# REPRODUCTIVE DEVELOPMENT IN *Pisum:* THE RÔLE OF GENES *Sn* AND *Lf*

by

Christiane DUCHÊNE, D.E.A. (Clermont Ferrand)

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## DECLARATION

To the best of my knowledge and belief, this thesis contains no material which has been submitted for the award of any other degree or diploma in any university. Nor does it contain any copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

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## LIST OF ABBREVIATIONS

DN Day neutral. The DN class is defined by Murfet (1984) and its characteristics are summarised on p. 1.

EI Early initiating. The EI class is defined by Murfet (1971a, 1984) and its characteristics are summarised on p.1.

FLR Flower leaf relativity as defined by Murfet (1984). FLR = NFD-LSF.

FLS Flower life-span. The number of days (or hours) between first opening and final closing of the standard petal (i.e., from stage 0.3 to stage 0.9 on the Maurer scale (Maurer *et al.*, 1966).

FT Flowering time. The number of days from sowing to the opening of the first flower (i.e., at NFD). A flower is considered open when the standard petal is fully expanded.

FT-TPI The time interval (in days) between flower initiation and flower opening at NFD.

L Late. The L class is defined by Murfet (1971a, 1984) and its characteristics are summarised on p. 1.

LD Long days (in this study LD = 24h in all experiments).

LS Leaf stage. The number of leaves expanded on the main stem counting from the lowest scale leaf as one. An arbitrary decimal system from 0.0 to 1.0 was used for the last expanding leaf (ves).

LSF Leaf stage at FT.

LSI Leaf stage at the time of flower initiation.

MB Maximum breadth (mm) as defined p.15 of the first developing pod.

MGR Maximum growth rate (cm $d^{-1}$) as defined p.17 for elongation of the first developing pod.

ML Maximum length (cm) as defined p. 17 of the first developing pod.

NAI Number of nodes in the apex at the time of flower initiation. The apex is the portion of stem above the last fully expanded leaf.

NFD Node of first developing flower. The number of the node on the main stem counting from the lowest scale leaf as one, at which the first flower opens.

NFI Node of flower initiation. The number of the node on the main stem counting from the lowest scale leaf as one, at which the first flower was initiated, regardless of the subsequent development of the flower primordium.

NFP Node of first pod. The number of the node on the main stem counting from the lowest scale leaf as one, at which the first pod developed.

PL Peduncle length. Distance (cm) from the leaf axil to the pedicel of the first flower in the raceme.

TFI Time to flower initiation. Number of days from sowing to initiation of the first flower primordium regardless of its subsequent development.

T95%ML Time to reach 95% of the maximum pod length, counted in days after the time of open flower.

TMB Time to reach the maximum breadth, counted in days after the time of open flower.

TMGR Time to reach the maximum growth rate, counted in days after the time of open flower.

TPI Time of floral primordium initiation. The number of days after sowing to the initiation of the floral bud for NFD.

SD Short days: (In this study, 8 to 12 hours of photoperiod depending on the experiment).

SW Final dry weight per seed (mg). For each plant, the 4 middle seeds of the first pod were weighed and averaged.

SWmax Final dry weight (mg) of the biggest seed enclosed in the first pod.

## ABSTRACT

The influence of the flowering genes *Sn/sn* and $Lf^{d}/Lf/lf/lf^{a}$ on post-initiation reproductive events in *Pisum* was examined using pure lines and two specially selected near-isogenic lines in the case of the *Sn/sn* difference and pure lines and segregating progenies in the case of the *Lf* series.

From a comparison of lines $299^{+}$ (*Sn*, early photoperiodic) and $299^{-}$ (*sn*, early day neutral) in short day conditions, it was found that gene *Sn* slowed the rate of flower bud, pod and seed development, prolonged flower life-span and increased the number of pods per raceme. *Sn* also promoted vegetative growth by delaying the onset of apical arrest and increasing the tendency to produce lateral branches. The delay in growth of the proximal pod at the first reproductive node remained even when the distal flower was excised at this and all subsequent nodes. The delay was therefore not a consequence of raceme ontogenesis. In contrast, removal of the apical bud immediately above the node bearing the first pod partly eliminated the effect of the *Sn/sn* difference on pod growth. By comparing genetically male sterile with fertile plants in a segregating progeny, it was found that flower life-span was substantially extended in the absence of fertilisation. However, when the growth of *Sn* and *sn* pollen tubes was examined *in vivo* with the aid of fluorescence microscopy, no difference in the rate of growth could be demonstrated for the two genotypes. Hence, the increased life-span in *Sn* flowers does not appear to be a consequence of slower pollen tube growth. Since short days increased the interval between flower initiation and open flower in *Sn* plants but not *sn* plants, this interval is neither independent of photoperiod nor genotype in *Pisum*.

The effect of the *Lf* series was examined on a day neutral background. The rate of flower bud development and pod growth was slightly promoted as the sequence $lf^{a}$, *lf*, *Lf* and $Lf^{d}$ was ascended, i.e., as the time to flower initiation was delayed, and the promotion in reproductive growth appears to be a consequence of the delay in time to initiation. It is suggested that the underlying metabolism is, at first, strongly geared toward vegetative growth but that it becomes more favourable to reproductive growth with the passage of time. In contrast, *Sn* appears to interfere with the metabolic pathway, probably by delaying the time at which autonomous changes in the underlying metabolism occur. *Sn* may act by controlling assimilate partitioning within the shoot system.

One of the crosses used to examine the effect of an *Lf*$^{d}$*/lf* segregation also permitted the effect of segregation for the flowering gene pair *Hr/hr* to be studied on an *Lf*$^{d}$ *sn* background for the first time. Although many of the *Lf*$^{d}$ segregates initiated their first flower at node 20 or higher, no significant effect of segregation for *Hr/hr* could be detected. It seems therefore that *sn* remains epistatic to *Hr* in these circumstances.

# CHAPTER 1 INTRODUCTION

## 1.1 The Genetic Control of Flowering

*Pisum* cultivars have been classified into 4 phenotypic classes regarding their flowering aptitudes characterised by the time at which the flower opens (FT), the position on the stem at which the first floral bud is initiated (NFI) and at which the first flower opens (NFD). The establishment of the 4 phenotypes Day neutral (DN), Early initiating (EI), Late (L) and Late high response (LHR) has been made possible by the use of controlled conditions which amplify the differences in plant response by favouring the expression of the genes conferring the response to photoperiod.

In the DN class, plants initiate their first flower as early as node 5 (Murfet, 1975a) and as late as node 28 (Murfet, 1984). NFI depends on the genetic background especially at the *Lf* locus as the ascending sequence of alleles, $lf^a$, *lf*, *Lf* and $Lf^d$ determines a minimum flowering node of 5, 8, 11 and 15, respectively (Murfet, 1975a, 1984; Yates and Murfet, 1978). Polygenic systems also contribute to variation in NFI (Murfet, 1975a; Murfet and Reid, 1974). In class DN, NFI and FT are both essentially day neutral (Murfet, 1971a,b). The day neutrality is conferred by the gene *sn* and/or *dne* (Murfet, 1971a,b, 1984). All floral primordia generally develop into a mature flower (and pod) and plants generally produce only a limited number of reproductive nodes in any photoperiod (Murfet, 1971a,b, 1982a; Reid, 1979b, 1980). The DN class includes the previously determined ED class (Murfet, 1971a,b, 1977).

In contrast, plants belonging to the classes EI, L and LHR all behave as long day plants, at least with respect to FT. All plants in these classes carry the gene combination *Sn Dne*. Plants of the EI class are day neutral with respect to NFI but FT can be slightly or greatly delayed in SD, depending on whether the plants carry *hr* or *Hr*, respectively. The NFI of the EI lines usually varies between nodes 9 to 12 but it can be extended up to node 16 (Murfet, 1971a,b). Very early lines, initiating at nodes 5 to 8 also belong to the EI class if the development of the lowest floral bud is retarded or aborted under SD (Murfet, 1971a, 1975a). In both EI and VEI lines, the number of leaves expanded prior to apical senescence is slightly or greatly increased under SD again according to whether the plants carry

gene *hr* or *Hr*, respectively (Murfet, 1971b, 1975b; Reid, 1979b). In the L class, plants flower at about node 15 under LD (Murfet, 1971a,b) but NFI is delayed by 5 to 15 nodes under SD. FT is consequently delayed in SD. Recently a line flowering at an early node (node 8) has been reported to show an L type response as the NFI was delayed by about 2 nodes under SD (Murfet, 1984). Plants of the LHR class flower at a similar node to L type plants in LD but SD can delay NFI by 25 to 50 nodes or more (Murfet, 1973a, 1975b). Thus late cultivars behave as quantitative LDP but LHR cultivars approach a near obligate LD requirement (Murfet, 1977).

The four flowering phenotypes are basically explained by the varying combinations of six major flowering genes: *Sn, Dne, Lf, E, Hr* and *Veg*. *Sn* and *Dne* jointly confer upon the plants a sensitivity to photoperiod (Barber, 1959; Murfet, 1971a,b, 1982b) probably by causing the production of a graft-transmissible inhibitor in the dark (Paton and Barber, 1955; Murfet, 1971c; Murfet and Reid, 1973). *Sn* and *Dne* are presumed to act at two different steps of the same metabolic pathway (Murfet and W.M. King, unpublished; Murfet, 1984). Thus, in this thesis, when *Sn* activity is mentioned, it is meant to infer activity of the *Sn Dne* system. The initiation of the first floral primordium is thought to be possible only when the balance between a flower promotor and inhibitor has reached a certain threshold level at the apex (Murfet, 1971c) as a consequence of the decrease of *Sn* activity with age (Murfet, 1971b; Reid, 1979a). The exact nature of the promotor and inhibitor is still unknown. *Sn* activity decreases with low temperature and increasing daylength (Murfet and Reid, 1974). However, *Sn* plants placed under LD may present a slightly higher NFI and FT than *sn* plants, possibly because of the remaining *Sn* activity in the buried cotyledons (Murfet and Reid, 1974). In addition, as the NFI and FT of some essentially DN plants can be delayed very slightly under SD, gene *sn* is thought to be a leaky mutant of *Sn* (Murfet, 1971b).

The effect of *Sn* on NFI is masked by the *E* gene in the EI cultivars. Gene *E* acts in the cotyledons where it probably reduces the level of the inhibitor which in turn leads to early flower initiation (Murfet, 1971c, 1973b). However, in SD inhibitor production by the shoot generally causes abortion or a delay in the development of the flower bud (Murfet, 1971a,b; Chapter 3). *Hr* amplifies and prolongs the effect of gene *Sn* in EI and LHR cultivars by blocking the effect of age on gene *Sn* (Murfet, 1973a; Reid,1979a). *Hr* activity is confined to the leaves and mature stem

(Reid, 1979a).

The 4 alleles at the *Lf* locus determine the length of the juvenile phase by controlling the minimum NFI (Murfet, 1975a). The alleles $Lf^d$ and *Lf* mask the effect of gene *E* probably because they delay flower initiation until cotyledonary influence is on the wane.

The recessive mutant *veg* completely prevents flowering regardless of the remaining genetic background and the promotory treatments applied to the plants (Reid and Murfet, 1984). In all the lines used for the present study, the dominant allele *Veg* is present.

It has been suggested recently (Murfet, 1984; Reid and Murfet, 1984) that flowering could occur when a specific target area of the apical meristem is triggered. The stimulus could be a hormonal factor and/or a specific balance of metabolites determined initially by *Sn* (Murfet, 1984). The *Lf* alleles would not interfere with the metabolic pathway but they are thought to control the sensitivity of the apex to the flowering signal (Murfet, 1971c, 1984). The *veg* gene appears to block the response of the apex to the flowering signal.

## 1.2 Influence of the Flowering Genes on the Post-initiation Stages of Reproductive Growth

The time of sowing throughout the year for 5 cultivars of pea ranging in maturity type from early (Alaska) to late (Mackay) was found to affect the growing period, i.e., the time from sowing to seed ripening, mainly by affecting TFI (Aitken, 1978). However, in this field study, photoperiod and temperature changed from the start to the end of the experiment and the environmental changes may mask differences between cultivars. Secondly, plant maturity may be influenced because of an effect on the duration of flower, seed or fruit development at each node and/or as a result of an increase in the number of reproductive nodes. Although these two components may be interrelated, it is necessary to distinguish between the number of reproductive nodes, which greatly influences the length of the reproductive phase, and the duration of flower and fruit development at a given reproductive node. Indeed, in photoperiodic pea cultivars, the number of reproductive nodes is increased in SD (Marx, 1968; Murfet, 1971a). *Sn* activity seems to have a direct delaying effect on apical senescence

as the effect is manifest in plants which never flower (Reid and Murfet, 1984) or from which flowers have been removed (Reid, 1980). In addition, *Sn* activity delays apical senescence indirectly by delaying flower and pod setting (Reid, 1979b, 1980; Gianfagna and Davies, 1981) since seeds may produce a senescence factor (Lockhart and Gottschall, 1961; Malik and Berrie, 1975; Wang and Woolhouse, 1982). The control of apical arrest, apical senescence and monocarpy and the role(s) of growing seeds on these events have been covered extensively in recent reviews (Leopold and Nooden, 1978; Wang and Woolhouse, 1982; Woolhouse, 1982).

Very little is known about the influence of the flowering genes on the development of the flower primordium into a mature flower and the development of the young carpel into a mature pod. Gene *Sn* was reported to slow the development of the flower primordium into a mature flower (Murfet, 1971a,b, 1977). In contrast, Berry and Aitken (1979) reported that photoperiod did not affect the duration of flower development in peas. These contrasting reports are treated in Chapter 3 where the development of the floral bud is specifically re-examined.

Some detailed studies of the rate and duration of pod and seed growth have been done in relation to the flowering genes on various cultivars of pea (Ingram, 1980; Gianfagna and Davies, 1981) which suggested that flowering genes, especially the *Sn Hr* combination, may have a pleiotropic effect on seed growth (Ingram, 1980). Indeed, the duration of seed-filling period was increased under SD for the photoperiodic line $I_2$ (*lf E Sn Dne hr*) and the cultivar Greenshaft (probably *Sn Dne*) whereas SD had little effect on the DN lines $I_1$ (*lf e sn Dne Hr*) and $I_3$ (*lf e sn Dne hr*). With respect to the growth rate of seeds, DN lines and the EI line $I_2$ (*Sn Dne hr*) were not affected by photoperiod whereas the rate for line G2 (*lf E Sn Dne Hr*) was significantly decreased under SD (Ingram, 1980). The delaying effect of SD on pod and seed growth in line G2 was also reported by Gianfagna and Davies (1981). In contrast, $F_2$ EI segregates with genotype *Sn Dne hr* (i.e., comparable to line $I_2$) showed an early delay in pod elongation (Murfet, 1984) like that reported for line G2 (Gianfagna and Davies, 1981). The behavioural characteristics of line $I_2$ (Marx, 1968; Murfet and Marx, 1976; Murfet, 1984) place it at the limit between photoperiodic and non-photoperiodic lines and this may explain the difference in results reported by Ingram (1980) and Murfet (1984). In addition, the observations were done at two different developmental (and thus chronological) stages of fruit

development as seed growth starts after the pod has finished elongating (see Section 1.3). Since *Sn* activity is supposed to decrease with time unless it is prolonged by *Hr* (Section 1.1) it is possible that, by the time seeds start to develop in line $I_2$, *Sn* activity has decreased to such an extent that seed growth is little affected. The effect of the *Sn/sn* gene difference on pod growth is investigated in Chapter 4.

Studies with other species lend support to the view that the genes which control flower initiation also influence the later developmental stages. In sorghum, four maturity genes have been identified (Quinby and Karper, 1945; Quinby, 1966, 1967). These four "flowering" loci, $Ma_1$, $Ma_2$, $Ma_3$ and $Ma_4$ also affect the response to photoperiod and temperature as in pea. Differences between genotypes in the duration of the panicle development as well as in the duration of seed-filling on the same panicle suggested that the four loci also influence the later reproductive stages (Quinby, 1972; Sorrells and Mayers, 1982). There was a significant and positive correlation between the time of initiation and the duration of seed-filling. However, two genotypes with an early phenotype showed an unexpectedly long seed-filling period given their small number of days to floral initiation. However, as pointed out by the same authors, the study was done in the field where photoperiod and temperature varied from the start to the end of the experiment so that early and late plants may not be in the same environmental conditions and the differences observed among genotypes may in fact be environmental effects. Similarly, in the field, the duration of pod-filling increased with late cultivars of soybeans (Johnson *et al.*, 1960). Daylength, which has a substantial effect on the spikelet initiation for barley, has been reported to influence the rate of spikelet development also (Cottrell, Dale and Jeffcoat, 1982). Thus, it appears that, in pea as in some other species, the development of the floral bud and of the fruit can be influenced by the genes controlling the transition between vegetative and reproductive growth, i.e., flower initiation.

## 1.3 The Growth Pattern of the Pod and its Components

In order to determine the genetic and physiological control of fruit (pod) growth, it is necessary to first establish its growth pattern. Various characteristics have been used to determine the fruit growth pattern

of pea or other leguminous plants, e.g., pod elongation (Linck, 1961; Gianfagna and Davies, 1981), pod inflation (Frank and Fehr, 1981; Gent, 1983 on soybeans), accumulation of fresh or dry matter of the pod, pericarp, seed and seed components (embryo, testa, endosperm) (Flinn, 1974; Ingram and Browning, 1979; Hedley and Ambrose, 1980) and finally changes in cell number and size (Davies, 1975; Hedley and Smith, 1985).

Considering fresh or dry weight changes over time, the growth of the pea pod can be divided into 3 phases. The first phase corresponds to the pericarp elongation stage during which the dry weight of the seeds is negligible since they have not started their main growth (Flinn, 1974). In the second phase, the pericarp inflates to its maximum as the seeds start to grow (Bisson and Jones, 1932; Flinn, 1974). Thus, the maximum pericarp size and fresh weight are reached well in advance of the maximum fruit weight. The third phase is characterised by the growth of the seeds so that pod weight increment is mainly attributable to the seed growth only (Bisson and Jones, 1932; Flinn, 1974). Seeds and pericarp maintain their fresh weight until a sharp decline is observed in the pericarp weight. McKee, Robertson and Lee (1955) interpreted this decline as the result of a translocation of starch and nitrogen from the pericarp to the seeds. However, as the weight increase in the seeds was 4 to 5 times the weight loss of the pod wall, McKee *et al.* concluded that there is a transport of metabolites from other parts of the plant. Experiments with radioactive tracers (Lowell and Lowell, 1970; Flinn and Pate, 1970; Harvey, 1973) support this view (see Section 1.5).

