temperature of 20° C. Batches of twelve plants were given continuous light for seven days at successive weekly intervals after germination. The results were similar in both varieties. Owing to poor survival of Telephone, only the results on Zelka are given in detail in fig. 4. It will be seen that treatment of seedlings with continuous light for the first ten days after germination has no effect on node of initiation of the first flower. At the end of the period the seedlings had one foliage leaf fully expanded with the second leaf beginning to expand. Treatment with continuous light during the next week, during which time a further two leaves expanded, gave a fully effective photoperiodic response. These plants flowered at a mean node of 16.9 whilst plants grown throughout their life in continuous light flowered at a mean node of 16.3. Later exposures to a week of continuous light lead to a gradual raising of node of first flower towards the first flowering node of 38 obtained when plants are kept throughout at P_8 .

As regards reversibility of photoperiodic induction, treatments 2, 3 and 4 all gave some plants which showed reversion to nodes producing vegetative axillary buds. In treatment 2, seven out of ten plants reverted, the mean node of reversion being 25.0. These plants produced 4.3 vegetative nodes and came into flower again at a mean node 29.3. There were one and two reversions in treatments 3 and 4. The behaviour of these plants was similar to those reverting after treatment 2 except that the node at which flowering first started was higher (node 19 after treatment 3, nodes 22 and 21 after treatment 4).

These results show that photoperiodic induction is completely reversible in late varieties of peas.

An interaction of photoperiod and vernalisation (or growth-temperature) in the sense observed in the late varieties of peas is unexpected on the Gregory-Melchers view of the relationship of these two processes. If the idea of a chain of reactions leading to the synthesis of a florigen or the removal of an inhibitor is adopted, prior vernalisation by providing the substrate for the photoperiodic reaction would be expected to enhance, not to decrease, the magnitude of the photoperiodic response. In peas the relationship between the two processes is competitive not complementary. In other words, a period of cold treatment partially replaces the necessity for exposure to long photoperiods.

How widespread this pattern of behaviour is, is still unknown. In only a few plants have the necessary experiments been done. The same pattern undoubtedly exists in *Trifolium subterraneum*, *Medicago* and other pasture legumes (Aitken, 1955). It probably exists in sugar

FIG. 4.—Diagram showing reversibility of photoperiodic induction. Plants grown in P_8 except for 1 week's continuous light. Abscissa gives time in days from start of germination to beginning of week's treatment with continuous light. Shaded area shows conditions and nodes where flowering occurs in all plants. Unshaded area outside curve gives non-flowering region. Unshaded area within curve gives mean behaviour of those plants which reverted to vegetative growth.

beet (*Beta vulgaris*, Owen *et al.*, 1940) and other related chenopods. If one reads between the lines of the many accounts of the experiments on vegetables reviewed by Thompson (1953), the same pattern may exist in Umbelliferæ, *e.g.*, celery, carrot, Cruciferæ, *e.g.* *Brassica*, *Raphanus*, etc.

It is, perhaps, worth discussing the possible adaptive value of this competitive (or replacement) pattern of the control of flowering as compared with the more popular and older idea of a phasic development. Phasic development assumes that a chain of reactions must occur in a set order before the plant can flower. Such a chain is obviously much less likely to be buffered against normal environmental fluctuations than is the replacement pattern. On the hypothesis of phasic development, as particularised by Melchers and Gregory, we can predict that a winter giving maximum vernalisation will give the most sensitive photoperiodic response. A warm winter may be followed by a weaker photoperiodic response. Any variation in degree of the completion of the first reaction of the chain may tend to be magnified by the subsequent reactions. Functionally, this seems a dangerous state of affairs capable of leading to an erratic control of time of flowering. The replacement pattern, on the other hand, is self-buffering. Two mechanisms have been developed by the plant either or both of which may give a time of flowering sufficiently stable for survival.

(b) *Node of formation of first leaf with more than two leaflets (C)*

The first formed foliage leaves of most varieties of peas have two large foliose stipules, two opposite pinnæ and then several pairs of opposite tendrils. Later in development a pair of tendrils is replaced by another pair of pinnæ to give a leaf with four leaflets. In some varieties a further pair of tendrils may be replaced by leaflets at nodes higher up the stem. The replacement of the tendrils is usually symmetrical but sometimes one-sided replacement does occur and there may be occasional reversion from a higher to a lower number of leaflets.

There is evidence that this pattern of leaf development is to some extent correlated with the flowering behaviour. The change to a four-leaflet leaf occurs at a higher node in the late-flowering varieties. The development of the leaf-lamina is to some extent controlled by purine derivatives (Bonner *et al.*, 1939). In view of these facts and of Highkin's suggestion that treatment with purine derivatives may control the position of the first flower, it seemed worthwhile to investigate, concurrently with flowering node, the effect of photoperiod and vernalisation on the node at which the transition from two to more than two leaflets first occurred. This node will be designated by C.