Carr and Skene (1961) studying the seed growth pattern in the haricot bean (*Phaseolus vulgaris*) in the glasshouse, reported that the curve of seed weight over time was a double-sigmoid. The lack of seed growth occurred early in seed development and lasted for only 2 days. Plotting the results obtained by other authors, Carr and Skene (1961) identified this lag phase for the two cultivars Telephone and Canner's Perfection. Telephone is a late cultivar (Murfet, 1977) while Canner's Perfection is a second early maturity type, i.e., almost insensitive to photoperiod with respect to NFI (Aitken, 1978). Several authors have reported a diauxic pattern of seed growth for DN cultivars (e.g., Alaska, Eeuwens and Schwabe, 1975) or for photoperiodic cultivars (e.g., line G2, Ingram and Browning, 1979). The duration and especially the timing of the lag phase varied according to the authors. However, Hedley and Ambrose (1980) were able to determine

two lag phases for both early and late cultivars which may correspond to one and the other reported lag phases. Hedley and Ambrose reported that the first lag lasted for only 2 days whereas the second lag lasted longer. By contrast, Bain and Mercer (1966) with the cultivar Victory Freezer, or Frydman, Gaskin and MacMillan (1974) with Progress No. 9 did not report a lag phase. It has to be kept in mind that the study of the seed growth pattern involves the successive removal of a certain number of pods. Therefore, the ability to recognise lag phases of short duration depends very much upon the experimental methods used (e.g., frequency and size of the samples). In addition, as pointed out by Hedley and Ambrose (1980), the seeds removed are not exactly at the same physiological stage, as seeds within a pod show a great variability in size (Linck, 1961; Hedley and Ambrose, 1981). These difficulties may explain the contradictory results found by the various authors. On the other hand, Pate and Flinn (1977) pointed out that seeds showing lag phase(s) may have been grown in environmental conditions conferring unusual growth patterns on the plants. In the present study, this aspect of seed growth is not studied in detail, precisely because of the nature of the controlled facilities (see Section 2.2.2).

Several interpretations of the lag phase(s) have been put forward. The lag phase coincided with the cessation of cell division in the embryo (Smith, 1973). In some cases it coincided with the disappearance of the endosperm (Carr and Skene, 1961; Eeuwens and Schwabe, 1975; Ingram and Browning, 1979). As the embryo had completely filled the space in the embryo sac at the time the lag phase was observable, Carr and Skene (1961) suggested that further growth of the seed would be restricted. Thus, the lag phase and the slower subsequent seed growth would correspond to a limited capacity of the embryo to expand. According to Hedley and Ambrose (1980) seed development would depend upon 3 levels of interaction in the plant: (a) the maternal parent, (b) the interaction between the various components of the seed and finally (c) the embryo genotype. As the difference in timing of lag phase occurring between genotypes appeared to be more related to the time from anthesis than any particular physiological state of the seed, the seed growth pattern would be mainly determined by the maternal parent. In addition, interactions might exist between the growth of the testa and embryo. It is also possible that competition exists between the embryo and the embryo sac for the assimilates contained in the endosperm (Hedley and Ambrose, 1981). Finally, the embryo genotype may interfere,

for example by limiting the seed size.

The lag phase has also been reported in cereals (Chevalier, 1983) and has been reported many times for fleshy fruit (see Nitsch, 1965, 1970). However, little interpretation have been put forward. The lag phase coincides with changes in hormonal content as it does in pea (Eeuwens and Schwabe, 1975; Ingram and Browning, 1979) but the causal relationship is still to be studied.

## 1.4 Genes Controlling Physical Characteristics of the Flower and Fruit

Numerous genes have been identified as controlling some physical characteristics of the inflorescence and the pod (pericarp and seed) in *Pisum*. The aim of this section is not to review all genes involved in reproductive growth but rather to give an idea of the complexity associated with pod and seed growth. The genetic control of the different traits has been reviewed by Yarnell (1962), Blixt (1972, 1977) and a list of genes in *Pisum* is given by Blixt, Marx and Murfet (1978).

The colour of seeds, mainly of the coat but also the hilum and cotyledons, is controlled by at least 27 different genes, including genes that affect the patterns appearing on the seed coat. The colour and striation patterns of the pericarp are probably controlled by about 10 different genes. The seed form, i.e., length, width and coat morphology is controlled by at least 19 genes. Eleven loci influence pod form and at least 6 genes are known to control the pericarp thickness and constitution. Various studies on seed size identified at least 3 genes as having a controlling effect. The number of flowers (and thus pods) per inflorescence is thought to be controlled by 3 different genes. As well, the length of the peduncle is reported to be under the control of 3 loci. The supposed identification of these various genes has often led to controversies, many of which are still not settled.

The above list refers only to proposed qualitative or major genes. However, many quantitative polygenic systems have also been proposed for the control of such traits. An example is given by the genetic control of the number of ovules per pod which seems to be under the control of an additive genetic system (Krarup and Davis, 1970). However, ovule number is

also correlated with the *bt* gene (Marx and Mishanec, 1967) which is responsible for the shape of the pod tip. This association could be due to linkage or it may result from a pleiotropic effect of gene *bt*. Experiments conducted on *Phaseolus vulgaris* suggest that the number of ovules per pod might be determined by the additive action of the alleles of a single major gene (Al-Murkhtar and Coyne, 1981). As well, the strong interaction with environmental conditions has made the genetic interpretation even more difficult.

Snoad (1972), from a 6 x 6 diallel cross, studied the genetic control of the number of flowers per inflorescence. Cultivars and wild strains seem to have developed different genetic systems. With domestic cultivars, the accumulation of dominant alleles results in an increased number of flowers, whereas this is a consequence of an accumulation of recessive alleles in the wild strains. Hole (1977) has shown that in cultivars which have 1 or 2 flowers per peduncle, the flower number is negatively correlated with temperature. By contrast, in plants exhibiting a multi-flowered raceme, the flower number is positively correlated with temperature. The parent, $F_1$, $F_2$ and backcross progenies of 3 lines with 1, 2 and 3 pods (flowers) per peduncle, respectively, have been studied in the field and in the glasshouse where the temperature was controlled (Ibarbia and Bienz, 1970a,b). Here again, the number of pods per peduncle increased with increasing temperature. It appeared that several genes control this character with a change of dominance from one experimental condition to the other (Ibarbia and Bienz, 1970a,b). Recently, it has been found for *Pisum* that the *Sn* and *Hr* flowering genes exert a strong influence on the number of flowers per inflorescence (Murfet, 1984;.S.C. Cayzer, unpublished). It was found that the *sn* segregates in an $F_2$ progeny had, at the most, 2 flowers per inflorescence, whereas *Sn Dne hr* segregates had 2 to 3 and *Sn Dne Hr* segregates had 3 to 4 flowers per peduncle (Murfet, 1984).

This example illustrates the complexity of the interactions involved in the control of reproductive growth and the necessity for working in strictly controlled environmental conditions. It also emphasises the necessity of using isogenic lines to study the effect of a given gene on a particular trait, although the same gene may have pleiotropic effects on other characters which in turn may influence the trait under consideration. For example, the flowering gene *Sn* influences the number of pods per plant which in turn may influence pod development, e.g., by increasing the

assimilate requirement (Chapter 5). In addition, knowledge of the chromosomal location of a gene can be used effectively to establish the phenotypic expression of the gene in circumstances where the effects might otherwise be obscured, e.g., marker gene *M* helps to recognise the allele at the *Hr* locus as both loci are tightly linked (Murfet, 1973a). *M* has been used to mark *Hr* in Chapter 7. Likewise gene *A* was used to mark *Lf* in Chapters 4 and 7.

## 1.5 Fruit Growth and Assimilate Distribution

There is no longer any doubt that the subtending leaf contributes greatly to the supply of carbon to the fruit (Flinn and Pate, 1970; Harvey, 1973, 1974; Szynkier, 1974) though cross nutrition from other leaves also occurs, e.g., a leaf may supply fruits situated above or below it and virtually any part of the plant including the roots can interfere with the nitrogen and carbon supply of a given fruit (see Harvey, 1977). The major contribution of the leaf occurs late in the reproductive growth period, i.e., during the seed-filling stage (Flinn and Pate, 1970 on pea; Stephenson and Wilson, 1977; Thorne, 1980 on soybean; Pate and Farrington, 1981 on lupin; Olufajo, Daniels and Scarisbrick, 1982, on haricot bean). Some authors report that the distribution of assimilates to the developing pod does not start immediately after fertilisation as the photosynthates would be kept in the stem between the pre-flowering and early maturity stages (Stephenson and Wilson, 1977). During the seed-filling period, the photosynthates move from the stem to the pod or they may move directly from the leaf to the subtended pod. Linck and Sudia (1962) established with *Pisum* that the $^{32}P$ distribution from the leaf to the pod situated in its axil started 4 to 6 days after fertilisation. Recent studies on tomatoes (Archbold, Dennis and Flore, 1982) and lupin (Pate and Farrington, 1981) gave similar results.

It appears that the pericarp is entirely committed to the supply of the seeds (Lowell and Lowell, 1970). The pericarp of the so-called "leafless"types, i.e., plants which have a chlorophyll area severely reduced, has a net uptake of $CO_2$ higher than plants with normal leaves (Harvey, 1978), whereas substantial changes in leaf morphology do not appear to have a marked effect on the photoassimilate export potential of the leaf to the pods (Harvey, 1974). The pericarp would mainly recycle the carbon lost from the seed respiration (Flinn and Pate, 1970). Seeds are also

supplied by the subtending leaf and the rest of the plant (Flinn and Pate, 1970). Inside the pod itself, the nutrient supply to the seed is made possible by the funicle and is transported through the phloem. By contrast, inside the seed, the cotyledons are deprived of vascular connections with the coat and thus the absorption of sucrose and other nutrients liberated by the internal part of the coat must involve specialised transport mechanisms (Thorne, 1980, on soybean). In pea, transfer cells occur at the cotyledon-testa interface (Marinos, 1970; Gunning and Pate, 1974). According to Murray (1980) the seed coat acts as the final arbiter of nutrient supply to the embryo.

No work has yet put forward conclusive interpretation concerning the causal relationships between the supplying sources and the growing fruits (i.e., sinks). If fruit growth depends upon assimilate supply and partitioning, the following questions are relevant. Firstly, does pollination and/or fertilisation *per se* act as a stimulus provoking the nutrient mobilisation which in turn allows fruit growth or does the metabolic activity attract the required assimilate, i.e., is the mobilisation of metabolites by the fruit a consequence or a cause of its growth? Secondly, is the delay in fruit development (e.g., in line G2 under SD; Ingram and Browning, 1979; Gianfagna and Davies, 1981) a result of a trophic competition with the various vegetative and/or other reproductive parts of the plant? In case of trophic competition between vegetative and reproductive parts, is the primary control exerted on vegetative or reproductive growth?

Archbold *et al.* (1982) reported for tomatoes that fruit set *per se* did not seem to be involved in the transport of $^{14}$C-sucrose applied on the subtending leaf. The translocation of radioactive elements from the leaf to the fruit was more closely associated with the growth of the fruit. It has been shown that the growing fruits influence strongly the photosynthetic activity of the subtending leaf and the direction in which the nutrients migrate (Tripathy, Eastin and Schrader, 1972 on corn; Flinn, 1974 on pea; Pate and Farrington, 1981 on lupin). Given a limited common assimilate source, the smaller sinks are competitively weaker in their ability to attract assimilates (Cook and Evans, 1978; Jaquiery and Keller, 1978). Thus, the growing fruit acts as a sink for metabolites. The translocation of metabolites to the fruit may be under hormonal control as the application of various growth substances causes the diversion of nutrients toward the application point (Seth and Wareing, 1967; see

Phillips, 1975). Thus, the growth substances naturally produced by the fruit tissue or diverted to the developing fruit could affect the movement of the metabolites into the developing fruits (see Bollard, 1970).

According to Bollard (1970), the developing seeds attract nutrients and thus compete with vegetative growth. Jaquiery and Keller (1978) reported that it is only when the growing activity of the apical bud has ceased that more nutrients are available for fruit growth in *Vicia faba*. As the removal of the young leaf promoted flower or pod development, it has been suggested that defoliation removed competing sinks for assimilates (Tsé, Romina, Hackett and Sachs, 1974 on *Bougainvillea;* Goh, 1977 on orchids; Kinet, 1977 on tomatoes; Carbonell and Garcia-Martinez, 1981 on pea). In all interpretations, a trophic competition between vegetative and reproductive growth is suggested regardless of whether vegetative growth deprives reproductive growth or vice versa. In pea, the flowering gene *Sn* has a direct delaying effect on apical senescence (Reid, 1980; Gianfagna and Davies, 1981) and it has been suggested that *Sn* activity may control apical arrest by controlling assimilate partitioning (Murfet, 1984; Reid and Murfet, 1984). Thus, the delay in pod growth may be, at least partly, a consequence of *Sn* activity on the enhancement of vegetative growth. The causal relationship between vegetative and reproductive growth in a photoperiodic plant is discussed in more detail in Chapter 5.

It has been shown in the above discussion that the reproductive growth depends upon the interaction of various genetical, biochemical and physiological factors which may vary with the developmental stages. This thesis considers in more detail the effects of the alleles at the major flowering loci *Sn* and *Lf* on various post-initiation stages of the reproductive phase, e.g., flower bud development (Chapter 3), flower life-span (Chapter 6) and fruit development (Chapter 4). Relationships between reproductive and vegetative growth are investigated in Chapter 5. Chapter 7 reports the effect of segregation for the *Hr/hr* pair of alleles on NFI in plants with an $Lf^{d}$ *sn Dne* background.

# CHAPTER 2 MATERIALS AND METHODS

## 2.1 Plant Material

The following lines from the *Pisum* collection at Hobart were used in this work:-

| Hobart Line No. | Genotype | Phenotype | Other Names |
|---|---|---|---|
| 53 | *lf e Sn Dne hr* | L | |
| 59 | *lf E sn Dne hr* | DN | |
| 65 | *Lf e sn Dne hr* | DN | |
| 69 | $lf^a$ *E sn Dne hr* | DN | |
| 89 | $Lf^d$ *sn Dne hr* | DN | |
| 94 | *lf e sn Dne Hr* | DN | Marx $I_1$ |
| 95 | *lf E Sn Dne hr* | EI | Marx $I_2$ |
| $299^+$ | *lf E Sn Dne hr* | EI | |
| $299^-$ | *lf E sn Dne hr* | DN | |

Lines 94 and 95 were developed by Professor G.A. Marx of Geneva, N.Y. (Marx, 1968). Lines 53, 59, 65, 69 and 89 were developed by Dr. I.C. Murfet from crosses performed at Hobart. Lines $299^+$ and $299^-$ are still under development as a pair of lines differing at the *Sn/sn* locus but otherwise isogenic. The author commenced the selection process from a single plant of genotype *lflf EE Snsn DneDne hrhr* in an $F_3$ of cross 60 (*lf E Sn Dne hr*) x 59 (*lf E sn Dne hr*) supplied by Dr. I.C. Murfet. Development has reached generation $F_7$ at the time of writing. The male sterile plants used for the study reported in Chapter 6 are descended from a mutant type which first appeared at Hobart in the $F_2$ of a cross between fertile lines L6 and L7. The mutation is inherited as a single gene recessive and the mutant plants are characterised by an absence of fertile pollen, profuse branching, formation of small parthenocarpic pods and prolonged growth (Murfet, unpublished). The relationship of this locus to several other loci known to control male sterility in *Pisum* (see Blixt, 1977) remains unknown and for this reason the gene concerned has not been symbolised in this thesis.

To assess the role of a specific gene on a certain trait, ideally isolines differing at the given locus should be used, since many levels of genetic interactions may be involved for the same trait e.g., for fruit

growth (see Section 1.4). Unfortunately, isolines take a long time to develop and this ideal condition is met only rarely. The EI and DN forms of line 299 have the advantage of initiating flowers at the same node because of the *E* background (see Section 1.1). In optimal environmental conditions, flowers and pods also develop at a similar node. Thus, the effect of the *Sn/sn* difference on reproductive growth can be studied at a similar chronological stage. This condition is especially required, considering the controlled facilities used, i.e., day temperatures and light intensity are not strictly controlled (see Section 2.2.2). When the selection program for lines $299^{+}$ and $299^{-}$ had reached $F_6$, seeds of the pure *SnSn* and *snsn* genotypes, respectively, i.e., seeds from non-segregating EI and DN $F_6$ families, were used as two nearly isogenic lines. For the study of other genetic differences (e.g., at the *Lf* locus), observations were made with segregating $F_2$ and $F_3$ progenies to randomise the genetic background. Finally, pure lines have been used when it was necessary to know the genotype of the plant from an early developmental stage (e.g., in Chapter 3).

## 2.2 Methods

### 2.2.1 Sowing Conditions

All plants were grown in 14 cm diameter pots (except in Experiment 4.4 where 3 litre cans were used) containing a 50:50 mixture of vermiculite and dolerite chips. To avoid damage to the apex during emergence of the shoot, the mixture was topped with 2 to 3 cm of sterile soil. The seeds, with nicked testa, were sown in the soil layer. These sowing conditions allow fast and homogenous germination. Pots were then placed at their appropriate place in the glasshouse and watered each day until approximately the day before the shoot was visible through the soil. Apices can be damaged by water at this early developmental stage. After the shoot had emerged, plants were watered each day and supplied twice a week with a nutrient solution (Aquasol). The potentiality of the plants may be better revealed when plants are grown one per pot. However, because of a space restriction in the glasshouse, they were sometimes grown two per pot. Details are given for each experiment.

### 2.2.2 Controlled Photoperiod Conditions

Experiments have been conducted in two glasshouses equipped with "dark" compartments inside which the temperature can be controlled within a margin of ±0.5 to 1.0°C. An automatic system allows the trucks carrying the pots to go in and out of the "dark" compartments at a set time. In Glasshouse B, the "dark" compartments are equipped with lights. The SD and LD plants both received the same period of natural light (8, 9 or 10h depending on the experiment). The photoperiod was then extended with low intensity artificial light to 24h for the LD plants and usually to 12h for SD plants. In all experiments, the light supplement was given by 6 x 25 W + 2 x 40 W tungsten filament incandescent bulbs giving a light intensity of 8.3 W $m^{-2}$ at seedling level. A low intensity of incandescent light was chosen because light of this quality prevents activity of the *Sn* gene (Reid and Murfet, 1977; Reid, 1979) while the quantity of light supplied was too low to add appreciably to the photosynthetic capacity of the LD plants. In Glasshouse B, night temperature could therefore be held identical for the LD and SD treatments. All experiments except Experiments 4.3, 4.4 and 6.1 were conducted in this glasshouse. In Glasshouse A, the dark chambers do not possess lights. Hence, the plants requiring LD were placed under natural light which was extended to 24h of light by a mixed incandescent fluorescent source consisting of 100 W incandescent bulbs and 40 W Mazda cool white fluorescent tubes arranged alternatively. This source gave an intensity of about 10 W $m^{-2}$ at seedling level. Plants requiring SD (usually 8h) were moved into the dark compartment which was maintained at 17.5°C±0.5°C. However, the temperature in the LD compartment (i.e., the glasshouse itself) could not be controlled as rigorously and therefore the temperature conditions for the LD and SD treatments were not necessarily the same during the night. However, the LD and SD trucks run parallel and close to each other (≃ 0.6 m apart) so that temperature and light conditions during the day were very similar for both treatments. The use of the trucks requires a split plot design and randomisation between SD and LD pots was not possible in either glasshouse.