The results are given in table 2 (a). Photoperiod has apparently little if any effect on C in Telephone and Massey and their F_1 . There is a small effect which is statistically significant in Alaska and Greenfeast. In these last two varieties reducing the photoperiod from P_{24} to P_{12} delays the production of leaves with more than two leaflets by 0.5-1 node. Vernalisation significantly alters C in all varieties except Alaska. In the late varieties, vernalisation reduces C by 1-2

nodes. In Massey vernalisation increases C. There is, thus, a parallelism in reaction to vernalisation in both flowering node and C.

TABLES 2 (a) and (b)

Vegetative responses of pure varieties to vernalisation and photoperiod

Table 2 (a) gives mean node, C, at which first leaf with more than 4 leaflets is produced in 6 treatments combining 2 vernalisations (UV and V) and 3 photoperiods. Table 2 (b) gives similar analysis for L in cm., the length of stem between nodes 5 and 9. Number of replications as in table 1. Since interactions are usually not significant, in both tables grouped means have been given.

TABLE 2 (a)

Variety	S.E. of treatment mean	Photoperiod			Significance	Vernalisation		Significance	Significance of PV interaction
		P ₂₄	P ₁₂	P ₈		UV	V		
<i>Early</i>									
Alaska .	0.156	9.10	9.50	9.45	0.05	9.37	9.33	N.S.	N.S.
Massey .	0.135	10.10	10.10	9.95	N.S.	9.90	10.20	0.01	N.S.
<i>Late</i>									
Greenfeast	0.270	11.05	12.35	(12.70)*	0.001	12.55	10.70	0.001	N.S.
Telephone	0.354	16.00	15.68	16.15	N.S.	16.27	15.62	0.05	0.05
F ₁ T × M .	0.698	12.80	14.40	14.00	N.S.	14.53	12.93	0.01	N.S.

TABLE 2 (b)

<i>Early</i>									
Alaska .	0.606	32.35	26.95	21.90	0.001	26.40	27.70	N.S.	0.001
Massey .	0.384	12.70	10.85	9.65	0.001	13.20	8.90	0.001	N.S.
<i>Late</i>									
Greenfeast	0.167	6.35	6.10	(4.96)*	N.S.	7.35	5.10	0.001	N.S.
Telephone	0.654	26.48	15.80	15.18	0.001	21.90	16.40	0.001	N.S.
F ₁ T × M .	1.342	26.00	20.50	16.80	0.001	25.80	16.40	0.001	N.S.

* Owing to poor survival of plants at P₈, full analysis of variance not possible for Greenfeast. S.E. of estimates of means of survivors under P₈ are : for C, 0.411 node and for L, 0.665 cm.

Where a reaction to photoperiod occurs it is also in the same sense as the reaction of the flowering node. The effects on C are smaller and there is not the strong competitive interaction between response to photoperiod and vernalisation. The absence of interaction has allowed the grouping of means in table 2 (a).

(c) Length of stem between nodes 5 and 9 (L)

As mentioned earlier, there have been numerous claims that auxins may affect the pattern of flowering. Gibberellic acid has been shown by several authors to affect flowering in several species of plants. Brian and Hemming (1955) have also shown that gibberellic

acid treatment will enable the recessive dwarf pea, *le*, to produce a phenocopy of the tall, *Le*. Thus, it seemed worthwhile to investigate the effects of photoperiod and vernalisation on growth in length of the stem. The distance, *L*, between node 5 and 9 was chosen because most of these nodes are laid down after germination and before flowering. The onset of flowering usually causes a sudden decrease in growth in length of internodes.

The results are shown in table 2 (*b*) which shows that both photoperiod and vernalisation have highly significant effects on length of the stem. In contrast to the effects on flowering there is no significant interaction. The effects are simply additive. For all varieties the shorter the photoperiod, the shorter the stem. There is some suggestion that plants of the *Le* phenotype are affected proportionally more than those of *le* phenotype. In Greenfeast and Massey reducing photoperiod from P_{24} to P_8 reduces stem length by 21.9 per cent. and 23.7 per cent. respectively whilst for the tall varieties, Telephone, Alaska and the F_1 of Telephone and Massey, the reductions are 42.5 per cent., 35.4 per cent. and 32.3 per cent.

Vernalisation, with the exception of Alaska, also reduces stem length. The reductions here seem to be the same whatever the genotype or phenotype with regard to the *Le* locus. They range from 25.1 per cent. to 36.0 per cent.

The data are scarcely extensive enough to show what the effect might be of substituting genes controlling late flowering for alleles controlling early flowering with regard to these growth patterns. The reactions appear to be the same in both early and late varieties. There is no explanation of the anomalous behaviour of the Alaska towards vernalisation.

4. GENETIC SEGREGATION UNDER CONTROLLED CONDITIONS

We have seen that the F_1 from Massey $\times$ Telephone behaves in all respects like the late parent Telephone, *i.e.* the late-flowering habit is dominant. Similar crosses of late $\times$ early have been made using Alaska $\times$ Telephone, Greenfeast $\times$ Massey, and Alaska $\times$ Greenfeast. In these cases the F_1 were grown only at Hobart. But in all cases the mean flowering node of the F_1 was very close to the late parent. In three of the four cases, the F_1 flowers at a slightly (0.25-0.75) lower node than the late parent grown under the same conditions. No reciprocal differences were noted.