### 2.2.3 Growth Measurements

In this work, the fruit is referred to as a pod, regardless of whether it contains seeds or not. The fruit envelop is referred to as pericarp. The measurements of the length and the breadth of the pod were done with

verniers to a precision of ±0.1 mm. The length of the pod corresponded to the length of the straight line between the base of the calyx and the pod tip. Pod breadth was measured at right angle of the length as the distance from wall to wall (not suture to suture). The verniers were placed at the widest part of the pod and tightened until a light resistance was felt. Length measurement started when the carpel emerged from the withered corolla and breadth measurement started at least two days later when the carpel could be lightly pressured with less risk of damage to the growing seeds. The frequency of measurement is given for each experiment in the appropriate chapters.

To determine the change over time for various organs, the following procedures were used:-

(a) Pericarp fresh weight: - 4 to 6 pods were removed regularly from the first reproductive node and each pericarp was weighed immediately. The mean of the sample represented the value for the pericarp fresh weight.

(b) Seed fresh weight: - The 4 central seeds only were taken from each of 4 to 6 pods as the distal and proximal seeds abort more frequently (Linck, 1961; Hedley and Ambrose, 1981; personal observations). The mean of the sample represented the seed fresh weight.

(c) Embryo fresh weight was determined similarly to the seed fresh weight following removal of the testa.

### 2.2.4 Analysis of the Results

For each plant, the changes in length with time were fitted to the logistic function (Erikson, 1976), also used by Gent (1983). This function has the following equation:

$$Y = \frac{ML}{1 + \exp\,[SGR(M-t)]} \quad \text{where}$$

Y is the length of the pod on day t

ML is the maximum length

SGR is the specific (or relative) growth rate measured during the early exponential phase (Gent, 1983)

M is the day at which 50% of the maximum length is reached.

The computer program used to calculate the 3 parameters ML, SGR and M is distributed by Dr. B.G. Cox; Otago University, New Zealand. Four parameters have been estimated from the equation to describe the changes in length over time. They are:-

(a) the maximum length: ML (the estimated value, ML, was very close to the actual measured value),

(b) the maximum growth rate: $MGR = \frac{ML \times SGR}{4}$,

(c) the time at which MGR is reached: TMGR = M, and

(d) the time at which 95% of ML is reached: T 95% ML

$$T\ 95\%\ ML = \frac{(SGR \times M) - Ln(ML/\ell)}{SGR} \qquad (\text{where } \ell = 95\%\ ML)$$

Since the curve theoretically approaches the assymptote gradually until $\infty$, it is not possible to calculate the time to maximum length. The four parameters were treated by analysis of variance and t-tests were performed for the differences between treatment means. The few plants whose data did not fit the logistic function were discarded.

The growth in breadth did not always fit the logistic equation. Thus, the growth rate was calculated as dB/dt and the maximum growth rate was the maximum dB/dt (dB/dt max). The breadth was considered to be at a maximum when the same value (±0.1 mm) was obtained on three consecutive measurements. The time to reach the maximum breadth (TMB) was the day the maximum breadth (MB) was reached. The changes in fresh weight per pericarp, per seed and per embryo were treated similarly to the data for pod breadth.

# CHAPTER 3 EFFECT OF PHOTOPERIOD AND GENETIC DIFFERENCES AT THE *Sn* AND *Lf* LOCI ON THE DURATION AND ONTOGENESIS OF THE FLORAL BUD DEVELOPMENT

## 3.1 Introduction

It has been reported for various cereals that photoperiod acted upon FT not only by affecting TFI but also by affecting the duration of flower development (Quinby, 1972; Aitken, 1974; Cottrell *et al.*, 1982; Sorrells and Myers, 1982). In contrast, with maize the time to silking (flowering) depended on the duration to initiation only, as the succeeding period was unaffected by photoperiod (Allison and Daynard, 1979). Results reported for peas are contradictory.

The flowering behaviour in the pea is controlled by the combination of alleles present at the 6 loci *Sn, Dne, E, Lf, Hr* and *Veg* described in Chapter 1. The photoperiod response is conferred by the joint presence of genes *Sn* and *Dne* and genotypes homozygous for *sn* and/or *dne* are essentially DN types. Whereas the FT of genotype *Sn Dne* is delayed under SD, NFI may or may not be altered by photoperiod, depending on the remaining background (see Section 1.1). An EI phenotype generally shows floral abortion at the lowest reproductive nodes in SD so that, even though NFI is unaffected, the node at which the first flower opens (NFD) is increased, and this leads to an increase in FT (Murfet, 1971a, 1977). However, even where NFD is the same in both LD and SD, FT is increased in SD since the flower bud development lags behind leaf expansion (Murfet, 1971a). It may be inferred that SD increase the time between flower initiation and opening of the first flower in EI plants but the results presented in this chapter represent the first direct observations in support of that conclusion. Thus, in quantitative LD plants, SD delay FT by delaying TFI and/or flower bud development.

Independently of photoperiod, NFI (and supposedly TFI) is controlled by the 4 alleles at the *Lf* locus. With the increasing order of dominance ($lf^a$, *lf*, *Lf* and $Lf^d$), the minimum NFI is respectively 5, 8, 11 and 15 (Murfet, 1975a; Yates and Murfet, 1978). No data are available on the duration of floral bud development but it has been reported that the floral bud of $Lf^d$ plants developed faster than its subtending leaf (Murfet, 1984).

Aitken (1974) and Collins and Wilson (1974) assumed that the time for the first floral primordium to develop is the time taken by the number of nodes (leaves) in the apical bud at initiation (NAI) to expand. Based on this assumption, Berry and Aitken (1979) concluded that the time interval between initiation and opening of the first flower was independent of genotype and photoperiod so that any differences in FT should be accounted for by the effect on time to initiation, apart from the fact that later flowering cultivars had more nodes in the apical bud at the time of initiation. However, this assumption implies that the flower opens at the same time that the subtending leaf reaches full expansion, i.e., the flower leaf relativity (FLR), as defined by Murfet (1984), equals zero. As it has been reported that, in some cases, a flower can open after or before its subtending leaf (Murfet, 1971a, 1982a, 1984), this assumption may be far from exact. The duration of flower bud development is therefore re-examined by direct observation of the dissected apices.

A detailed study of the period between initiation and open flower would not only clarify the situation but would also establish more conclusively the effect of genotype and photoperiod on the rate of the bud development. The latter information is of great physiological interest. For example, is the action of the flowering genes *Sn* and *Lf* on initiation (see Section 1.1) extended also to the course of floral bud development *per se*?

This chapter considers the effect of photoperiod and genetic differences at the *Sn* and *Lf* loci on the ontogenesis and the rate of development of the floral bud, i.e., the duration of the period between initiation and opening of the first developing flower. The duration of flower bud development was also examined for the other buds growing along the stem in the case of the *Sn/sn* gene difference.

## 3.2 Materials and Methods

### 3.2.1 Materials

The effect of the *Sn/sn* difference on flower bud development was studied with the help of two near-isogenic lines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*), two photoperiodic lines (*Sn*, lines 53 and 95) and four DN lines (*sn*, lines 69, 59, 65 and 89). The effect of allelic differences at the *Lf* locus was studied

by comparing flower development in the DN lines 69 ($lf^a$), 59 ($lf$), 65 ($Lf$) and 89 ($Lf^d$) in SD. Further information on the lines used is given in Section 2.1. As discussed previously, the effect of allelic differences is better studied using segregating progenies to randomise the genetic background. However, the need to know the plant genotype in the early developmental stage rules out the use of such material.

Experiment 3.1

Plants of lines $299^+$ and $299^-$ were grown two per pot in Glasshouse B and placed either under LD (24h) or SD (12h). All plants received 8h of natural light. The plants were then moved to a "dark" chamber where the photoperiod was extended by either 16h or 4h of artificial light (for details, see Section 2.2.2). Temperature in the "dark" chamber was 15±0.5°C and ranged from 18 to 25°C during the exposure to natural light.

Experiment 3.2

Plants of lines 53, 95, 59, 65, 69 and 89 were grown two per pot in Glasshouse B and exposed either to LD = 24h or SD = 10h of light. All plants received 10h of natural light and the LD plants received, in addition, 14h of artificial light (for details, see Section 2.2.2). Temperature in the "dark" chamber was 15±0.5°C and ranged from 19 to 25°C during the exposure to natural light.

### 3.2.2 Measurement of the Interval FT-TPI and of Variables Influencing FT-TPI

The duration of the development of the first floral bud is the time between the initiation and the opening of that flower. Since abortion may occur at NFI (in EI plants, for example), the time of initiation is calculated for NFD and symbolised TPI (time of primordium initiation). The duration of the floral bud development at NFD is referred to as FT-TPI.

The apices of 4 to 6 plants per line were dissected regularly from the start of shoot emergence until some time after flowering, under a stereo-microscope equipped with a camera-lucida. The number of leaves

expanded (LS) and the total number of nodes (leaves and leaf primordia) present (TN) were recorded. The 3 variables NFD, FT and LSF (leaf stage at the time of open flower) were scored on a sample of at least 10 plants for each line. The flower leaf relativity (FLR) equals NFD-LSF. The rate of leaf expansion (RLE) was given by the slope of the linear regression of LS versus time. RLE was calculated for the period between initiation and open flower as it can vary during plant growth (Aitken, 1974; Berry, 1981). The number of nodes (leaves) in the apex at initiation of the first developing flower (NAI) equals TN-LSI. LSI is calculated from LS = f(t). TPI is obtained by substituting NFD in the equation TN = f(t) (the linear regression of TN versus time) and is subtracted from FT to give the time interval: FT-TPI. There was a good agreement between the calculated value of TPI and the observed time of initiation in the dissected apices. The duration of flower bud development at various positions along the main stem was obtained similarly by replacing the number of the node for the given primordium in the equation TN = f(t) and by subtracting the value obtained from the time of opening of the given flower. The regressions LS = f(t) and TN = f(t) were tested for linearity and in all cases, the linear regression explained the variation by more than 97.5%. The 95% confidence limits were calculated for FT-TPI. In line 69, flowers at nodes 5, 6 and 7 often opened simultaneously (Figure 3.1). However, in some plants, the flower at node 7 opened before the flowers at lower nodes (Figure 3.1). For this reason, the flower at node 7 was treated as the first flowering node and FT-TPI was calculated for that node.

## 3.3 Results

### 3.3.1 Effect of Genetic Differences at the *Sn* and *Lf* Loci on FT-TPI

#### 3.3.1.1 FT-TPI measured in days

The duration of flower bud development at NFD was prolonged by the *Sn* x SD interaction (Tables 3.1 and 3.2). In SD, FT-TPI was 6 days longer for line $299^{+}$ (*Sn*) than for line $299^{-}$ (*sn*), whereas in LD, no significant difference existed between the two lines (Table 3.1). Moreover, FT-TPI was prolonged in SD compared to LD by 5.5 and 4 days, respectively for the *Sn* lines 95 (EI type) and 53 (late type) (Table 3.1). The results obtained with the DN lines 59, 69 and 65 confirm that photo-

period has little effect on FT-TPI in *sn* plants (Table 3.2). A one day increase in FT-TPI was the maximum variation between LD and SD for these lines and line $299^-$ (Tables 3.1 and 3.2). Line 89 (*sn*) showed a slight response to photoperiod with respect to NFI and FT (Table 3.2) which was also noticed in other experiments (see Chapter 7). However, for this line, FT-TPI was slightly shorter in SD than LD (difference not statistically significant; Table 3.2) which contrasts with the longer interval observed in lines carrying gene *Sn*.

Independently of photoperiod, the NFI of lines 69 ($lf^a$), 59 (*lf*), 65 (*Lf*) and 89 ($Lf^d$) varied dramatically, as expected since the 4 alleles govern the minimum flowering node. FT-TPI decreased with increasing NFI from 29 days in line 69 (NFI = 5 to 7) to 23 days in line 65 (NFI = 16) (Table 3.2). However, although line 89 initiated floral buds more than 6 nodes later than line 65 in SD the first flower bud took a similar time to develop in both lines (Table 3.2).

3.3.1.2 FT-TPI counted as number of plastochrons

As also observed by Aitken (1974), RLE increases as the upper leaves expand in late-flowering lines, e.g., RLE increased for line 53 in SD where NFI was delayed ($P<0.001$; Table 3.3) and for lines 65 and 89 compared to the early lines 69 and 59 ($P<0.001$; Table 3.4). Changes in RLE are likely to affect FT-TPI. As pointed out by Aitken (1974), the interval between initiation and open flower may be measured in terms of the number of leaves (nodes) that are expanded during the development of the floral bud, i.e., the number of plastochrons occurring between TPI and FT which is given by the expression NAI-FLR. In the photoperiodic lines $299^+$, 95 and 53, FLR decreased to a negative value in SD whereas NAI increased with NFD, i.e., the number of plastochrons increased in SD for lines with *Sn* (Table 3.5). In contrast, when the *Lf* series was ascended on an *sn* background, NAI and FLR both increased with increasing NFD and consequently the number of plastochrons is not necessarily increased in late-flowering cultivars (Table 3.6).

In conclusion, SD prolonged FT-TPI in photoperiodic lines (*Sn*) regardless of the measure used (days or plastochrons). On an *sn* background, the increase in NFI caused by the ascending sequence $lf^a$, *lf*, *Lf* and $Lf^d$,

is associated with a decrease in the time interval between TPI and FT, due partly to the faster RLE. Nevertheless, the number of plastochrons occurring between initiation and opening of the flower is not necessarily increased with the late-flowering cultivars on *sn* background and the delay occurring in the very early DN line ($lf^a$) is visible microscopically (Section 3.3.3).

### 3.3.2 Variation of FT-TPI up the Stem

The simultaneous flowering pattern of the DN line 69 ($lf^a$ *sn*) indicates that the delay in bud development is most marked at the lowest reproductive nodes, i.e., the duration of bud development decreases with increasing node number (see Section 3.3.3). Is this also the case in *Sn* lines where FT-TPI is controlled by photoperiod?

The duration of the development of the buds growing at different nodes along the stem was studied for lines $299^+$ and $299^-$ under SD in Experiment 3.1. In this photoperiod, line $299^-$ produced 2 to 3 flowers whereas line $299^+$ produced 5 to 8 developed flowers in addition to the first 4 flower buds which aborted (Table 3.1). For line $299^+$, the observations were limited to the nodes producing pods, i.e., a maximum of 6 nodes, even though the sixth position was not necessarily the last node to bear an open flower. The buds of line $299^-$ developed slightly faster at each succeeding node as the time interval between initiation and opening of the flowers was reduced by 2 days from the first to the third position (Table 3.7). Likewise for line $299^+$, this time interval declined with increasing node number, gradually at first and then more rapidly for the fifth and sixth flowers (Table 3.7). At the sixth position, the time interval lasted only 35.5 days compared to 39.3 for the first node. Even at the sixth position on line $299^+$, the time interval was still larger (35.9 days) than for the first flower on $299^-$ plants (33.3 days). However, as mentioned previously, the observations on line $299^+$ were confined to 6 nodes and it is possible that, at the uppermost nodes, the time interval declined to the point where it overlapped the range for line $299^-$.

### 3.3.3 Floral Bud Ontogenesis

Microscopic observation of the apex revealed interesting ontogenetic

differences underlying the variation in FT-TPI. With the exception of lines with genotype *lf*$^a$ and early photoperiodic lines in SD when the lowest floral primordia aborted (e.g., line 299$^+$), an axillary primordium was recognisable as a flower primordium from the moment that node became distinguishable from the apical dome because of its precocious inception and fast development relative to the subtending leaf primordium. This was true for DN lines 59 (*lf sn*) and 89 (*Lf*$^d$ *sn*) early photoperiodic lines 299$^+$ and 95 (both *lf Sn*) in LD, and in line 95 in SD. Thus, inception of the axillary primordium occurred in the axil of the last (nth) formed leaf primordium, its differentiation (considered at a morphological level; Figure 3.2c) into a floral bud occurred when two further primordia had appeared (i.e., the n-2nd primordium differentiated when the nth primordium was formed) and the various floral parts of the bud were soon visible.

In contrast, for the very early DN line 69 (*lf*$^a$), the n-4th axillary primordium (i.e., the primordium at node 6) had not differentiated into a flower bud when the nth primordium (i.e., the primordium at node 10) was formed (Figure 3.2d(1)). Because of the slow development of the floral primordium in the pre-differentiation stage, it was not possible to recognize it as a flower primordium before it had shown the first signs of floral differentiation as slow development usually characterises a vegetative primordium (Vescovi, 1968). The four lowest primordia differentiated at a similar time (and size) and they then continued their development into a mature flower at a similar rate (Figure 3.2d(2)) which explains the simultaneous flowering behaviour of line 69. Therefore, it appears that in comparison to genotype *lf* (e.g., line 59), differentiation in genotype *lf*$^a$ is held back until a certain number of primordia have been formed or until the primordia have reached a certain size and the delay in differentiation appears to account for the increase in FT-TPI.

In line 299$^+$ (*Sn*) in SD, the differentiation of the first initiated floral primordium (at NFI) was delayed and the primordium failed to develop into an open flower. However, regardless of the photoperiod and genotype, once floral differentiation has occurred for the first time in an apex, it then occurs at the same rhythm, i.e., the n-2nd primordium differentiates when the nth primordium has been formed. Thus, as opposed to the situation in line 69, the increase in FT-TPI observed at NFD (4 nodes higher than NFI) in the early photoperiodic lines in SD (e.g., lines 299$^+$ and 95) appears to be due to a slower development of the differentiated

floral primordium rather than a delay in the time of differentiation.

Line 89 (*Lf*$^{d}$ *sn*) showed a pattern of development for vegetative axillary primordia which differed from all the other lines examined. After some 14 or 15 nodes had been laid down, the newly formed vegetative axillary primordia showed precocious swelling and development almost akin to that of flower primordia (Figure 3.2e). This contrasts with the usual pattern for a vegetative apex where no primordium is generally visible in the axil of the last formed leaf primordium (Figure 3.2a) since the inception of a vegetative primordium occurs usually in the axil of the 4th to 5th leaf primordium below the apical meristem (Vescovi, 1968; Neville and Afzal, 1972).