F_2 were also raised at Hobart in the garden in summer and under the short-day conditions of the winter in a heated glasshouse with large gradients in temperature and light intensity. In neither case was a discontinuous segregation in type of flowering behaviour obtained. In summer the segregations were usually bimodal and could be interpreted as showing both a segregation of a major gene giving three late : one early and associated modifiers and polygenes (*cf.* Tedin, 1923, and Pellew, 1940). There is at least a 10 per cent. error possible in

classification solely on phenotypes. Winter sowings of smaller numbers of plants gave a continuous unimodal segregation. The difference between summer and winter sowings showed that genes controlling

FIG. 5.—Histograms showing behaviour of Massey and Telephone, their F_1 and F_2 when grown under the four environments: continuous light, unvernalised and vernalised, and photoperiod of 8 hours, unvernalised and vernalised. Number of plants in samples given in top right and left hand corners of histograms.

the development of vernalisation and photoperiodic reactions were segregating in F_2 .

In order to clarify the genetic system further, parental, F_1 and F_2 seed were sown in the constant environments of the Earhart Plant Research Laboratory, California Institute of Technology. The data are given in fig 5. and table 3. The cross Massey $\times$ Telephone gave the fullest data and will be dealt with first. In this cross the *Le : le* pair of alleles which control internode length, is also segregating. It

will be seen that discontinuous segregations with regard to node of first flower are obtained when the F_2 is grown under short days. Under

TABLE 3
Genetic segregations in F_2

Gross environment		Phenotypes					Total planted	χ^2
P	V	<i>Le, Sn</i>	<i>Le, sn</i>	<i>le, Sn</i>	<i>le, sn</i>	T		
M × T (homozygous for <i>r</i>)								
24	UV	70	30	39	5	144	144	...
12	UV	87	27	19	7	140	144	...
8	UV	80	25	23	4	132	144	...
Totals . . .	UV	237	82	81	16	416	432	4.21
24	V	(65 <i>Le</i>)		(24 <i>le</i>)		89	92	...
12	V	41	19	16	4	80	92	...
8	V	45	20	18	3	86	92	...
Totals . . .	V	86	39	34	7	166	184	3.95
Grand Totals . . .	UV + V	323	121	115	23	582	616	6.61
A × T (homozygous for <i>Le</i>)		<i>Sn, R</i>	<i>Sn, r</i>	<i>sn, R</i>	<i>sn, r</i>			
24	UV	(52 <i>R</i>)		(24 <i>r</i>)		68	76	...
12	UV	33	19	12	4	68	76	...
8	UV	33	12	13	3	61	76	$R^* \quad r^*$
Totals . . .	UV	66	31	25	7	129	152	0.30 0.88
24	V	(52 <i>R</i>)		(24 <i>r</i>)		70	76	...
12	V	32	14	18	7	71	76	...
8	V	35	14	19	7	75	76	...
Totals . . .	V	67	28	37	14	146	152	6.21† 1.56
Grand Totals . . .	UV + V	133	59	62	21	275	304	4.79† 0.07
M × G (homozygous for <i>le, r</i>)			<i>Sn</i>	<i>sn</i>				
24	UV	...	unclassifiable		...	72	72	...
12	UV	...	54	17	...	71	72	...
8	UV	...	54	14	...	68	72	...
Totals . . .	UV	...	108	31	...	139	144	0.54
24	V	...	unclassifiable		...	70	72	...
12	V	...	48	18	...	66	72	...
8	V	...	52	15	...	67	72	...
Totals . . .	V	...	100	33	...	133	144	0.00
Grand Totals . . .	UV + V	...	208	64	...	272	288	0.31

* Seeds were selected for *R* : *r* before planting. χ^2 refer to *Sn* segregation within *R* plants and *r* plants.

† Significant at $P < 0.05$.

continuous light and with unvernalsed seed a continuous bimodal distribution is obtained which, with a small chance of misclassification

(about 5 per cent.), can be interpreted as a segregation of three lates : one early. Prior vernalisation and continuous light gives an almost unimodal segregation. Under short days, whether the progeny is vernalised or not, a discontinuous segregation of three late : one early is obtained. Change of photoperiod from P_8 to P_{12} and prior vernalisation simply increases or decreases the size of the discontinuity. These treatments do not break up the late segregates into definite groups some reacting to vernalisation but not to photoperiod and *vice versa*. The main genetic difference between Massey and Telephone thus resides in a major dominant gene which apparently causes the late-flowering habit by the induction of vernalisation and photoperiodic responses. Following Wellensiek (1925) I propose to designate this locus with the symbol *Sn*.

TABLE 4
Analysis of χ^2 for Sn/sn segregation

Source of χ^2	M $\times$ T		M $\times$ G		A $\times$ T			
	d.f.	χ^2	d.f.	χ^2	uncorrected		corrected	
					d.f.	χ^2	d.f.	χ^2
Genetic deviation	1	0.02	1	0.31	1	3.94*	...	...
V treatment	1	1.09	1	0.23	1	3.74*	1	3.41
P treatment	2	0.41	1	0.60	1	0.07	1	0.07
P and V treatment	4	1.44	3	0.86	3	3.88	3	3.45

Table 4 gives analysis of χ^2 for the crosses M $\times$ T, M $\times$ G and A $\times$ T. χ^2 significant at 0.05 level shown by *. In the cross A $\times$ T there is a significant overall excess of *sn* plants with an apparent effect of V treatment. However, after correcting for the excess *sn* plants ($P = 0.302$) the effect of V treatment becomes non-significant at the 0.05 level ($P = 0.08$).

A χ^2 analysis is given in table 4. It shows that segregation at the *Sn* locus is independent of segregation at the *Le* locus. There is a slight but not significant deficiency of double recessives, *le, sn* ($\chi^2 = 6.61$, $P = 0.10$). The analysis of χ^2 can be followed further and used as a test for heterogeneity in segregation under five of the six different treatments. For the *Sn* locus this analysis is given in table 4. There are no significant χ^2 either for genetic deviation or for treatments or their interaction.

The results for the F_2 of Massey $\times$ Greenfeast and Alaska $\times$ Telephone are given in table 3. No F_1 seed was available for growth under constant environments. Under continuous light whether vernalised or not, accurate classification in late and early segregates is impossible. In the case of Greenfeast $\times$ Massey the only major locus segregating is *Sn*. Tables 3 and 4 show that segregation at this locus agrees in all respects with the segregation in Massey $\times$ Telephone F_2 . There is again no suggestion that vernalisation or change of photoperiod splits the late segregations up into groups differing in response to these

environmental changes. Similarly the χ^2 analysis gives no significant effect on the segregations of either vernalisation or photoperiod.

In the cross Alaska $\times$ Telephone, the two loci, *R* (round : wrinkled seed) and *Sn*, were segregating. There was a good 3 : 1 segregation at the *R* locus in F_2 and segregation at this locus is independent of segregation at the *Sn* locus although after vernalisation there seems to be a significant excess of *sn* plants in the *R* class. Unlike the other two F_2 , there is an overall deficiency of late segregates in this F_2 ($\chi^2 = 3.94$, $P < 0.05$). An analysis of χ^2 for heterogeneity shows that the deficiency is associated with vernalisation ($\chi^2 = 3.74$, $P < 0.05$). If the analysis of χ^2 for heterogeneity is carried out correcting for the overall deficiency of lates, the heterogeneity due to vernalisation is just non-significant at the 5 per cent. level ($\chi^2 = 3.41$, $P = 0.08$). Further work is required using F_3 progeny to decide whether the deficiency of lates after vernalisation is caused by the appearance of late segregates in F_2 which show a greater response to vernalisation than the late parent.

Table 5 gives estimates of means and variances of the distributions of flowering node in parents F_1 and F_2 . The variability of the early parents is unaffected by change of environment whilst for the late parent variability is greatly reduced by vernalisation and by long days. Similarly the variances of the early segregates in F_2 are relatively unaffected by environmental change whilst the late segregates behave very similarly to the late parent. It is noteworthy that the estimates of variance in the F_1 of Massey $\times$ Telephone, although based on only five plants per sample, are always less than the corresponding variance of the late parent. Variability in F_1 is probably only slightly greater than in the early parent. Since the F_1 is phenotypically late in all other respects, the reduction in variability as compared with the late parent is to be interpreted as an example of genetic developmental homeostasis (Lerner, 1954), the homeostasis showing itself not as a reduction in total response to environmental variables but in a more *regular* response in the heterozygote than in the homozygote. As will be seen later the *Sn* gene has pleiotropic effects on node of formation of first leaf with four leaflets and also on stem length. There is no evidence of homeostasis with regard to these effects.

A comparison of the variances of the early and late segregates with the parental variances shows that in addition to segregation at the *Sn* locus, modifiers or polygenes or both must also be segregating. Let us compare the means and variances of the early segregates with those of the early parent (table 5). In all cases the early segregates flower at a higher node than the early parent. The difference is about 2-3 nodes. The variances of the distribution of flowering node of the segregates are over ten times larger than the variances of early parent with the distributions of the segregates regularly transgressing the upper but not the lower parental limits. It is also clear that neither vernalisation nor change in photoperiod from P_8 to P_{12} alters the mean

flowering node or the variance of the early segregates. These facts show that several polygenic loci are segregating in F_2 . The polygenes cannot be considered as modifiers of the physiological system on which the *Sn* locus works. They presumably work through some physiological system of flower-control other than the system controlling the development of responses to vernalisation and photoperiod.