## 3.4 Discussion

The results show firstly, that the duration of flower bud development differs with photoperiod and genotype and secondly, that independently of photoperiod, on an *sn* background, the late-flowering cultivars may present the same if not a shorter FT-TPI than early cultivars (e.g., compare FT-TPI in lines 89 and 69). The difference between these results and those of Aitken (1974) and Berry and Aitken (1979) is fully explained by the fact that these authors omitted to consider the leaf stage at the time of open flower, i.e., they ignored FLR. However, FLR by itself does not always fully account for a difference in FT-TPI as RLE and NAI may vary. NAI increases with NFI as also reported by Berry and Aitken (1979), i.e., it increases with the ascending sequence *lf*$^{a}$, *lf*, *Lf* and *Lf*$^{d}$ since these alleles determine the minimum NFI (Murfet, 1975a; Yates and Murfet, 1978) and in SD for L type plants since NFI is delayed by the *Sn* x SD interaction (Murfet, 1971a). RLE increases during the reproductive phase for the late-flowering cultivars so that the reproductive nodes of the late-flowering cultivars develop faster than those of the early-flowering ones (Aitken, 1974). Variation in RLE complicates the interpretation of the variation in FT-TPI. For example, in line 53, the *Sn* x SD interaction increased the number of plastochrons occurring between TPI and FT; however, the difference in FT-TPI between LD and SD was not as much as expected because plants grew slightly faster under SD than under LD, at least during the period of bud development. In the case of the *Lf* allelic series, lines 69 and 89 took the same number of plastochrons to develop; however, FT-TPI was shorter for line 89 probably because of the increase in RLE

for the late-flowering line 89. Thus, the *Sn* x SD interaction increases FT-TPI by slowing the ontogenesis of the floral bud whereas the promotion in the time interval between TPI and FT observed with *Lf* and $Lf^d$ is mainly a consequence of the high NFI. However, $lf^a$ also slowed the ontogenesis of floral buds thereby causing the simultaneous flowering pattern attributed to this allele (Murfet, 1984).

Although the differencesreported in FT-TPI are relatively small compared to the differences in TPI, their physiological meaning is important. For example, Nitsch (1965) raised the question as to whether or not the two developmental stages (i.e., initiation and flower development) were controlled by the same "mechanisms" since photoperiod requirements for initiation are not necessarily similar to those for flower development. Indeed, although photoperiod generally affects both initiation and flower development in the same way, numerous examples are given in the literature where this is not so (Nitsch, 1965; Evans and Wardlaw, 1976).

The fact that the *Sn* x SD interaction prolonged FT-TPI at NFD and that this period decreased with increasing node number fits the hypothesis that *Sn* activity has a delaying effect on flower bud development in addition to its effect on NFI (Murfet, 1971a,b; see Section 1.1). At first, *Sn* activity may prevent or retard development of the floral bud (Murfet, 1971c) but by the time the uppermost flower buds become mature, less and less inhibitor (and/or more and more promotor) would be produced in the shoot since *Sn* activity decreases with age (Murfet, 1971b; Reid 1979a) and since the upper leaves may develop with a more promotory hormonal balance (Reid, 1979a), allowing faster and faster bud development. The fact that floral buds of *sn* plants also showed a promotion in FT-TPI with increasing node number is compatible with this hypothesis as the upper leaves of *sn* plants may also develop with a more promotory ratio.

It has been suggested that *Sn* product may direct the flow of metabolites towards vegetative growth and away from reproductive growth (Murfet, 1984; Reid and Murfet, 1984). In *Lathyrus odoratus*, a similar pathway has been put forward to explain the control of flowering (Ross, 1983). In SD, floral buds of *Sn* plants face more competition from vegetative growth than those of *sn* plants as more nodes developed in *Sn* plants after floral initiation and developing floral buds may compete with vegetative parts of the apical bud for common assimilates. The hypothesis that floral development

is prevented by an actively growing apex through assimilate competition has been suggested by various authors (Jeffcoat and Harris, 1972; Tsé *et al.*, 1974; Kinet, 1977; Sachs and Hackett, 1977; Leonard *et al.*, 1983). Evidence of direct competition between floral axillary buds and the apical bud for available assimilates is provided by Leonard *et al.* (1983) for tomatoes and it was suggested that floral bud development may depend on the partitioning of assimilates, itself being influenced by the synthesis and distribution of endogenous hormones e.g., cytokinins (Tsé et al., 1974; Leonard *et al.*, 1983). However, as underlined by Leonard *et al.*, to prove that assimilate supply limits inflorescence growth, it has to be shown that the enhanced metabolite supply precedes the increased metabolic activity in the inflorescence. It would certainly be of interest to measure hormonal activity and partitioning of assimilates in the two near-isogenic lines $299^{+}$ and $299^{-}$ in relation to nuclear activity changes in the leaf axil.

The possibility that *Sn* activity may delay the rate of flower bud development by a direct hormonal effect cannot be discarded. The slower rate of bud development could be caused by the presence of an inhibitor in the inflorescence as suggested by Cottrell *et al.* for barley (1982).

The result that, on an *sn* background, FT-TPI was slightly promoted with increasing NFI, i.e., with increasing dominance order of the alleles at the *Lf* locus, supports the assumption that, by the time flowering occurs in *Lf* and $Lf^{d}$ plants, a plentiful supply of nutrients should be available to promote reproductive growth (Murfet, 1984). In contrast, in very early plants (e.g., line 69), $lf^{a}$ allows floral evocation so early that the plants may not be able to provide the necessary resources to the floral axillary bud as the underlying metabolism is still geared toward vegetative growth at the time the first floral buds develop. The result that buds of plants carrying *Lf* or $Lf^{d}$ presented a similar development could mean that the sequential events leading to the stage of open flower have now plateaued at a minimum time. In addition, it is possible that, in $Lf^{d}$ plants, the precocious and fast developing vegetative axillary buds compete with the floral buds. Alternatively, as discussed earlier, since lines 89 and 65 are two pure lines, genes other than $Lf^{d}/Lf$ may interfere directly or indirectly with bud formation.

The two lines 69 and 89 present contrasting characteristics of interest for the study of the developmental sequence of floral evocation. The

precocious inception of the axillary initials of both lines and their transformation into floral and vegetative organs, respectively, raises the question as to when the signal for floral evocation reaches the apex and what are the essential events of floral evocation. For example, plants of line 89 generally flowered at node 20, but they occasionally formed a flower at node 15 (the stage at which the first precocious axillary buds occurred) and reverted to the vegetative state at node 16 to 19. As the precocious inception of the axillary initials is the most universal sign associated with floral evocation (Bernier, Kinet and Sachs, 1981), this raises the question as to whether evocation has occurred and that subsequent flower formation is prevented in most plants, or whether vegetative axillary buds initiate and develop more rapidly in line 89 than in lines with the lower order alleles *lf*$^{a}$ and *lf*.

The behaviour of line 89 lends support to the interpretation that, if the precocious inception is necessary, it is not a sufficient component for evocation (Bernier *et al.*, 1981). It has been suggested that precocious initiation of axillary primordia is a consequence of a loss of apical dominance (Thomas, 1962). The latter may thus represent a causal factor for inflorescence development. However, the apex of *lf*$^{a}$ plants seems to show a strong apical dominance over the axillary bud, in the early stage of the bud formation at least. If the apex of *Lf*$^{d}$ plants is not evoked at the time the first precocious axillary buds occur it would appear that the factors controlling the precocious inception are different, at least partially, to the ones controlling the outgrowth of the axillary vegetative buds, as suggested by Thomas (1962). Since an evoked meristem presents specific histological characteristics (i.e., nuclear activity in the leaf axil; Bernier *et al.*, 1981) it would be of interest to follow the histological configuration of the apices of lines 89 and 69.

TABLE 3.1 Effect of photoperiod on the node of flower initiation and development (NFI and NFD), time of initiation for NFD (TPI), flowering time (FT) and duration of flower bud development for NFD (FT-TPI) for lines $299^+$ and $299^-$ (LD = 24h; SD = 12h) and for lines 95 and 53 (LD = 24h; SD = 10h). Data from Experiments 3.1 and 3.2.

| Treatment | NFI | | | NFD | | | TPI | FT | | | | FT-TPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | | $\bar{x}$ | ±SE | n | | |
| $299^+$LD | 10.50 | 0.15 | 12 | 10.50 | 0.15 | 12 | 10.18 | 42.70 | 0.39 | 12 | ) | 32.5 (30.2; 34.5)† |
| | | | | | | | | | | | )*** | |
| $299^+$SD | 10.36 | 0.13 | 14 | 14.36 | 0.29 | 14 | 20.89 | 60.10 | 1.34 | 14 | ) | 39.2 (36.1; 41.6) |
| $299^-$LD | 9.70 | 0.12 | 14 | 9.70 | 0.12 | 14 | 7.41 | 39.80 | 0.32 | 14 | ) | 32.4 (29.1; 35.3) |
| | | | | | | | | | | | )NS | |
| $299^-$SD | 10.00 | 0.10 | 14 | 10.00 | 0.10 | 14 | 7.42 | 40.70 | 0.42 | 14 | ) | 33.3 (29.8; 35.7) |
| 95 LD | 10.35 | 0.15 | 17 | 10.35 | 0.15 | 17 | 10.48 | 37.00 | 0.44 | 17 | ) | 26.5 (24.6; 28.4) |
| | | | | | | | | | | | )*** | |
| 95 SD | 10.47 | 0.14 | 19 | 10.47 | 0.14 | 19 | 10.75 | 42.68 | 0.32 | 19 | ) | 31.9 (30.2; 33.7) |
| 53 LD | 14.50 | 0.14 | 18 | 14.50 | 0.14 | 18 | 17.24 | 41.90 | 0.18 | 18 | ) | 24.7 (23.7; 25.6) |
| | | | | | | | | | | | )*** | |
| 53 SD | 19.52 | 0.22 | 25 | 19.52 | 0.22 | 25 | 27.18 | 56.10 | 0.27 | 25 | ) | 28.9 (28.0; 29.9) |

*** Means are significantly different at the 0.001 probability level. NS: non significant.
† The 95% confidence limits are shown in parentheses.

TABLE 3.2 Effect of photoperiod on the node of flower initiation and development (NFI and NFD), time of initiation for NFD (TPI), flowering time (FT) and duration of flower bud development for NFD (FT-TPI) for 4 lines 69, 59, 65 and 89. LD = 24h; and SD = 10h. Data from Experiment 3.2.

| Treatment | NFI = NFD | | | TPI | FT | | | FT-TPI | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | | $\bar{x}$ | ±SE | n | | |
| 69 LD | 7 † | | | 1.8 | 30.0 | 0.18 | 20) | 28.3 | |
| | | | | | | | )NS | | |
| 69 SD | 7 | | | 1.7 | 30.9 | 0.19 | 22) | 29.2 | |
| 59 LD | 9.1 | 0.08 | 17 | 6.0 | 32.6 | 0.43 | 17) | 26.6 | (25.4; 28.1) ø |
| | | | | | | | )NS | | |
| 59 SD | 9.3 | 0.13 | 15 | 6.9 | 32.6 | 0.37 | 15) | 25.7 | (25.0; 27.0) |
| 65 LD | 15.6 | 0.22 | 21 | 19.4 | 41.7 | 0.44 | 21) | 22.3 | (20.5; 24.1) |
| | | | | | | | )* | | |
| 65 SD | 16.1 | 0.31 | 18 | 20.0 | 43.0 | 0.45 | 18) | 23.0 | (21.6; 24.3) |
| 89 LD | 19.8 | 0.24 | 15 | 26.2 | 52.7 | 0.54 | 15) | 26.5 | (25.4; 27.6) |
| | | | | | | | )* | | |
| 89 SD | 22.5 | 0.46 | 17 | 31.6 | 55.2 | 1.04 | 17) | 23.6 | (20.2; 26.0) |

† See text for explanation of NFI of L 69.

ø The 95% confidence limits are shown in parentheses.

* Means are significantly different at the 0.05 probability level;

NS: not significant.

TABLE 3.3 Regression coefficient (RLE) and SE of the regression coefficient for the plot of leaf stage versus time for lines $299^+$ and $299^-$ (LD = 24h and SD = 12h) and for lines 95 and 53 (LD = 24h and SD = 10h). $r^2$ = coefficient of determination. Data from Experiments 3.1 and 3.2.

| Treatment | b = RLE† | $r^2$ |
|---|---|---|
| $299^+$ LD | 0.25±0.02 | 0.983 |
| $299^+$ SD | 0.27±0.02 | 0.983 |
| $299^-$ LD | 0.26±0.03 | 0.986 |
| $299^-$ SD | 0.26±0.02 | 0.978 |
| 95 LD | 0.29±0.02 | 0.971 |
| 95 SD | 0.27±0.01 | 0.997 |
| 53 LD | 0.31±0.01 | 0.998 |
| 53 SD | 0.37±0.01 | 0.992 |

† RLE (rate of leaf expansion) measured over the interval FT-TPI

TABLE 3.4 Regression coefficient (RLE) and SE of the regression coefficient for the plot of leaf stage versus time for the lines 69, 59, 65 and 89 grown under LD (24h) and SD (10h). $r^2$ = coefficient of determination. Data from Experiment 3.2.

| Treatment | b = RLE † | $r^2$ |
|---|---|---|
| 69 LD | 0.29±0.01 | 0.998 |
| 69 SD | 0.29±0.01 | 0.993 |
| 59 LD | 0.29±0.01 | 0.995 |
| 59 SD | 0.30±0.01 | 0.994 |
| 65 LD | 0.36±0.01 | 0.997 |
| 65 SD | 0.37±0.01 | 0.995 |
| 89 LD | 0.32±0.01 | 0.998 |
| 89 SD | 0.40±0.03 | 0.989 |

†RLE (rate of leaf expansion) is measured over the interval FT-TPI

TABLE 3.5 Number of nodes in the apex at the initiation of the first developing flower (NAI) and the flower leaf relativity (FLR) for lines $299^+$ and $299^-$ (LD = 24h and SD = 12h) and for lines 95 and 53 (LD = 24h and SD = 10h). Data from Experiments 3.1 and 3.2.

| Treatment | NAI | FLR | | | NAI-FLR |
|---|---|---|---|---|---|
| | | $\bar{x}$ | $\pm$SE | n | |
| $299^+$LD | 7.9 | -0.24 | 0.07 | 12 | 8.14 |
| $299^+$SD | 8.8 | -1.64 | 0.11 | 14 | 10.44 |
| $299^-$LD | 7.9 | -0.14 | 0.05 | 14 | 8.04 |
| $299^-$SD | 8.1 | -0.13 | 0.05 | 14 | 8.13 |
| 95 LD | 7.3 | +0.21 | 0.07 | 17 | 7.09 |
| 95 SD | 7.3 | -1.43 | 0.12 | 19 | 8.13 |
| 53 LD | 8.7 | +0.45 | 0.06 | 18 | 8.25 |
| 53 SD | 10.2 | -1.08 | 0.10 | 25 | 11.28 |

TABLE 3.6 Number of nodes in the apex at the initiation of the first developing flower (NAI) and flower leaf relativity (FLR) and duration of flower bud development (FT-TPI) for the 4 lines 69, 59, 65 and 89 grown under SD (10h).

| Line | NAI | FLR | | | FT-TPI | NAI-FLR |
|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $\pm$SE | n | | |
| 69 | 7.0 | -1.97 | 0.06 | 22 | 29.2 | 9.0 |
| 59 | 7.4 | -0.30 | 0.05 | 15 | 25.7 | 7.7 |
| 65 | 9.6 | +0.45 | 0.06 | 18 | 23.0 | 9.1 |
| 89 | 11.4 | +1.72 | 0.19 | 17 | 23.6 | 9.7 |

TABLE 3.7 Effect of flower position on the stem on the duration of flower bud development (FT-TPI), for the near-isogenic lines $299^+$ (*Sn*) and $299^-$ (*sn*) in SD (12h). The number of replicates is shown in parentheses. Data from Experiment 3.1.

| Line | Position | (FT-TPI) |
|---|---|---|
| $299^-$ | 1 † | 33.3 (14) |
| | 2 | 32.5 (14) |
| | 3 | 31.3 (13) |
| $299^+$ | 1 | 39.3 (13) |
| | 2 | 39.0 (13) |
| | 3 | 38.4 (12) |
| | 4 | 37.9 (9) |
| | 5 | 36.6 (7) |
| | 6 | 35.5 (4) |

† Position 1 = NFD. In line $299^-$ NFD = NFI.
In line $299^+$ NFD = NFI + 4.

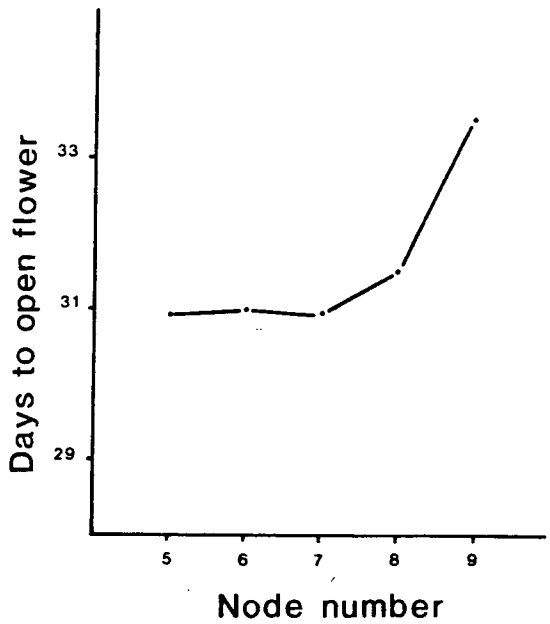



FIGURE 3.1 Time at which flower opens at nodes 5 to 9 for line 69 grown in SD (10h)

FIGURE 3.2 Drawings of the apical bud

(a) A vegetative apex, 8 days after sowing, of a line 95 plant grown in LD (24h) showing the youngest leaf primordia. No axillary initials are visible. m indicates the apical meristem and n, n-1 and n-2 the three uppermost leaf primordia.

(b) A reproductive apex, 12 days after sowing for a line 95 plant grown in LD (24h), showing a precocious floral primordium (f.p.) in the axil of the last formed leaf primordia (nth and n-1st l.p.).

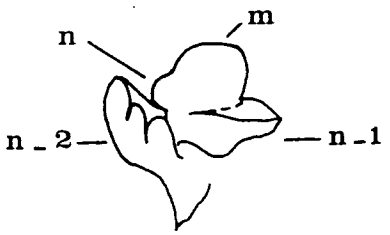
(c) An older reproductive apex of a line 95 plant grown in LD (24h). Note the fast development of the axillary floral primordia compared to that of the subtending leaf primordia. The n-2nd f.p. starts to show the configuration of a floral bud.

(d) Apex and floral buds of a line 69 plant grown in SD (10h).

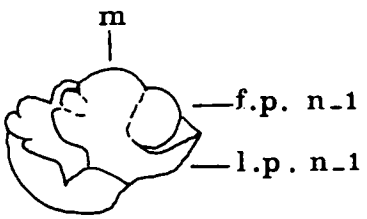
(1) 8 days after sowing, the floral primordia in the axil of the 6th, 7th and 8th leaf primordia were the same size. The 6th and 7th l.p. were removed to show the axillary initials.

(2) 16 days after sowing, the 5th, 6th, 7th and 8th floral buds were a similar size.

(e) Apex of a line 89 plant, 20 days after sowing (SD = 12h) showing the youngest leaf primordia. Note the precocious but slow growing axillary primordia in the axil of the 15th and 16th leaf primordia.