TABLE 5
Mean and variance of flowering node, F , in parents F_1 and F_2

	Vernalisation	Variety or Segregate	Photoperiod					
			P_{24}		P_{12}		P_8	
			$\bar{x}$	Var.	$\bar{x}$	Var.	$\bar{x}$	Var.
<i>sn</i> EARLY VARIETY OR SEGREGATE	UV	Massey . . .	9.10	0.10	9.10	0.10	9.10	0.10
		F_2 segregate $M \times T$	11.50	0.74	11.26	1.29	11.04	2.13
		Alaska . . .	10.00	0.00	10.20	0.18	10.20	0.18
		F_2 segregate $A \times T$	...	...	13.25	2.86	12.38	2.78
		F_2 segregate $M \times G$	...	...	11.18	1.28	11.50	1.18
	V	Massey . . .	9.60	0.27	9.80	0.18	10.00	0.00
		F_2 segregate $M \times T$	...	...	11.83	1.06	11.26	0.86
		Alaska . . .	10.30	0.23	10.20	0.18	10.10	0.10
		F_2 segregate $A \times T$	...	...	12.04	1.04	12.15	0.70
		F_2 segregate $M \times G$	...	...	11.39	1.31	12.20	2.16
<i>Sn</i> LATE VARIETY OR SEGREGATE	UV	Telephone . .	18.00	0.74	23.95	2.06	29.00	7.68
		$F_1 M \times T$. .	17.60	0.30	24.00	0.50	26.00	0.00
		F_2 segregate $M \times T$	16.56	1.56	23.19	10.50	26.99	21.48
		F_2 segregate $A \times T$	(15.88)	(6.95)	21.88	3.36	23.84	11.43
		Greenfeast . .	16.50	0.50	25.70	2.68	29.43	3.62
		F_2 segregate $M \times G$	(15.70)	(7.58)	25.71	16.84	27.81	17.06
	V	Telephone . .	15.80	0.27	20.10	0.94	21.35	1.40
		$F_1 M \times T$. .	15.00	0.00	19.80	0.70	20.80	0.70
		F_2 segregate $M \times T$	(13.29)	(3.73)	19.47	3.22	21.17	7.43
		F_2 segregate $A \times T$	(14.07)	(3.21)	18.63	2.15	20.16	4.01
		Greenfeast . .	14.90	0.32	19.80	0.92	22.00	2.00
		F_2 segregate $M \times G$	(13.47)	(3.58)	21.60	5.07	23.27	9.04

Figures in brackets give mean and variance of total F_2 distribution where progeny is not classifiable into *Sn* and *sn*.

If we consider the late segregates, it is clear that the means of the distributions of flowering node are affected by photoperiod and vernalisation in the same way as the means of the late parents. The distributions as a whole respond to vernalisation and change of photoperiod in the same way and to approximately the same extent as the late parent. The variances of the distributions of the late segregates are greater than the variances of the late parents, the F_2 distributions tending to transgress both upper and lower parental limits. The simplest interpretation of these facts is that we are dealing with segregation of a system of genes modifying the action of the *Sn* gene. Whether

there is also a system of polygenes, segregation of which is capable of changing the node of first flower independently of the action of the *Sn* gene, is at present impossible to demonstrate directly. However, by comparison with the distributions of the early segregates it is probable that a polygenic system independent of the system of modifiers does exist. Further tests in F_3 are required to identify and separate genetically the two systems.

These simple experiments thus afford proof that the position of first flower in these varieties depends on at least three identifiable systems of genes. Without the design of experimental tests based on detailed knowledge of the pattern of behaviour of the parental varieties, this analysis could not have been made. The analysis of parental behaviour enabled the environment which maximises the parental differences to be selected for testing the genetic segregations. This precaution seems an essential one for experiments of this kind. Thus, Davern *et al.* (1957) have attempted to analyse genetically the differences in flowering behaviour of varieties of subterranean clover without taking this precaution. They state: "By manipulation of the sowing date one should be able to obtain discrete segregations into flowering and non-flowering types superficially suggesting major gene inheritance." It is not quite clear what the word "superficially" means in this connection. They then discuss the conflicting studies on the inheritance of spring and winter habit in cereals and suggest that "This confusion could be largely explained by the theory that a continuous variable, the genetic control of cold requirement for competence to flower, underlies the two phenotype classes, spring and winter habit." There seems little doubt that, in the diploid cereal rye, segregation at a single locus of a pair of alleles determining a major requirement for vernalisation, does occur (Purvis, 1939). We could hardly expect to demonstrate the existence of this major gene by sowing an F_2 in the autumn. Spring sowings in long days are necessary to allow the effect of the major gene-difference to appear. In the hexaploid cereals, the genetic situation will undoubtedly be more complex; but experiments planned (*a*) to reduce environmental fluctuations to a minimum and (*b*) to allow the difference in pattern of behaviour best to express itself, are necessary before concluding that a continuous genetic variable for cold requirement underlies the difference between spring and winter flowering habit.

This problem is, perhaps, not so academic as might at first sight appear. A strain of subterranean clover corresponding to the early pea varieties would be a strain with some considerable agronomic potentiality. The only way to select it from a segregating F_2 would be to grow the F_2 under conditions which would prevent (or delay) the flowering of all segregates with a requirement for vernalisation or long days. Aitken's work shows the type of environment required. Study of F_2 segregation at high temperatures and in short days will probably bring to light variation in subterranean clover homologous

genetically and physiologically with that present in these varieties of peas.