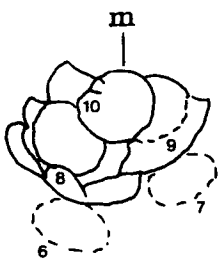



(a) x625

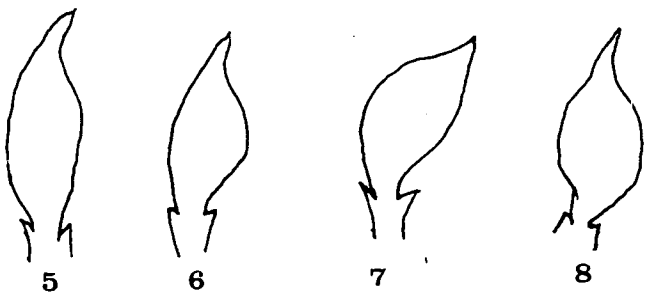



(b) x625


(c) x625

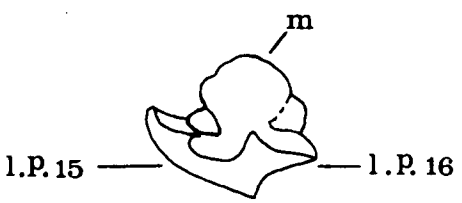



(1) x625




(2) x150

(d)




(e) x625

# CHAPTER 4 THE EFFECT OF GENETIC DIFFERENCES AT THE *Sn* AND *Lf* LOCI ON POD AND SEED DEVELOPMENT

## 4.1 Introduction

Alleles at the *Sn* and *Lf* loci control FT not only by governing NFI and/or NFD but also by affecting the course of the flower bud development. It has been shown conclusively in Chapter 3 that *Sn* activity increased the duration of flower development at NFD, whereas the increasing dominance order of the *Lf* allelic series was associated with a decrease in the duration of flower bud development, at least for $lf^a$ to *Lf*. Is the effect of the genetic differences at the *Sn* and *Lf* loci on flower development extended to pod and seed development?

It has been suggested that some flowering genes could have a pleiotropic effect on fruit growth (Ingram, 1980; Gianfagna and Davies, 1981; Murfet, 1984). Ingram showed that although the early part of seed growth of both DN plants (e.g., lines $I_1$: *lf e sn Dne Hr* and $I_3$: *lf e sn Dne hr*) and EI-type plants (e.g., lines $I_2$: *lf E Sn Dne hr* and G2: *lf E Sn Dne Hr*) was significantly delayed under SD, the rate of seed filling was substantially decreased for line G2 only. Gianfagna and Davies (1981) also reported for line G2 that SD conditions caused a delay in early growth and a decrease in the growth rate for pod elongation, fruit weight accumulation and seed weight accumulation. Because DN lines and line $I_2$ were unaffected by photoperiod with respect to the rate of pod development and because a late line (line 53: *lf e Sn Dne hr*) behaved similarly to the DN line 58 (*lf e sn Dne hr*), Ingram concluded that *Sn* in combination with *Hr* may have a pleiotropic effect on seed development but that *Sn* alone (i.e., on an *hr* background) had either no effect or a minor effect on seed development. By contrast, with an $F_2$ progeny segregating at the *Sn/sn* locus, the pod elongation of *Sn* segregates was found to be delayed compared to *sn* segregates, at least in the early stage of elongation (Murfet, 1984). The difference in results could be explained by the fact that observations were made at two different stages of fruit development as seed expansion generally starts after pod elongation has finished (see the fruit growth pattern in Chapter 1, Section 1.3). It is thought that *Sn* activity, which appears to decrease with time (Murfet, 1971b; Reid, 1979b), is prolonged by the gene *Hr* (Murfet, 1973a; Reid, 1979a), and that gene *E* decreases the level of inhibitor produced by *Sn* in the cotyledons (Murfet, 1971c; 1973b).

Therefore, if *Sn* activity is directly implicated in fruit growth, the difference in results could be explained by a change in activity of the *Sn* gene with time.

On the other hand, it has been found that the late line 53 and the DN line 58 showed the same response to photoperiod with respect to seed growth (Ingram, 1980). However, as emphasized in Chapter 1 (Section 1.4), numerous genes could conceivably influence fruit growth, or at least fruit characteristics, and the differing genetic backgrounds of the two pure lines could mask the effect of the flowering genes. Thus, the effect of a given gene should be studied at least on a randomized background (i.e., with an $F_2$ or $F_3$ progeny) or better, with isogenic lines differing only at the given locus.

No information is available on the effect of the alleles at the *Lf* locus on pod growth though it is reported that $Lf^d$ plants (on an *sn* background) appeared to develop their pods at a relatively fast rate (Murfet, 1984).

Chapter 4 reports the effect of genetic differences at the *Sn* and *Lf* loci on the course of fruit development, that is to say, the development of the carpel inside the pollinated flower into a mature pod, at the first pod bearing node (NFP). The effect of the *Sn/sn* difference on pod growth (i.e., pod elongation, inflation and fresh seed weight accumulation) has been studied using two near-isogenic lines $299^+$ and $299^-$ (Experiments 4.1 and 4.2); the effect of the 3 alleles *lf*, *Lf* and $Lf^d$ has been examined on pod size only using segregating progenies (Experiments 4.3 and 4.4).

## 4.2 Materials and Methods

### 4.2.1 Details of Experiments

Experiment 4.1 was designed originally to investigate the influence on pod growth of the *Sn/sn* gene difference under LD and SD using two near-isolines $299^+$ (*Sn*) and $299^-$ (*sn*). However, in SD, the first pod on line $299^+$ plants set on average 20 days later than on line $299^-$ plants as a consequence of successive abortion of the lowest floral buds. Temperature records showed that in the 20 days between the start of pod development on $299^-$ and $299^+$, the day temperature fluctuated only between 18 to 20°C,

whereas after pods started to develop on $299^+$ plants it fluctuated daily from 20 to 25°C. Thus, pods of line $299^-$ developed under a lower temperature than pods of line $299^+$. In addition, the low temperatures during this period were associated with poor light quality. Thus, the comparison of lines $299^+$ and $299^-$ for the SD treatment is not valid, as plants were not placed in comparable conditions. The effect of only slight differences in temperature on seed growth has been demonstrated (Robertson, Highkin, Smydzuk and Went, 1962). The effect of the *Sn/sn* gene difference in SD was therefore re-examined when favourable conditions prevented flower abortion in line $299^+$ (Experiment 4.2). However, the comparison of lines $299^+$ and $299^-$ in LD remains valid as pods developed in similar light quality and temperature conditions. In Experiments 4.3 and 4.4, these conditions fluctuated from day to day, due to the nature of the controlled environment facilities (Section 2.2.2) but no major changes occurred during the course of the experiments.

## Experiment 4.1

Observations were made on plants used as part of Experiment 3.1 and details are given in Section 3.2.1.

## Experiment 4.2

The same near-isolines $299^+$ and $299^-$ as in Experiment 4.1 were used. Plants were grown one per pot in Glasshouse B, under a 12h photoperiod (9h of natural light plus 3h of low intensity incandescent light; see Section 2.2.2). Temperature was 15±0.5°C in the "dark" compartment and fluctuated within the range 20 to 25°C during the exposure to natural light.

## Experiment 4.3

The effect on pod development of segregation for $Lf^d/lf$ was studied in the $F_2$ of the cross 94 x 89 (*a lf e sn Dne Hr* x *A* $Lf^d$ *sn Dne hr*). The experiment was conducted under both LD and SD but only the results for the SD plants are given here. SD = 8h of natural light. Night temperature was 17±0.5°C. Day temperatures varied from 18°C to 23°C. Plants were

grown one per pot in Glasshouse A. The genotype at the *Lf* locus was identified by growing the $F_3$. The cross 94 x 89 is reported in detail in Chapter 7, but only 43 healthy plants were observed in this experiment.

Experiment 4.4

The effect on pod development of segregation for $Lf^d/Lf$ was examined in the $F_3$ descended from DN $F_2$ segregates of cross 110 x 31 (*a Lf E Sn dne hr* x *A* $Lf^d$ *E Sn Dne hr*). Plants were grown 3 per can and placed under 24h of light in Glasshouse A, where the temperatures generally fluctuated between 16 to 28°C. (For details, see Section 2.2.2.) The gene marker *a* generally helped to identify the *LfLf* segregates which were expected mostly to have white flowers since loci *a* and *Lf* are closely linked (White, 1917; Murfet, 1971b, 1975a; Chapter 7), and *a* and *Lf* are in the coupling phase. Plants with flowers and pods at the lowest nodes (11 and 12) were assumed to be homozygous for the *Lf* allele as they all had white flowers (*aa*). Red flowered plants with the highest NFP (18 to 20) were considered probably homozygous for allele $Lf^d$. The distribution of NFI for each $F_3$ family did not always allow conclusive determination of the 2 genotypes $Lf^dLf^d$ and $Lf^dLf$. *LfLf* plants were therefore compared to $Lf^d-$ plants.

## 4.2.2 Criteria of Measurement and Sampling

The criteria used to study pod growth were mainly determined by the plant source. To measure the weight of the pod and/or its components, a great number of plants is needed and the limited number of $F_2$ and $F_3$ seeds available for Experiments 4.3 and 4.4 did not allow successive pod removal. Therefore, observations were confined to measurements of pod length and breadth. Because a greater number of plants was available for lines $299^+$ and $299^-$, changes in pericarp and seed weight were also examined.

Pod lengths were measured each second day from day 3 after open flower for Experiments 4.2 and 4.3, each day from day 2 for Experiments 4.1 and 4.4 although some measurements were missed at day 4 in Experiment 4.4. Pod breadths were measured each second day for all experiments. Measurements of fresh weight accumulation were done each second day in Experiment 4.1 and each 3 to 4 days in Experiment 4.2. All definitions relative to sampling and the analysis of the data are described in Chapter 2.

When there were 2 pods per raceme, as in line $299^+$ under SD in Experiment 4.2, measurements were made on the proximal pod since distal pods do not always develop (Hole and Scott, 1983; Jaquiery and Keller, 1978; personal observations). The effect of the number of pods per raceme is studied in Chapter 5.

## 4.3 Results

### 4.3.1 Effect of the *Sn/sn* Gene Difference in Two Photoperiods

#### 4.3.1.1 Effect on pod elongation and inflation

The results show that the *Sn/sn* gene difference has little effect on the sigmoidal pattern of pod elongation and inflation in LD (Table 4.1; Figure 4.1) whereas it has a marked effect in SD (Table 4.2; Figure 4.2a). Indeed, in LD, the maximum growth rate (MGR), time to reach MGR (TMGR) and the maximum length (ML) which characterised the elongation curve were not significantly different between lines $299^+$ and $299^-$ (Table 4.1). Likewise, the changes in pod breadth over time were very similar in LD for both lines $299^+$ and $299^-$ as shown by the near superimposition of the inflation curves (Figure 4.1). By contrast, in SD, pods of line $299^+$ were significantly shorter than those of line $299^-$ (Table 4.2) but reached 95% of their ML two days later than pods of line $299^-$. This was due to a delay of about two days in TMGR and a slower MGR (MGR = 1.35 and 1.58 cm $d^{-1}$ for line $299^+$ and $299^-$, respectively; $P \leq 0.05$; Table 4.2).

The delay in TMGR for the $299^+$ SD plants was due to a delay in the onset of pod elongation, i.e., an early lack of pericarp growth. This was visually noticeable as, in SD, the carpel of $299^-$ flowers started to emerge from the withered corolla 2 to 3 days after the day of open flower, whereas in $299^+$ plants, at this stage, the carpel was still enclosed in the open flower. The flower life span (FLS) (i.e., the time interval between the first opening and final closing of the standard, corresponding to stages 0.3 to 0.9 on the Maurer scale (Maurer *et al.*, 1966)) of $299^+$ plants was significantly prolonged by an additional 2 days compared to $299^-$ plants ($P<0.001$; Table 4.2). In LD, there were no significant differences in FLS and in TMGR between lines $299^+$ and $299^-$ (Table 4.1).

The *Sn/sn* difference, in SD, also affected the pattern of pod inflation which showed an early delay in line $299^+$ (Figure 4.2a). In addition,

maximum pod breadth, though narrower, was reached later than for line $299^-$ (Figure 4.2b). However, the *Sn/sn* difference did not affect the maximum inflation rate (see Figure 4.2b).

4.3.1.2 Effect on seed weight

The *Sn/sn* difference had very little effect on seed growth in LD (Figure 4.3) whereas it had a pronounced effect in SD (Figure 4.4a). In both LD and SD, seed growth showed a general sigmoidal pattern. However, in Experiment 4.1 (i.e., in LD) where the seed weight was measured each second day, the growth curves presented 1 or 2 more or less accentuated "shoulders" (Figure 4.3). These "shoulders" corresponded to phases where the seed growth rate failed to increase or decreased sharply (Figure 4.5a). They may be due to experimental artefacts (e.g., sample size). However, they could be interpreted as lag phase(s) as described by Carr and Skene (1961) and Hedley and Ambrose (1980) (see Section 1.3). In line $299^-$, a first supposed lag phase would occur between days 13 and 15, and a second, lasting for 4 days, between days 19 and 23 after open flower (Figure 4.5a). The second lack of growth occurred simultaneously with a lack of growth of the embryo (Figure 4.5a,b). In line $299^+$, suggested lag phases of 2 days only, could occur between days 19 and 21 and between 25 and 27 after open flower (Figure 4.5a), both occurring simultaneously with a lack of growth of the embryo (Figure 4.5a and b). Nevertheless, there was little difference in the absolute fresh weight between seed of lines $299^+$ and $299^-$ in LD apart from a significant increase at about day 25 for line $299^+$ (Figure 4.3). Seeds of both lines started and finished their growth at a similar time (Figure 4.3).

In contrast, in SD, seed growth of $299^+$ plants was delayed from the very early stage of growth as the absolute weight was significantly lower than that of $299^-$ plants, 13 days after open flower (Figure 4.4a). Seeds of $299^-$ plants reached an average MGR of 61 mg $d^{-1}$ between days 16 and 20 after open flower, whereas the average MGR for $299^+$ plants was only 24 mg $d^{-1}$ between days 20 and 24. In addition, the maximum seed weight for line $299^+$ was reached later than for line $299^-$ though it represented only 75% of the maximum seed weight of $299^-$ plants. As measurements were done each 3 to 4 days in this experiment any supposed lag phase of 2 to 3 days would not be detectable.

Thus, pod and seed growth of the photoperiodic line $299^+$ (*Sn*) was delayed relative to that of line $299^-$ (*sn*) in SD but not in LD. The delay was characterized by an early lack of pod (seed) expansion and a lower rate of pod elongation and seed growth. Such an effect of the *Sn* x SD interaction on pod growth was observed consistently in other experiments (Tables 5.1, 5.3 and 5.5).

#### 4.3.1.3 Relationship between pod inflation and pericarp and seed growth

In SD, the *Sn/sn* difference did not significantly affect the maximum inflation rate of the pod (see Section 4.3.1.1; Figure 4.2b). This is possibly due to the fact that pod breadth depends on pericarp and seed growth. For example, between days 13 and 16, when the average breadth increment per day was maximal for both lines (Figure 4.2b), pericarp weight of $299^+$ plants increased on average by 130 mg $d^{-1}$ and the seed weight by only 15 mg $d^{-1}$, whereas pericarp weight of $299^-$ plants increased by only 66 mg $d^{-1}$ and the seeds increased by 38 mg $d^{-1}$ (see Figure 4.4a,b). Later in the inflation phase, between days 21 and 25, the average breadth increment per day decreased steadily for line $299^-$ but not for line $299^+$ (Figure 4.2b). During this period, the seed growth rate and possibly the pericarp weight started to decrease for line $299^-$ (see Figure 4.4a,b). In contrast, for line $299^+$, pericarp weight was still constant (as the increase in absolute weight at day 27 is most likely an artefact of sampling considering the large S.E.) but the seeds reached their phase of highest growth rate (see Figure 4.2a,b). Thus, the difference in timing and rate at which both components of the pod (pericarp and seed) increase, certainly affects the resulting breadth. Late in the inflation phase in line $299^+$, it is possible that the breadth increment is mostly due to the enlarging seed as reported by Ingram and Browning (1979) for line G2.

### 4.3.2 Effect of Allelic Differences at the *Lf* Locus

#### 4.3.2.1 Effect of segregation for $Lf^d/lf$ on an *sn Dne* background

In Experiment 4.3, NFP varied from node 10 to node 26 with an average of 10.5, 17.5 and 25.0 for genotypes *lflf*, $Lf^dlf$ and $Lf^dLf^d$, respectively (Table 4.3). There were substantial differences between the 3 genotypes

with respect to both, pod elongation and inflation (Table 4.3; Figure 4.6). The pods of *lflf* segregates reached their MGR one day later than $Lf^dLf^d$ pods and they elongated more slowly as their MGR reached only 60% of that of $Lf^dLf^d$ pods (0.79 cm $d^{-1}$ and 1.34 cm $d^{-1}$, respectively; Table 4.3). Pods of heterozygous segregates ($Lf^dlf$) elongated at a significantly slower rate than $Lf^d$ homozygotes and they also started to grow significantly later even though the difference was very slight (i.e., 0.5 day; Table 4.3). There was very little difference between the 3 genotypes with respect to final pod length (Table 4.3), but nevertheless the difference between genotypes *lflf* and $Lf^d-$ was significant at the 0.01 level ($t_{43} = 2.82$). The average maximum inflation rate (dB/dt max) and maximum breadth (MB) increased with increasing NFP (Table 4.4). This indicates that the highest pods ($Lf^dLf^d$) reached the largest breadth and they inflated at the fastest rate. They also finished their expansion earlier as the time to reach MB decreased with increasing NFP (Table 4.4). The final seed dry weight of the lowest pods (genotype *lflf*) was smaller than for genotypes $Lf^dLf^d$ and $Lf^dlf$ (Table 4.3).

#### 4.3.2.2 Effect of segregation for $Lf^d/Lf$ on an *E Sn dne hr* background

In Experiment 4.4, NFP varied from node 11 to node 20 with an average of 12.00 for the *LfLf* segregates and 16.37 for the $Lf^d-$ (homozygous and heterozygous) segregates (Table 4.5). Like in Experiment 4.3, TMGR was significantly hastened for $Lf^d-$ pods, but they did not elongate at the highest rate (1.81 and 2.15 cm $d^{-1}$ for $Lf^d-$ and *LfLf* segregates, respectively; Table 4.5). However, there was no correlation between NFP and the average inflation rate (-0.012; Table 4.6) and pods of both genotypes inflated at about the same rate (2.10 and 2.36 mm $d^{-1}$ for $Lf^d-$ and *LfLf*, respectively; $t_{39} = 1.47^{NS}$). The maximum breadth increased with increasing NFP whereas time to reach maximum breadth decreased with increasing NFP (Table 4.6) indicating that the highest pods finished inflating first, though they were larger. Seeds of the lowest pods (i.e., in genotype *LfLf*) had a smaller final dry weight than seeds of the upper pods (i.e., in genotype $Lf^d-$), (Table 4.5).