5. PLEIOTROPIC EFFECTS OF THE *Sn* GENE

We have seen already that the dominant *Sn* gene has three pleiotropic effects on flowering—a delaying action and the induction of competence to respond to vernalisation and photoperiod. The data available also allow some consideration of the pleiotropic effect of this gene on growth in length of stem and on leaf pattern. The Massey $\times$ Telephone F_2 allows us to test the action of this gene on both an *Le*

TABLE 6
Pleiotropic effects on vegetative characteristics of segregation at Sn locus

Cross	Genetical background	C					L				
		UV			V		UV			V	
		P ₂₄	P ₁₂	P ₈	P ₁₂	P ₈	P ₂₄	P ₁₂	P ₈	P ₁₂	P ₈
M $\times$ T	<i>Le, Sn</i>	13.63	14.78	15.52	14.38	15.41	25.49	19.69	19.01	17.12	14.24
	<i>Le, sn</i>	11.38	11.04	10.50	11.27	10.95	27.83	25.59	26.12	19.68	18.00
	<i>le, Sn</i>	13.30	13.16	15.42	13.79	15.83	8.44*	7.20*	6.79	6.32*	4.58*
	<i>le, sn</i>	10.60	10.57	10.50	11.25	9.67	8.80	8.57	10.13	7.25	5.67
A $\times$ T	<i>Le, Sn</i>	...	13.43	13.73	11.70	12.29	...	22.16	17.16	24.26	18.51
	<i>Le, sn</i>	...	10.71	10.88	10.24	10.58	...	25.76	24.16	28.28	21.19
M $\times$ G	<i>le, Sn</i>	...	15.50	15.05	13.23	...	...	7.09	6.08	5.27*	4.99
	<i>le, sn</i>	...	10.41	10.66	10.67	10.85	...	8.41	8.50	5.78	6.03

Table 6 gives mean values of C (node of first four-leaflet leaf) and L (length in cm. of internodes 5-9) on F_2 progenies segregating for *Sn/sn* grown under six different environments.

All differences ($C_{Sn} - C_{sn}$) on both *Le* and *le* background are significant at $P < 0.001$ (*t* test).

All differences ($L_{Sn} - L_{sn}$) on *Le* background are significant at $P < 0.01$; on *le* background, unstarred differences are significant at $P < 0.02$, starred differences * are not significant at $P = 0.05$ in most cases owing to small numbers of *le, sn* plants available for comparison. In all cases $L_{Sn} < L_{sn}$.

and an *le* background. Similar comparisons are available from the Alaska $\times$ Telephone cross (*Le*) and the Massey $\times$ Greenfeast (*le*).

Table 6 gives the mean values for C and L in the three F_2 grown in those environments where classification with regard to *Sn/sn* is possible. There is no doubt that *Sn* raises the node of formation of the first leaf with four leaflets in all F_2 grown under all environments. The size of the rise varies between crosses and in the different environments. In Alaska $\times$ Telephone, substitution of *Sn* for *sn* gives a rise of under 3 nodes in unvernalsed plants in short days. In the other crosses the rise is about 5 nodes. As would be expected from the behaviour of the pure varieties, photoperiod and vernalisation have no effect in *sn* plants. In *Sn* plants, however, there is a small response to photoperiod and to vernalisation, the responses, as in the pure varieties, being smaller than those of the flowering node. Similar responses occur in both *Le* and *le* plants.

As regards *L*, the recessive *sn* plants in all comparisons available are taller than *Sn*. The effect is apparently present on both *Le* and *le* backgrounds although owing to the small numbers of double recessive plants available in the Massey $\times$ Telephone cross, only one of the differences is significant at the 0.05 level in the individual comparisons available in this F_2 . As in the pure varieties, *L* decreases with decrease in photoperiod and with prior vernalisation except in the case of Alaska $\times$ Telephone where the vernalisation response seems to be anomalous (*cf.* behaviour of Alaska).

Summing up, the dominant *Sn* allele in comparison with the *sn* allele has the following five functions: (a) it delays flowering to a higher node; it induces competence to respond to (b) photoperiod and (c) vernalisation; (d) it delays the appearance of first leaf with

TABLE 7
Effect of segregation at Le locus on node of first flower

Genetic background	UV			V	
	P ₂₄	P ₁₂	P ₈	P ₁₂	P ₈
<i>Le Sn</i>	16.41	23.42	27.81	19.63	21.29
<i>Le sn</i>	11.48	11.07	11.44	11.89	11.40
<i>le Sn</i>	16.82	21.87	23.83	19.00	20.69
<i>le sn</i>	11.60	12.00	11.00	11.50	10.33

Table 7 gives mean node of first flower, *F*, in F_2 of Massey $\times$ Telephone grown under different environments.

four leaflets and (e) it reduces growth in length of stem. Although the genetic tests for these five pleiotropic functions are not complete, there is at present no evidence that the apparent pleiotropy is due to tight linkage of several loci each with a single function.

6. MODIFYING EFFECTS OF THE *Sn* GENE OF SEGREGATION AT OTHER LOCI

We have seen that there is evidence of (1) a system of polygenes controlling node of first flower by a mechanism other than that controlled by the *Sn* gene and (2) a system of genes modifying the effect of *Sn* and presumably ineffective in the absence of *Sn*. The crosses allow us to test the effects of segregation at two other major loci on *Sn/sn*. They are the *Le* and *R* loci. Rasmussen (1935) has claimed that the *le* gene is dominant for a small delay in time to flower.