In summary, for both experiments, the maximum pod size (length and breadth) was larger and the onset of pod elongation was hastened for the $Lf^d-$ (homozygous and heterozygous) segregates. $Lf^d-$ pods developed

faster than the *lflf* pods situated at the lower nodes. However, there was little difference in the rate of development of $Lf^{d}$— and *LfLf* pods in the cross used.

## 4.4 Discussion

Results showed firstly, that the *Sn/sn* gene difference affected fruit development only in SD and secondly, that independently of the *Sn* (and *Dne*) gene, significant variation in pod growth was associated with genetic differences at the *Lf* locus for the 3 alleles studied.

The use of the two near-isolines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*) under two photoperiods demonstrates that the *Sn* gene, which confers photoperiod sensitivity on the plants (Barber, 1959; Murfet, 1971a,b) has a pleiotropic effect on pod and seed growth. The two lines $299^{+}$ and $299^{-}$ showed a similar pattern of fruit growth under LD, but under SD, the onset of both pod elongation and seed growth was delayed and the rate of pod elongation and seed growth was decreased in line $299^{+}$ compared to line $299^{-}$. It could be argued that the use of two near-isogenic lines does not exclude the possibility that the retardation and delay in pod (seed) growth may be due to a separate gene closely linked to *Sn*. However, if this was so, it would be expected that the linked gene would affect pod (seed) growth similarly in both photoperiods. Since no difference occurred in LD, it is reasonable to conclude that gene *Sn* has a pleiotropic effect, direct or indirect, on fruit development in addition to its effect on flower initiation (Murfet, 1971a,b), flower development (Chapter 3), fruit setting (Reid, 1979b, 1980) and apical senescence (Murfet, 1971a,b; Reid, 1979b, 1980; Reid and Murfet, 1984).

Photoperiod has been reported to affect pod (seed) growth in line G2 (Ingram and Browning, 1979; Gianfagna and Davies, 1981). In these studies, where the LD plants received a longer exposure to light than the SD plants, the question arises as to whether the retardation of fruit development observed under SD was the result of a difference in photosynthetic activity i.e., in assimilate availability (Gianfagna and Davies, 1981). The present work using two near-isolines under the same photoperiod indicates that the effect of the *Sn* x SD interaction is a true photoperiodic effect brought about by gene *Sn* (Reid, 1979a). Light is presumed to act by

reducing activity of the *Sn* gene (Murfet and Reid, 1974).

Because of the presence of gene *E*, flowers (and pods, in favourable conditions) appeared at the same node for both lines $299^{+}$and $299^{-}$. Gene *E* is supposed to allow early flower initiation by lowering the level of *Sn* product (inhibitor) in the cotyledons (Murfet 1971c, 1973b, see Section 1.1). However, by the time the pod develops, the activity of gene *Sn* in the shoot (Murfet, 1971c) may increase the level of inhibitor which in turn could act to delay the pod growth. However, the question arises as to whether the *Sn* output has a direct or indirect effect on pod and seed growth. In SD, plants of line $299^{+}$ produced a greater number of nodes than plants of line $299^{-}$ and they almost invariably produced two pods per raceme whereas plants of line $299^{-}$ produced only one. These results are in accord with previous reports on the effect of *Sn* on these characters (Murfet, 1971a, 1982a, 1984; Reid, 1979b; S.C. Cayzer unpublished). Thus, for line $299^{+}$, pods on the same raceme could compete for a common nutrient source and in addition, vegetative growth could compete with fruit growth. These two aspects are studied in more detail in Chapter 5.

The onset of pod and seed growth and the rate of pod elongation and seed growth weremarkedly affected by the *Sn* x SD interaction. However, even in LD, slight differences might exist between lines $299^{+}$ and $299^{-}$ in the timing and duration of phases during which the seed growth rate declines. These phases could be interpreted as lag phases as described by several authors (Carr and Skene, 1961; Burrows and Carr, 1970; Eeuwens and Schwabe, 1975; Hedley and Ambrose, 1980; see Section 1.3). However, as noted earlier the conclusive determination of lag phase(s) depends very much on the experimental conditions and sampling procedures used. Nevertheless, it is possible that seed growth in both lines showed a tri-phasic pattern with three phases of high growth rate being separated by two phases of less active growth as reported by Hedley and Ambrose (1980). The difference in timing and duration of the supposed lag phases varied between lines $299^{+}$ and $299^{-}$ in LD, and it is possible that this difference is due to the *Sn* gene. *Sn* could be active in the seed as it does not receive direct light. Such an action of the *Sn* gene in the maturing embryo has been put forward previously (Ingram, 1980). According to Hedley and Ambrose (1980) seed growth may depend on the interaction of three major factors: the maternal influence, the embryo genotype and the interaction between seed components. In addition, lag phases have been found to be

associated with changes between the cell division and the cell enlargement stages (Smith, 1973), two factors which could regulate seed growth and size as suggested by Hedley and Ambrose (1981). Thus, it would certainly be of great interest to study seed growth at a tissue and cellular level in two isolines such as $299^{+}/299^{-}$ using more strictly controlled conditions to minimize experimental artefacts. Such studies would enable a deeper insight into the influence of the *Sn* gene on seed growth.

In line G2, the much slower rate of seed growth observed in SD was associated with an increase in the $GA_{20}$ level in the seed (Ingram and Browning, 1979) which suggests a role for GAs in the inhibition of seed growth by SD. However, although quantitative and qualitative GA changes occurred between treatments,a causal relationship between the increase of $GA_{20}$ and the delay in seed growth was not clear (Ingram and Browning, 1979).

The effect of the 3 alleles, *lf*, *Lf* and $Lf^{d}$, was studied in the absence of *Sn* activity (i.e., *Sn Dne* activity; see Section 1.1) on an *sn* or *dne* background. In both experiments, the onset of pod elongation and pod inflation was hastened for $Lf^{d}$ plants, and their seeds were larger. Though observations were made on segregating $F_2$ and $F_3$ progenies, it could be argued that the promotion associated with the $Lf^{d}$ gene is in fact due to a gene or genes closely linked to $Lf^{d}$. However, as the promotion was shown to depend on whether 1 or 2 dominant alleles were present at the *Lf* locus (Experiment 4.3), the above supposition would imply that the linked gene also showed partial dominance as did $Lf^{d}$ (see Chapter 7). Therefore, it is likely that the effect on pod size growth is caused by the genetic difference at the *Lf* locus, or at least is a consequence of this genetic difference.

With the lines and environmental conditions used, the differences between *lflf* and $Lf^{d}-$ plants were more marked than between *LfLf* and $Lf^{d}-$ plants, although in both experiments, a large difference existed in NFP. This tends to indicate that although the $Lf^{d}/Lf$ gene difference has a marked effect on flower initiation, it has only a small effect on pod growth as it likewise had only a small effect on flower development (Chapter 3). On the other hand, it is possible that the genetic background masked the effect of $Lf^{d}/Lf$ difference in Experiment 4.4.

For example, plants segregating for *Lf*$^d$*/Lf* carried the *Le* allele responsible for tall phenotype (White, 1917) and the author has observed, using two pure lines, that pod elongation of a tall phenotype carrying gene *lf* was faster than pod elongation of a dwarf phenotype carrying *Lf*$^d$ (unpublished data). The particularly high MGR (an average of 2 cm $d^{-1}$) measured on the *Lf*$^d$*/Lf* segregates compared to all other lines (all dwarfs) used in this work may be a direct consequence of the presence of the *Le* gene. It is possible that in the tall phenotype, pod growth is increased to a maximum point, thus masking the effect of *Lf*$^d$*/Lf* difference. It would be of interest to study the effect of *Lf*$^d$*/Lf* difference on a dwarf phenotype and/or study the effect of *Le/le* difference on pod growth.

Allelic differences at the *Lf* locus did not affect the number of reproductive nodes nor the number of pods per raceme. Thus, unlike the *Sn/sn* result, the retardation in pod development and the smaller seeds observed with genotypes *lflf* and *LfLf* could not be accounted for by differing trophic competition between vegetative and/or reproductive growth. However, segregation for *Lf*$^d$*/lf* and *Lf*$^d$*/Lf* leads to a change in the first pod-bearing node. The effect of each allele was therefore narrowly associated with the length of the pure vegetative phase, i.e., variation in photosynthetic capacity as more leaves per plant were available to produce photosynthate in plants with a higher NFP. It has been demonstrated that the onset of fruit development in peas leads to a rapid increase in photosynthetic activity in the whole plant (Lawrie and Wheeler, 1974). On the other hand, Flinn (1974) demonstrated that, in the early stages of pod development (i.e., during the elongation and early inflation phases), the pod might be supplied mainly by its subtending leaf, whereas at the later developmental stage, i.e., seed expansion, the assimilates are also supplied by the pericarp (Flinn and Pate, 1970; Lowell and Lowell, 1970) and from other parts of the plant (Flinn and Pate, 1970). Thus, the genetic difference at the *Lf* locus may influence pod growth indirectly through its effect on photosynthetic area. However, the area of photosynthetic activity not only depends on leaf number but also on leaf size (see Harvey, 1977). A characteristic of *Lf*$^d$ plants is to produce pods grouped at the top of the plants since the internode and peduncle lengths are dramatically reduced in this part of the plant. The size of the leaves is also reduced (Murfet, 1984). Thus, even though a greater capacity for photosynthesis appears to be available from the vegetative parts of the *Lf*$^d$ plants, photosynthetic capacity may actually be reduced in the area where

pods are produced. It would be rewarding to examine in more detail the relationship between photosynthetic activity and pod (seed) growth in progenies differing at the *Lf* locus.

It has been suggested that the allelic series $lf^a$, *lf*, *Lf* and $Lf^d$ delays NFI by decreasing the sensitivity of the apex to the flowering factor(s). The promotion of the onset of pod elongation, the larger seeds and the faster rate of inflation and elongation in the presence of the higher order alleles *Lf* and $Lf^d$, support the hypothesis that, by the time pods develop in the late region of these plants, the underlying metabolic processes would be heavily geared towards reproductive growth (Murfet, 1984).

TABLE 4.1 Effect of the *Sn/sn* gene difference in LD (24h) on 3 parameters[1,2,3] characterizing the curve of elongation of the pod growing at the first reproductive node and the flower life-span (FLS) for lines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*). Data from Experiment 4.1. n = number of replicates.

| | $299^{+}$ | | $299^{-}$ | | t-test |
|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | $\bar{x}$ | ±SE | |
| ML[1] | 7.90 | 0.18 | 7.79 | 0.18 | NS[†] |
| MGR[2] | 0.96 | 0.54 | 1.03 | 0.05 | NS |
| TMGR[3] | 6.02 | 0.21 | 5.60 | 0.21 | NS |
| FLS | 1.58 | 0.19 | 1.41 | 0.15 | NS |
| n | 12 | | 14 | | |

[1] Maximum length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to reach maximum growth rate (day)

[†] NS: means are not significantly different at the 0.05 probability level

TABLE 4.2 Effect of the *Sn/sn* gene difference in SD (12h) on 4 parameters[1,2,3,4] characterizing the curve of elongation of the pod growing at the first reproductive node and on the flower life-span (FLS) for lines $299^+$ (*Sn*) and $299^-$ (*sn*). Data from Experiment 4.2. n = 10.

| | $299^+$ | | $299^-$ | | t-test |
|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | $\bar{x}$ | ±SE | |
| ML[1] | 7.76 | 0.22 | 9.49 | 0.17 | ***[†] |
| MGR[2] | 1.35 | 0.09 | 1.58 | 0.07 | * |
| TMGR[3] | 7.31 | 0.18 | 5.34 | 0.12 | *** |
| T95%ML[4] | 11.65 | 0.24 | 9.80 | 0.23 | *** |
| FLS | 3.71 | 0.21 | 1.90 | 0.10 | *** |

[1] Maximum length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to reach maximum growth rate (day)

[4] Time to reach 95% of the maximum length (day)

[†]: *,*** Means significantly different at the 0.05 and 0.001 probability level, respectively

TABLE 4.3 Effect of segregation for $Lf^d/lf$ in the $F_2$ of cross 89 x 94 on the node of the first pod (NFP), final dry weight per seed (SW,mg) and on 3 parameters[1,2,3] characterizing the curve of elongation of the pod growing at NFP. SD (8h). Data from Experiment 4.3. n = number of replicates.

| | *lflf* | | $Lf^d lf$ | | $Lf^d Lf^d$ | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | $\bar{x}$ | ±SE | $\bar{x}$ | ±SE |
| NFP | 10.5 | 0.94 | 17.5 | 0.58 | 25.0 | 1.12 |
| SW | 296.0$^a$ | 22.33 | 373.7$^b$ | 13.84 | 380.2$^b$ | 26.7 |
| ML[1] | 5.32$^a$ | 0.22 | 6.03$^b$ | 0.13 | 5.93$^{ab}$ | 0.26 |
| MGR[2] | 0.79$^a$ | 0.05 | 1.09$^b$ | 0.03 | 1.34$^c$ | 0.06 |
| TMGR[3] | 5.38$^a$ | 0.16 | 4.66$^b$ | 0.10 | 4.08$^c$ | 0.19 |
| n | 10 | | 26 | | 7 | |

[1] Maximum length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to reach maximum growth rate (day)

$^{a,b,c}$ Means followed by the same letter within rows are not significantly different at the 0.05 probability level.

TABLE 4.4 Correlation between the node of the first pod (NFP) and the maximum pod breadth (MB), the time to reach the maximum breadth (TMB) and the average maximum inflation rate (dB/dt max) in the $F_2$ of cross 89 x 94 in SD (8h). Data from Experiment 4.3. n = 43.

| | MB | TMB | dB/dt max |
|---|---|---|---|
| NFP | 0.650*** | -0.609*** | 0.553*** |

*** Significant at the 0.001 probability level

TABLE 4.5 Effect of segregation for $Lf^{d}/Lf$ in the $F_3$ of cross 31 x 110 on the node of first pod (NFP), final dry weight per seed (SW,mg) and on 3 parameters[1,2,3] characterizing the curve of elongation of the pod growing at NFP. LD (24h). Data from Experiment 4.4. n = number of replicates.

| | *LfLf* | | $Lf^{d}$– | | t-test |
|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | $\bar{x}$ | ±SE | |
| NFP | 12.00 | 0.15 | 16.37 | 0.97 | *** |
| SW | 232.00 | 8.68 | 255.59 | 4.30 | * |
| ML[1] | 5.91 | 0.10 | 6.35 | 0.06 | *** |
| MGR[2] | 2.15 | 0.08 | 1.81 | 0.03 | *** |
| TMGR[3] | 3.13 | 0.05 | 2.74 | 0.007 | ** |
| n | 10 | | 31 | | |

[1] Maximum length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to reach maximum growth rate (day)

*,**,***: Differences between means are significant at the 0.05, 0.01, 0.001 probability level, respectively

TABLE 4.6 Correlation between the node of first pod (NFP) and the maximum breadth (MB), time to reach the maximum breadth (TMB) and the average maximum inflation rate (dB/dt max) for segregates in the $F_3$ of the cross 31 x 110. LD (24h). Data from Experiment 4.4. n = 41.

| | MB | TMB | dB/dt max |
|---|---|---|---|
| NFP | 0.492*** | -0.513*** | -0.012 |

***: Significant at the 0.001 probability level

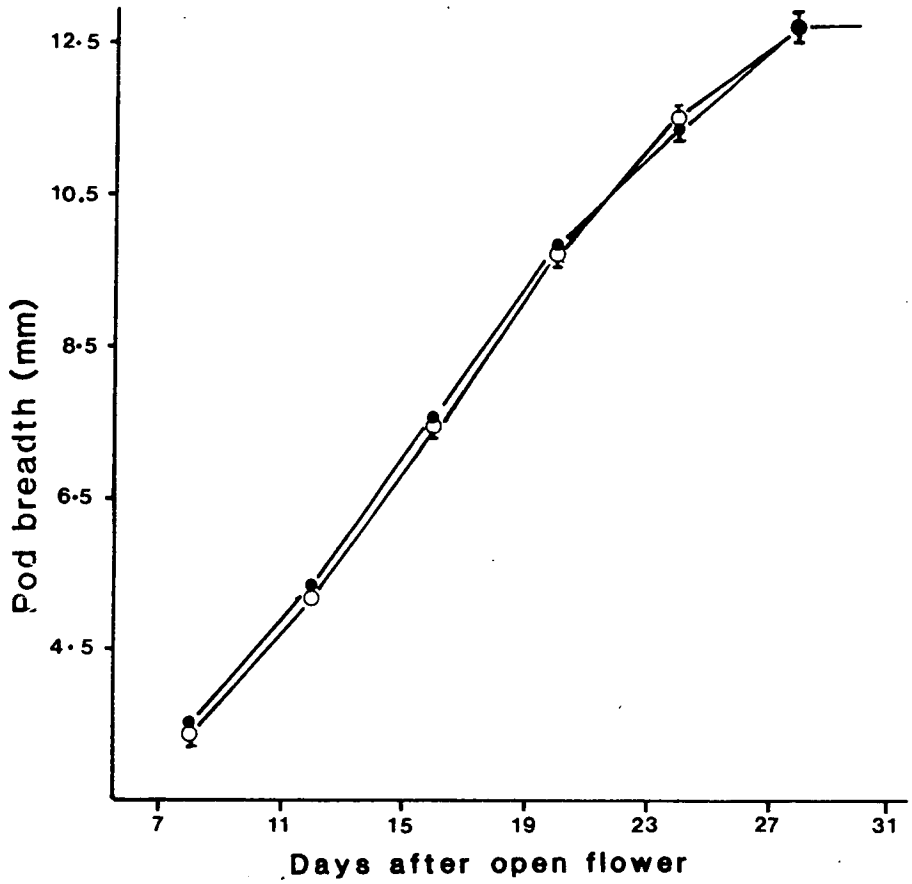
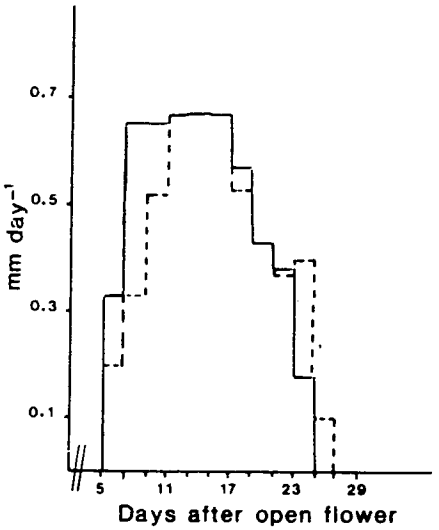



FIGURE 4.1 Changes in pod breadth over time for two near-isogenic lines $299^{+}$ (○) and $299^{-}$ (●) grown in LD (24h). SE>$\pm$0.2 mm are shown as vertical bars. Number of replicates = 12 and 14, respectively. Data from Experiment 4.1.