R, segregating in Alaska $\times$ Telephone, has no effect detectable in the experiments reported here on flowering node.

Table 7 shows that in the Massey $\times$ Telephone cross there is some slight evidence that *Le* on a background of *Sn* may slightly raise the node of first flower. In four out of the five comparisons possible, *Le*

plants flower later than *le*. The difference only reaches statistical significance in one case (P_{12} , UV, $P < 0.01$). On an *sn* background segregation at the *le* locus has little if any effect.

The comparisons in this cross suffer because of the great increase in variance on flowering node in the F_2 segregates. Two F_2 progenies from Telephone $\times$ Greenfeast and Alaska $\times$ Massey grown in the winter of 1954 at Hobart are available for a more precise comparison. In a progeny of 42 plants from T $\times$ G, the mean flowering node for *Le* (31 plants) was 22.84; for their 11 *le* sisters, the mean flowering node was 22.18. The difference is just significant at the 0.05 level. For the A $\times$ T cross corresponding figures are *Le* (34 plants) mean flowering node = 10.03; *le* (14 plants) mean flowering node = 9.93; the difference is not significant.

It is thus possible that the genes at the *Le* locus in addition to their major effect of stem length, are modifiers of the action of the *Sn* gene, having no effect on the *sn* allele. Further tests are being carried out to clarify this point.

7. PHYSIOLOGICAL GENETICS OF FLOWERING

It remains to attempt to correlate all these observations into a coherent scheme. Let us start our physiology from the genetical facts. The *Sn* gene is dominant for later flowering and it is dominant for competence to respond to photoperiod and vernalisation. The easiest way of looking at these facts is to suggest that the *Sn* gene provides the substrate for the photoperiodic and vernalisation reactions. If this substrate is a colysanthin, all these effects of the *Sn* gene are simply explained. Vernalisation and long days destroy colysanthin and so flowering is made earlier after cold treatment or growth in long days. This scheme enables us to predict that the vernalisation and photoperiodic responses should be competitive (as has been found) and not complementary as would be expected on the older ideas of a chain of reactions, leading to the synthesis of a positive flower hormone with an early link in the chain temperature-sensitive and a later link light-sensitive.

This simple picture enables us to explain the results of grafting and leaching experiments. The grafting experiments are of three types: (a) *sn-Sn* grafts as reported in Paton and Barber (1955) and interpreted as a transfer of a colysanthin from a late stock to an early scion; (b) the demonstration that the amount of colysanthin in a vernalised *Sn* stock is less than in an unvernalsed stock by use of *sn* scions for bio-assay; (c) the induction of a small photoperiodic response in *sn* scions grafted into leafy *Sn* stocks, the composite plants being grown in long (no delay in flowering *sn* scion) and short days (1-2 node delay in flowering) (Barber and Paton, unpublished). Similarly as shown by Sprent and Barber (1957) the earlier flowering of young *Sn* cuttings soaked in water is easily explained by the leaching out of colysanthin (or possibly its destruction during metabolism under conditions of

semi-starvation). Scions of early varieties are unchanged by the leaching.

It has so far not been possible to isolate and chemically characterise this gene-product, a colysanthin. However, recent experiments have greatly improved the possibilities of efficient bio-assay. Barber *et al.* (1958) have shown that 1 μ g. of gibberellic acid in alcoholic solution applied to the dry testa of intact seeds of the early variety Massey will cause a delay in flowering of 10-20 per cent. in terms of node of first flower. The delay is unaffected by vernalisation and photoperiod, so that although gibberellic acid is a colysanthin it cannot be chemically identical with the colysanthin produced by the *Sn* gene. However, the existence of a pleiotropic effect on stem length at the *Sn* locus, may mean that colysanthin is metabolically related to gibberellic acid in some way. Haupt (1957), using the technique of aseptic culture of embryos without cotyledons, has shown that yeast extract contains a substance capable of inducing a photoperiodic response in embryos of an early variety. Under short days the treated embryos flower at a later node than embryos grown in total darkness or in long days. Haupt attempts to explain his results on the basis of a "Blüh-hormon". They seem more easily explicable (as do the similar results of Tashima (1953) on radish) on the basis that yeast extract contains a substance which in short days acts as a precursor of a colysanthin. Further experiments with diffusates, macerates of peas, yeast, etc., and leach-waters are continuing.

There remain two further problems. The first is the question of whether flower-stimulating substances are also present in peas. This problem may be connected with the existence of a polygenic system controlling flowering by means other than the *Sn* system of inhibitors. The second problem is the wider question of the amount of variation in the genetics and physiology of control of flowering which we may expect in different species of flowering plants.