(b)




(a)

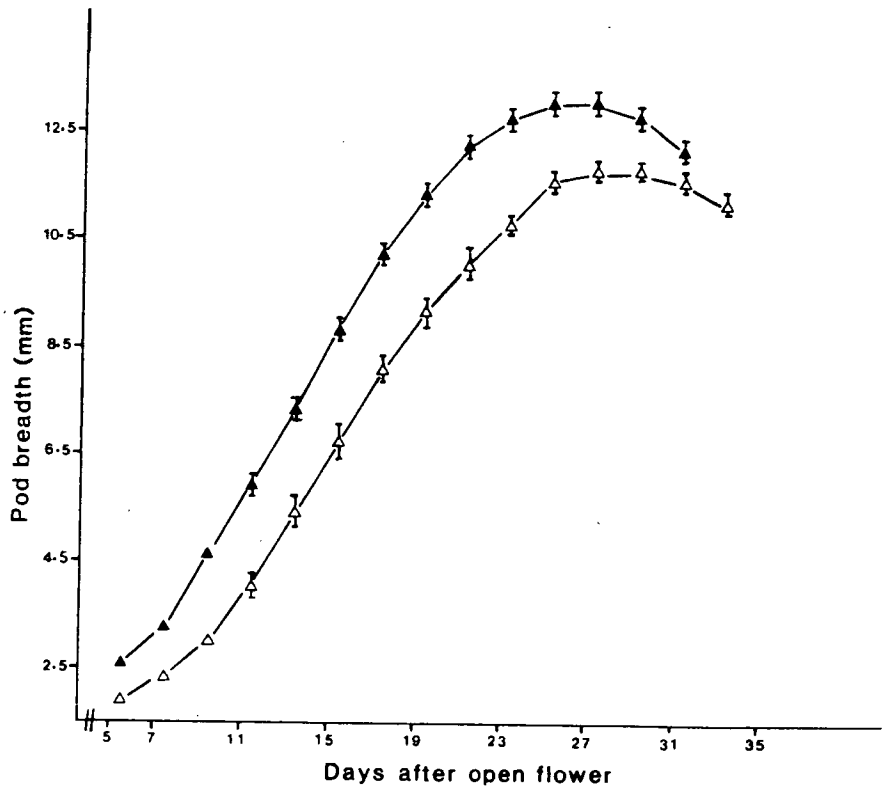



FIGURE 4.2 Pod breadth for two near-isogenic lines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*) grown under a 12h photoperiod. (n = 10). Data from Experiment 4.2.

(a) Changes in pod breadth over time. Line $299^{+}$ (△); line $299^{-}$ (▲). SE$\pm$0.2 mm are represented as vertical bars.

(b) Changes in the average growth rate ($mmd^{-1}$) over time. Line $299^{+}$ (---); line $299^{-}$ (—).

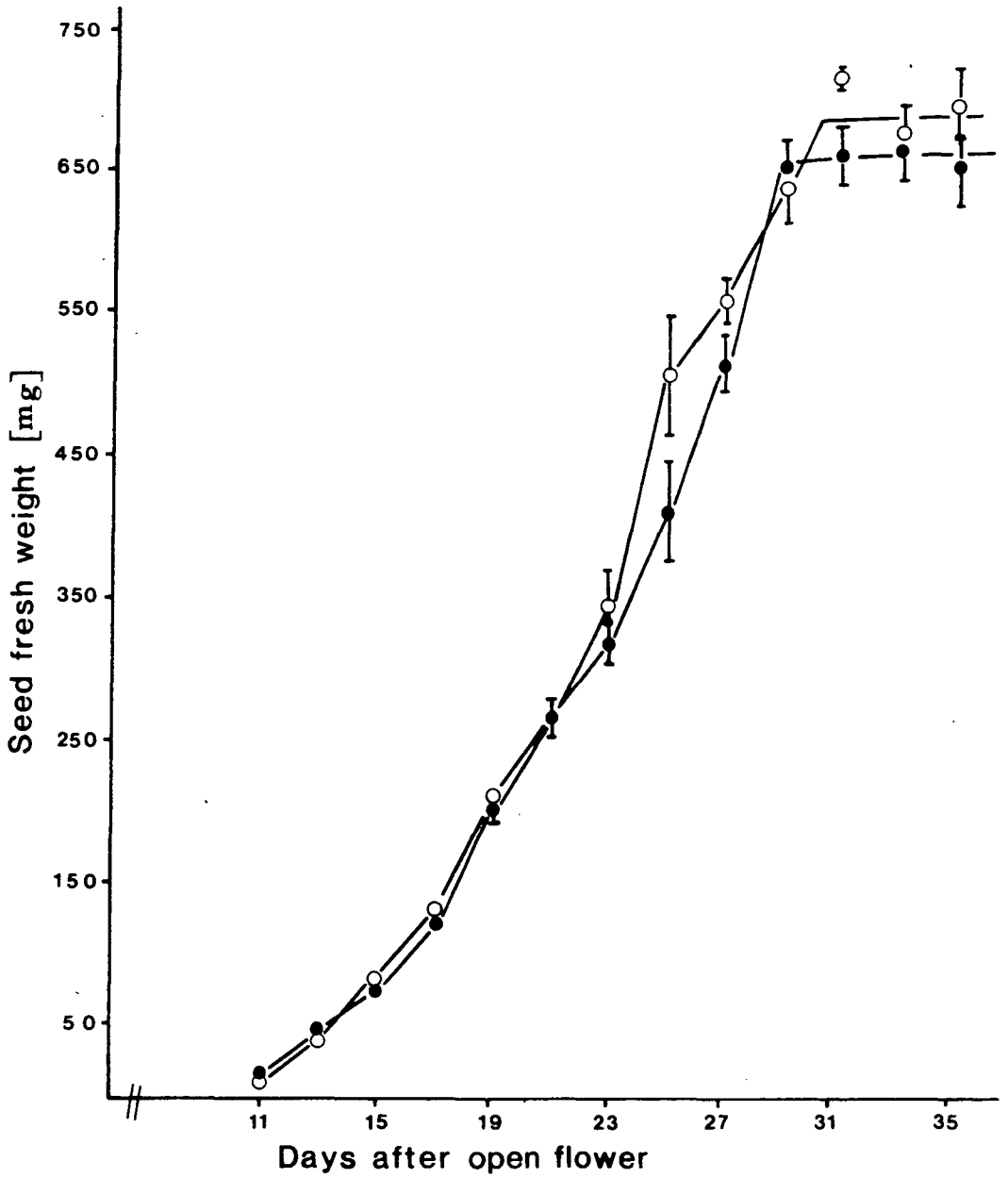



FIGURE 4.3 Changes in seed fresh weight over time for two near-isogenic lines $299^{+}$ (○) and $299^{-}$ (●) grown under LD (24h). SE$\geq\pm$10 mg are represented as vertical bars. Minimum number of seeds weighed per sample = 12. Data from Experiment 4.1.

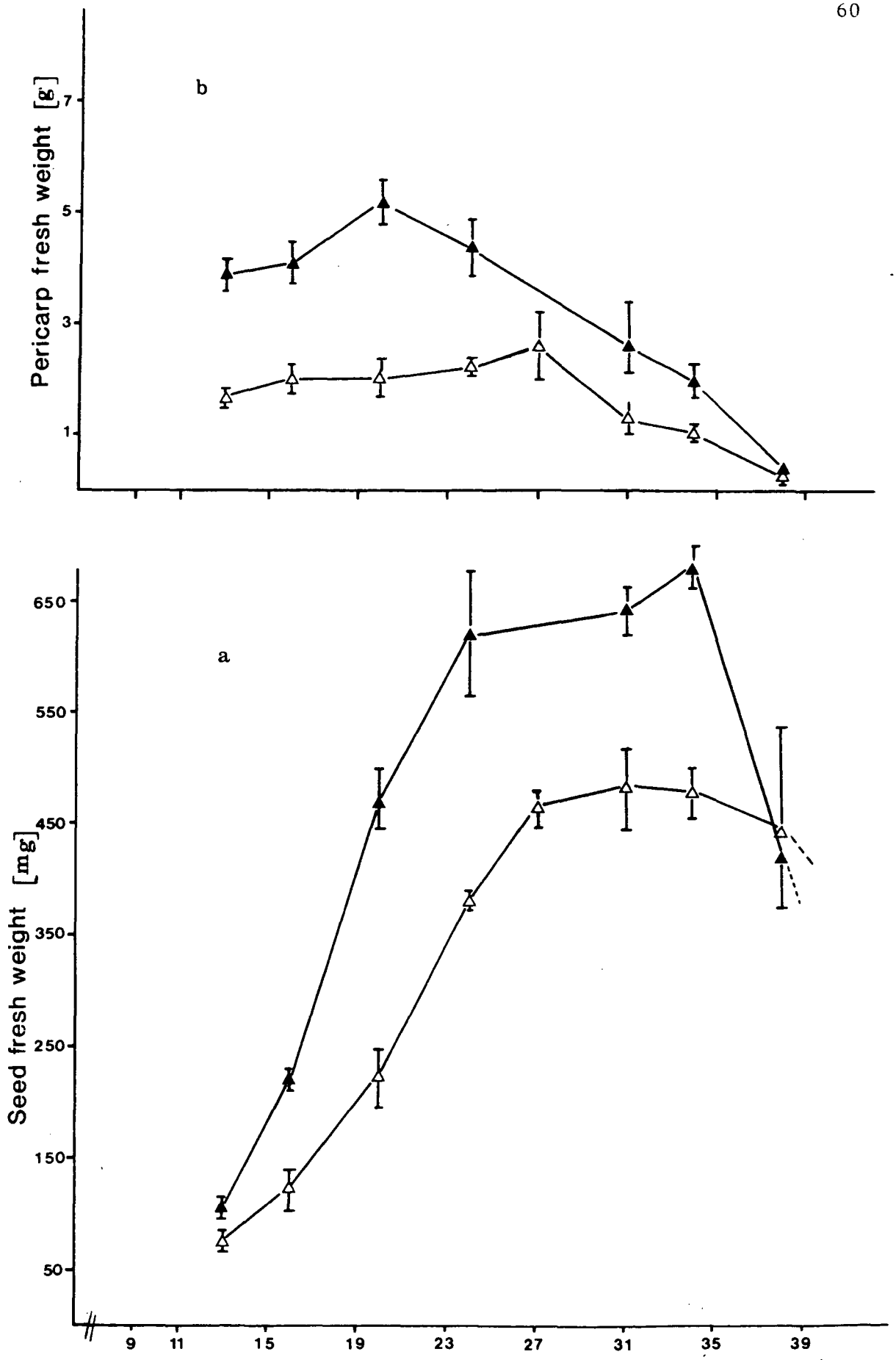



FIGURE 4.4 Changes in fresh weight over time of seed (a) and pericarp (b) for two near-isogenic lines $299^{+}$ (△) and $299^{-}$ (▲) grown under a 12h photoperiod. SE$\pm$10 mg are shown as vertical bars. For (a), n = 16; for (b), n = 4. Data from Experiment 4.2.

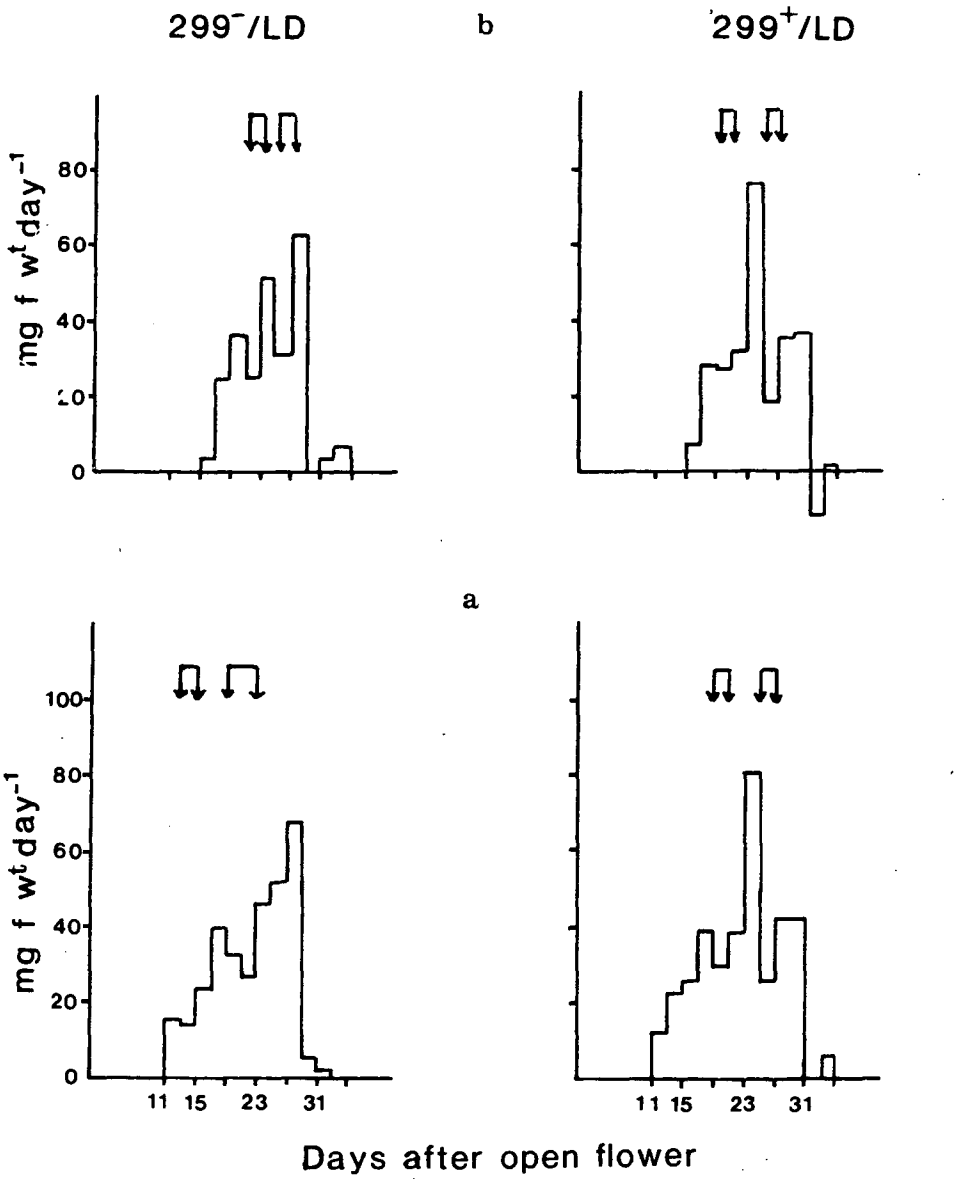



FIGURE 4.5 Average changes in fresh weight day$^{-1}$ for the seed (a) and embryo (b) for the two near-isogenic lines 299$^{-}$ and 299$^{+}$ grown in LD (24h). Minimum number of seeds weighed per sample = 12. Data from Experiment 4.1.

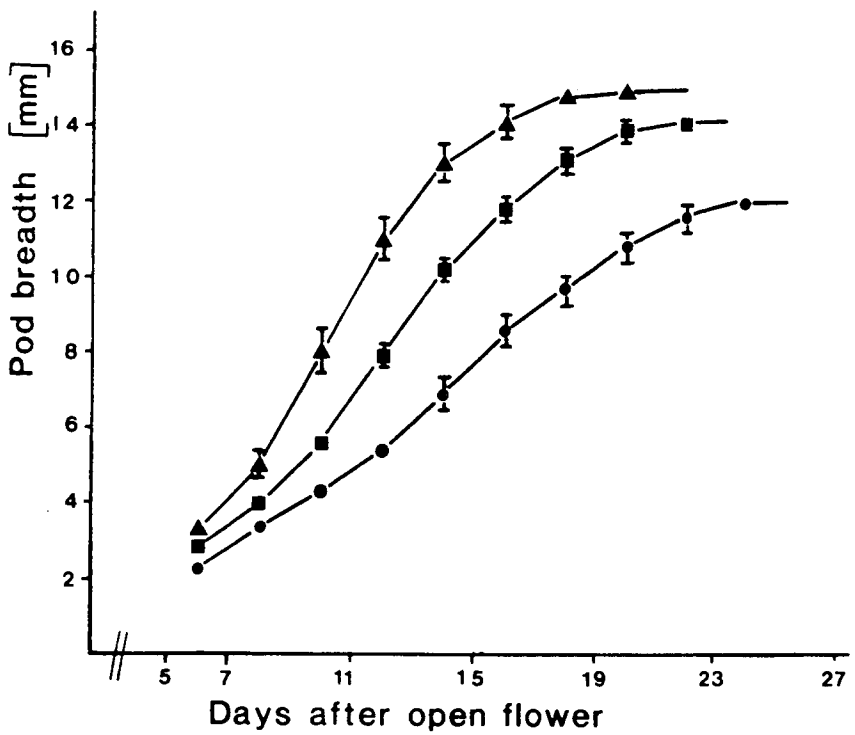



FIGURE 4.6 Effect of segregation for $Lf^d/Lf$ on the pod breadth changes over time. $Lf^dLf^d$ (▲); $Lf^dlf$ (■); $lflf$ (●) with n = 10, 26 and 7, respectively. SE>$\pm$0.4 mm are shown as vertical bars. SD = 8h. Data from Experiment 4.3.

# CHAPTER 5 POD AND SEED DEVELOPMENT: INTERACTION BETWEEN VEGETATIVE AND REPRODUCTIVE GROWTH IN *Sn* AND *sn* PLANTS

## 5.1 Introduction

SD delayed the onset and decreased the rate of pod and seed growth in an EI line (*Sn*) and not in its equivalent DN line (*sn*), indicating that *Sn* activity is causally implicated in fruit growth (Chapter 4). The slower fruit growth was associated with several morphological changes, e.g., a higher number of pods per node and enhancement of vegetative growth since the total number of nodes was increased. Effects of the *Sn* gene on the number of flowers (thus pods) per raceme (S.C. Cayzer, unpublished; Murfet, 1984) and on cessation of apical growth (Murfet, 1971a,b; Reid 1979b, 1980) have been described previously. Because of the narrow interrelationship between vegetative and reproductive growth, the question arises as to whether the *Sn* "flowering" gene exerts a direct effect on pod growth and/or whether it has an indirect action through its effect on vegetative growth and number of pods per raceme.

Hole and Scott (1983) reported that increasing number of pods per raceme was associated with decreasing pod and seed weight. The same authors reported that there seemed to be more competition between fruits at the same node than at successive nodes. Competition for nutrients and/or hormones could cause the abortion of the most distal young pod on a multiflowered raceme (Kambal, 1969; Jaquiery and Keller, 1978). As DN plants usually develop only one pod per node, or at least less pods than EI and L plants, such a competition would be eliminated or reduced in DN plants. Experiment 5.1 examines the effect of number of pods per node on pod growth to see whether *Sn* activity could retard fruit growth via an effect on inflorescence ontogenesis.