As regards the question of a "florigen", Haupt (1952) and Cruickshank (unpublished) have shown that pea embryos of early varieties, when grown without cotyledons on a medium unsupplemented with yeast extract, may flower at a higher node than intact plants. Haupt has concluded that this is evidence for the presence of a florigen. However, growth is very slow and poor and the effect may be a secondary one. There is, also, the more general argument for the presence of stimulators in the widely-held doctrine of organ-forming substances deriving from Sachs' speculations. An early variety of pea produces 5 to 7 vegetative nodes whilst growing in the pod. On germination it produces a further 3 to 5 vegetative nodes and then suddenly initiates flowers at the 8-10th node. It is still an attractive speculation to explain this pattern of development by postulating that a florigen is formed soon after germination.

We have seen that polygenes can cause a considerable variation in node of first flower in early non-photoperiodic varieties and segregates.

Extremes vary from node 6 (in an *Acacia* stock) to at least node 13 or 14. Is this variation controlled by the timing of florigen synthesis? There is scarcely any direct evidence either way. But the genetics and physiology can easily be linked up in a scheme such as is given in fig. 6 where florigen is shown as a precursor of colysanthin. It may be that vernalisation and long-day treatment act by reversing the action of the *Sn* gene. The possibilities are too numerous to specify in detail. However, further combined genetic and physiological work will undoubtedly limit the possibilities. If the relationship is as shown, with polygenes largely concerned with the synthesis and use of precursors of florigen and florigen itself a precursor of colysanthin, we can predict that the effect of the *Sn* gene will vary according to the polygenic background. With the scheme shown we would expect the photoperiod and vernalisation responses of *Sn* to be smaller when the

FIG. 6.—Scheme showing one of the many possible relationships of genes, gene-products and environment in the control of flowering in peas. π_n are precursors of florigen, ϕ , which is assumed to be a precursor of colysanthin, κ . Long days and vernalisation are assumed to reverse the action of *Sn*. The polygenic system, P_n , is assumed to regulate the supply of π_n by controlling utilisation of π_n in other metabolic processes. The modifiers of *Sn* including the *Le* locus may act by utilising some of κ or by altering the mechanism of responses to vernalisation and photoperiod.

gene is introduced into an *sn* plant flowering at a high node than when it is introduced into an *sn* flowering at a low node. It is hoped to select such stocks from F_3 progenies of early $\times$ late.

As regards the physiological fate of colysanthin during its destruction by vernalisation and by long days, it may be possible to isolate genes controlling these reactions. This would help greatly in understanding the physiology.

As regards the second problem of the variation in the genetical and physiological control of flowering within the Angiosperms, it is a natural first hypothesis to assume a single system of control (*cf.* Lang, 1952). However, the evidence is becoming overwhelming that fundamental differences exist between different species. As we have seen there is little doubt that the system in peas is based on a colysanthin; in other species of plant the evidence is almost as strong for a florigen base. Recent work has shown that the same substance (gibberellic acid) can act as a florigen (or vernalin) in plants with a

vernalisation requirement and in plants with an obligate or facultative long-day requirement. It can act as a colysanthin in short-day plants and also in facultative long-day plants. There is, thus, an almost complete variation in competence to respond to the one substance within four or five species of flowering plant.

How many other substances exist which have been used for the control of flowering in the genetically different mechanisms of different species is unknown. But it is unlikely that all the different mechanisms rely on a single substance. The control of time of flowering is a control of prime adaptive value. It is a control which must be sufficiently flexible to cope with the irregularities of seasons as well as with secular changes in climate. Evolutionary changes in control to meet the secular changes have been brought about by natural selection working on adaptively random gene-mutation and recombination. The evolutionary changes in gene-frequency must be expected to control both the chemical type of evocator and the competence (whether inhibition or stimulation) to respond to a specific evocator. As in so many adaptive responses we must expect the same end-result—the adaptive control of flowering—to have been arrived at in several genetically and chemically different ways (Barber, 1956).

8. SUMMARY

1. Early varieties of *Pisum sativum* are non-photoperiodic and non-vernalisable (or slightly negatively vernalisable). Late varieties are quantitative long-day plants with a positive reaction to vernalisation. The reactions to photoperiod and vernalisation compete with one another. Prior vernalisation reduces the photoperiodic response.

2. The interaction of genetic segregation and environment shows that the late varieties differ from the early by possessing a dominant, *Sn*, gene which causes later flowering by inducing a response to photoperiod and vernalisation. It is suggested that the *Sn* gene causes these effects by producing a flower-delaying substance (or *colysanthin*). This gene-product must be destroyed before flowering can take place. Colysanthin is preferentially destroyed at low temperatures and in long days.

3. The *Sn* gene has several other pleiotropic effects. It raises the node at which the first leaf with four leaflets is produced. It also slightly reduces growth in length of the stem, the reduction being about 10 per cent. of the reduction brought about by genes at the *Le* locus.

4. In addition to the major control by the *Sn* locus, there are two other gene systems controlling flowering. The first is a system of genes modifying the action of the *Sn* gene. The second is a system of polygenes which alter the node of first flower by a physiological mechanism other than by way of colysanthin.

5. Segregation at the *R* locus has no effect on flowering. The *Le* locus may be a modifier of *Sn*, the *Le* allele causing a slight delay in flowering when associated with the *Sn* gene.

6. There is some evidence that heterozygous *Sn, sn* plants show a more regular homeostatic response to environmental changes than the *Sn* parent.

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