The fact that deflowering, depoding and/or deseeding stops or delays apical senescence of plants has led many authors to conclude that growing fruits and especially seeds are able to produce a hormonal senescence factor (Lockhart and Gottschall, 1961; Malik and Berrie, 1975; Davies *et al.*, 1977; Woolhouse, 1982) or that they represent a sink which attracts nutrients and/or hormones to the detriment of the vegetative growth (Bollard, 1970; Davies *et al.*, 1977). Thus, *Sn* activity would delay

apical arrest by delaying fruit setting (Reid, 1979b, 1980). On the other hand, there is evidence that the flowering inhibitor produced by the *Sn* gene directly delays apical senescence (Reid, 1980; Gianfagna and Davies, 1981) and it has been put forward that *Sn* activity may delay apical senescence by diverting the metabolism towards vegetative growth (Reid and Murfet, 1984). On the contrary, in a DN phenotype (*sn*) and/or in photoperiodic plants under LD, resources would be directed away from vegetative growth after a short growth period (Reid and Murfet, 1984). Thus, it is possible that pods on *Sn* plants in SD are delayed in their development because they must compete with vegetative growth. The growing apex, and especially the young developing leaves, are reported to inhibit fruit growth on a pea plant (Carbonell and Garcia-Martinez, 1980; Garcia-Martinez and Carbonell, 1980) either by acting as a source of inhibitor, or as a competitive sink. In Experiment 5.2, pod growth was compared in decapitated EI and DN plants. If the delay in pod growth in *Sn* plants is a consequence of the growing activity of the apex, then pods of decapitated plants of both DN and EI type should show the same growth.

Vegetative growth in DN plants is less competitive with pod growth since the terminal bud stops growing early in the reproductive phase and the lateral buds generally stay inhibited. In contrast, both EI and L type plants have a marked tendency to produce basal laterals under SD regardless of whether they flower at a low node (EI) or a high node (L) (Murfet and Reid, 1985). The effect of lateral growth on pod growth of both DN and EI plants was studied by allowing the lateral buds, released from apical dominance after removal of the apex, to grow (Experiment 5.3). In this experiment, the effect of the *Sn/sn* gene difference on branching in decapitated plants was also examined.

## 5.2 Materials and Methods

### Experiment 5.1

The effect of the number of pods per raceme on pod growth was studied on the late line 53 (*lf e Sn Dne hr*). Under LD, this line produces one flower per inflorescence and generally 2, rarely 3 flowers under SD. Plants were grown one per pot and exposed either to LD (24h of light) or SD (10h of natural light) in Glasshouse B. Details about light conditions are given in Section 2.2.2. Temperature was 16±0.5°C in the "dark" chambers

and 20 to 25°C during the exposure to natural light.

Treatments were as follows:

T24: LD, plants intact.
T10: SD, plants intact.
T10.1: The distal flower bud at NFP was removed the day the proximal flower on the same node opened, i.e., at FT.
T10.2: The distal flower bud was removed on each raceme the day the proximal flower of that inflorescence opened.

The length and the breadth (as defined in Section 2.2.3) of the remaining pod for the first reproductive node (NFP) were measured each day, from day 3 and 4 after open flower for length and breadth, respectively. Details of the analysis of the growth curves are given in Section 2.2.4.

Experiment 5.2

The two lines $299^-$ (*sn*) and $299^+$ (*Sn*) were compared under SD. The comparison between near-isolines rather than between photoperiods overcomes the problem of the possible difference in photosynthetic activity between LD and SD. Seeds of lines $299^-$ and $299^+$ were planted one per pot. The experiment was conducted under 12h of light in Glasshouse B (10h of natural light + 2h of artificial light; for details, see Section 2.2.2). Temperature in the "dark" chambers was 15±0.5°C and 20 to 26°C during the exposure to natural light.

Treatments were as follows:

$299^+$I = line $299^+$, plants intact.
$299^+$D = line $299^+$, plants decapitated.
$299^-$I = line $299^-$, plants intact.
$299^-$D = line $299^-$, plants decapitated.

For both lines, the apex was removed the day the first flower opened (i.e., FT) by cutting the shoot about 1 cm above the first reproductive node. The distal flower, in line $299^+$, was removed at the same time. Removing the apex at this late stage of flower formation overcame the problem of flower bud abortion in the EI plants and thus, decapitation was

rarely done above a node at which the flower ultimately abscised. Moreover, in the conditions used only a small number of flowers aborted. All laterals were removed as soon as they appeared in decapitated and intact plants. Pod length and breadth (as defined in Section 2.2.3) were measured each second day from day 3 and 6 after open flower, respectively.

Experiment 5.3

The same lines, growing conditions and procedures were used as in Experiment 5.2 with the following difference: in a third group of plants, all laterals were allowed to grow once the apex had been removed, i.e., two additional treatments were included:

$299^{+}$DLAT and $299^{-}$DLAT.

The length of the laterals was measured on days 7 and 14 after open flower (i.e., after decapitation) and at the time pods started to dry. Pod length and breadth (as defined in Section 2.2.3) were measured each second day from day 3 and 5 after open flower, respectively. The data were analysed as described in Section 2.2.4.

## 5.3 Results

### 5.3.1 Effect of Pod Removal

In SD, in Experiment 5.1, the removal of the distal flower at either the first or all reproductive nodes of line 53 (treatments T10.1 and T10.2), respectively) had only a small effect on development of the proximal pod at NFP. It caused a slight increase in the rate of pod elongation since MGR of T10.2 pods was significantly higher than that of T10 pods (1.02 cm $d^{-1}$ and 0.89 cm $d^{-1}$, $P<0.05$, Table 5.1). The effect was similar when the distal flower was excised only at the first or at all reproductive nodes (0.98 cm $d^{-1}$ for T10.1 and 1.02 cm $d^{-1}$ for T10.2). However, even in the T10.1 or T10.2 treatments, MGR was still lower than in treatment T24 (LD). The removal of the distal flower did not affect the onset of pod elongation, at least in the experimental conditions used, since TMGR was the same for T10, T10.1 and T10.2 (Table 5.1). This is supported by the fact that FLS was unchanged for any of the SD treatments T10, T10.1 and T10.2 (Table 5.2). Likewise, the removal of the distal flower at

either the first or all reproductive nodes did not affect either the pattern of pod inflation (see Figure 5.1) or the final dry weight of the largest seed per pod (Table 5.2).

The removal of the distal flower caused a decrease in the peduncle length which, in intact plants, was about twice as long under SD as under LD (Table 5.2). The longer peduncle on *Sn* plants under SD was observed in all experiments where EI and/or L type plants were used and has been reported earlier (Murfet, 1984). Thus, the number of flowers per raceme affects the length of the peduncle, at least when the number of flowers is associated with the *Sn* gene.

Thus, in SD, the number of growing pods per raceme had very little effect on the growth of the proximal pod at NFP and even when only one pod was allowed to grow at each reproductive node, pod development at NFP was still retarded compared to the LD treatment. However, the number of flowers per inflorescence greatly affected the peduncle length of the same inflorescence.

### 5.3.2 Effect of the Removal of the Apical Bud

Decapitation tended to promote pod growth in both lines $299^+$ and $299^-$ as shown by the larger ML and MGR (Tables 5.3 and 5.5), the larger pod breadth during the whole pod inflation phase (Figure 5.2) and the larger final dry seed weight (SW; Tables 5.4 and 5.6) for treatment $299^+$D (decapitated) than $299^+$I (intact) and for treatment $299^-$D than $299^-$I. However, for line $299^-$ the difference in MGR did not reach the 5% probability level in Experiment 5.3 (Table 5.5). In line $299^-$, decapitation had little effect on TMGR and T95%ML (Tables 5.3 and 5.5). By contrast, in line $299^+$, there was a significant decrease in TMGR and T95%ML in $299^+$D compared with $299^-$I in Experiment 5.3 (Table 5.5) but this difference did not exist in Experiment 5.2 (Table 5.3). However, of more interest is the fact that removal of the apex tended to eliminate the effect of the *Sn/sn* gene difference on pod growth, e.g., ML and MGR were similar for treatments $299^+$D and $299^-$D in Experiment 5.2 (Table 5.3), although a significant difference still occurred with respect to ML in Experiment 5.3 (Table 5.5). The pod inflation curve of $299^+$D plants was displaced towards the curve for line $299^-$D plants although pod breadth remained narrower in $299^+$D plants than in $299^-$D plants

(Figure 5.2). The final dry weight of the seed remained statistically different between treatments $299^{+}D$ and $299^{-}D$ in Experiment 5.2 (Table 5.4); however, in Experiment 5.3, the difference in final seed weight was not statistically different though it remained nearly as large as the one between treatments $299^{+}I$ and $299^{-}I$ (Table 5.6). The removal of the apex also reduced the seed abortion generally noticeable in EI and L type plants in SD, so that the number of developing seeds per pod (DS/P) was similar in treatment $299^{+}D$ and the two $299^{-}$ treatments (Table 5.4).

### 5.3.3 Effect of the Growing Laterals

In the decapitated EI and DN plants, the *Sn/sn* gene difference influenced the outgrowth of the lateral buds (Experiment 5.3; Table 5.7). The total length of laterals was similar for both lines until 14 days after decapitation. However, after day 14, the difference between treatment $299^{+}$DLAT and $299^{-}$DLAT started to be displayed and by the time the pod became yellow (on average 42 and 38 days for line $299^{+}$ and $299^{-}$ plants, respectively), $299^{+}$DLAT plants had developed a total lateral length 3 times greater than that of $299^{-}$DLAT plants (Table 5.7). The total length of laterals on $299^{-}$DLAT plants resulted almost entirely from the outgrowth of the axillary bud situated closest to the decapitated end of the main shoot. In contrast, for $299^{+}$DLAT plants, the total length of laterals resulted from the outgrowth of the axillary bud on the same upper-most node plus the outgrowth of axillary buds situated at the lowest nodes, i.e., nodes 1, 2 and 3 mainly.

The growing laterals did not affect greatly the pod growth of the decapitated $299^{-}$ plants whereas they retarded pod growth of the decapitated $299^{+}$ plants (Table 5.5; Figure 5.3). MGR, TMGR, T95%ML and SW were similar in $299^{-}D$ and $299^{-}$DLAT plants (Tables 5.5 and 5.6) and there was only a slight decrease in ML and pod breadth for $299^{-}$DLAT plants compared to $299^{-}D$ plants (Table 5.5 and Figure 5.3). Moreover, pod breadth was affected only in the late part of the inflation phase (from day 20 after open flower (and decapitation) onward; Figure 5.3). In contrast, MGR, pod breadth and SW were significantly decreased in the treatment $299^{+}$DLAT compared to the treatment $299^{+}D$ (Tables 5.5, 5.6 and Figure 5.3). The effect of the growing laterals on pod breadth started at least from day 7 after open flower ($P \leq 0.05$) and continued until maximum breadth was reached

(Figure 5.3). However, the growing laterals did not affect TMGR in the elongation phase (Table 5.5) nor the number of developing seeds per pod (Table 5.6).

In summary, the effect of the *Sn/sn* gene difference on pod growth in SD remained even after all distal flowers were removed. In contrast, the effect of this genetic difference on pod growth and seed abortion was partly eliminated when both the apex and the laterals were removed, but differences tended to remain with respect to seed weight. The growth of the laterals (which was greater for the photoperiodic line $299^{+}$ (*Sn*) than for the DN line $299^{-}$ (*sn*)) and the presence of the apical bud had an inhibitory effect on pod growth for both lines but to a far greater extent in *Sn* than *sn* plants.

## 5.4 Discussion

Removal of one or all distal flower(s) had approximately the same effect on pod growth indicating little competition between pods at the same or at successive nodes. Thus, the increased number of pods per raceme does not appear to be the reason why pods on *Sn* plants develop more slowly in SD than in LD. It has been reported for peas that the total number of fruits on the stem slightly affected the growth of the remaining pod at NFP (Hole and Scott, 1983). The same authors concluded that there seems to be more competition between fruits at the same node than between fruits at successive nodes. However, they suggested that, as the distal pods are more inclined to abort (Jaquiery and Keller, 1978; Snoad, 1974), it is possible that they accumulate less dry matter because the abscission phenomenon is already taking effect at the tissue level, rather than because of a competition with the other pods of the inflorescence. Examples are given in the literature where an increase in seed size was blocked *in situ* and not *in vitro* showing that abscission deprives the fruit of assimilate supplies (see Nitsch, 1970). In photoperiodic lines, the distal pods commonly abort, but the abortion rate appears to decrease with increasing plant vigour (personal observation). The lack of apparent competition between pods at successive nodes could be explained by the fact that the increase in number of pods is also associated to an increase in leaf number (because of an increase in node number) which in turn increases the photosynthetic area. Thus, it is possible that plants, in line 53 at least, may be able to supply a larger number of pods and the restriction in assimulates, if

any, could be compensated for by pod and seed abortions.

The slower pod growth of EI and L type plants in SD may be more dependent on metabolite partitioning between the different parts of the plant than on the absolute amount of available assimilates. It has been suggested that apical senescence or at least cessation of apical activity was delayed partly through a direct action of *Sn* activity (Reid, 1979b, 1980; Gianfagna and Davies, 1981) as confirmed in a recent study (Reid and Murfet, 1984). The *Sn* flowering gene may act by diverting the metabolites (hormones and/or nutrients) to the apex allowing vegetative growth but depriving reproductive growth (Murfet, 1984; Reid and Murfet, 1984). The results of Experiment 5.2 lend support to this hypothesis since, although the apex had a correlative inhibitory effect on fruit growth in both photoperiodic and DN lines, it had more effect on the former. In the case of the *sn* plants, metabolites would be geared towards reproductive growth earlier than in *Sn* plants. In contrast, for the *Sn* plants, at the time the first pod develops, the metabolic process would still be geared largely in favour of the vegetative growth, and thus, the removal of the apical bud (i.e., a competitive sink) would have more effect than in *sn* plants. The hypothesis of a competition for metabolites between vegetative and reproductive growth is also supported by the results of Experiment 5.3 where the limited vegetative growth of *sn* plants had little effect on pod growth whereas with *Sn* plants, laterals developed profusely and the pod growth was delayed. However, the possibility that the apex exerts an effect on pod growth by acting as a source of inhibitor is not excluded.

In addition to its effect through the actively growing main and lateral apices, *Sn* may have a direct effect in pods and/or seeds. Seed weight was still different after decapitation in Experiment 5.2 and differences in seed growth pattern were found in LD between *Sn* and *sn* plants (Chapter 4). In addition, the fact that the number of growing pods per stem had only a small effect on pod growth at NFP may indicate that *Sn* also prevents the developing pod from metabolising the extra-nutrients available. The genotype of the embryo or testa could impose a limit in seed size. Such a consequence of the embryo genotype has been put forward by Hedley and Ambrose (1980) and it would be rewarding to study the growth of *Sn* and *sn* embryos and seeds *in vitro*, i.e., away from any maternal influences.

Generally, seeds of EI and L type plants (e.g., line $299^{+}$ and line 53) have a tendency to abort under SD, whereas DN lines are little affected by photoperiod. When the apex was removed, the number of developing seeds was similar in both line $299^{-}$ and line $299^{+}$ and the growing laterals did not reverse this effect. This tends to indicate that the high frequency of ovule abortion generally exhibited in photoperiodic types under SD is not due to a lack of pollination and/or fertilisation as reported by Linck (1961) and suggested by Pate and Flinn (1977). In contrast, it appears that the developing seeds are dependent on the amount of assimilates available in the early stage of seed growth, as suggested by Hedley and Ambrose (1981). The fact that lateral growth affects final seed dry weight without affecting the number of developing seeds could suggest that the inter-seed competition occurs from an early stage. For example, in Experiment 5.3 seeds of decapitated plants would have a strong enough sink-activity to get their fair amount of nutrients by the time developing laterals play their antagonistic rôle. By contrast, in intact *Sn* plants, only the seeds with the greatest growth rate or greatest sink-activity would be able to compete for more limited nutrient resources. Seeds situated at both ends of the pod are generally the most susceptible to abortion (Linck, 1961; Hedley and Ambrose, 1981; personal observations), which means that the seeds most capable of competition are situated in the middle of the pod. The cause for such a pattern within the pod remains unexplained.

It appears possible that seed number is associated with seed size. When young seeds were surgically aborted, the size of the remaining seeds was significantly increased (Harvey, cited by Hedley and Ambrose, 1981). In the same way, there was a negative correlation between seed number per pod and seed weight in *Phaseolus vulgaris* (Al-Mukhtar and Coyne, 1981). Secondly, it is suggested that the strength of a sink is determined by its size and its activity, i.e., its relative growth rate (Warren-Wilson, 1972). Therefore, an increased percentage of ovule abortion would certainly decrease the size of the sink capacity. The mechanism(s) implicated in the sink-activity are not well understood but it may be possible that hormone-directed transport is playing an important role in the redistribution of assimilates (see Phillips, 1975). It has been suggested that fertilized ovules and growing seeds synthesize hormones (Eeuwens and Schwabe, 1975; see Wang and Sponsel, 1985) which would divert the metabolite transport towards the fruit (Seth and Wareing, 1967). Thus a decrease in the number of growing seeds, i.e., hormone-synthesisors, may lead to

a decrease in physiological sink activity. Thereby, the growing apex may have an additional effect on pod growth through an indirect and early effect on seed setting.

The study of the effect of the actively growing shoots (e.g., laterals and apical bud) on pod growth in EI and DN lines complements the information already available on the effect of developing pods and seeds on apical arrest and senescence, where it was suggested that growing seeds restrain apical growth by causing nutrient drainage and/or by producing an hormone-like senescence factor (Lockhart and Gottshall, 1961; Malik and Berrie, 1975; Davies *et al.*, 1977; Reid, 1979b, 1980; Gianfagna and Davies, 1981; Wang and Woolhouse, 1982; Woolhouse, 1982). Thus, the two vegetative and reproductive parts seem to interact with each other both ways and the question remains as to where the primary control is exerted, although it has been conclusively shown on *veg* plants that gene *Sn* affects apical senescence independently of flowering (Reid and Murfet, 1984).

Decapitation, in both EI and DN plants caused the outgrowth of the highest axillary bud, i.e., the bud situated immediately underneath the node at which the pod developed. The outgrowth of the most proximal axillary bud after the release of apical dominance in pea is well documented (see Champagnat, 1965). In the DN line $299^{-}$, lateral outgrowth was largely limited to the uppermost axillary buds. On rare occasions, a non-vigorous lateral developed from a basal node. By contrast, in the EI line $299^{+}$, the stem produced many laterals at the lowest nodes in addition to laterals arising near the top of the stem. It is possible that in the DN plants, the outgrowth of the lowest axillary buds was inhibited by the actively growing uppermost shoot as suggested by Champagnat (1965). In contrast, in the EI type, the uppermost shoot did not have such a strong effect and the lowest axillary buds were able to develop.

The differences in the pattern of branching between the DN and EI types are consistent with those reported by Murfet and Reid (1985) for intact plants where the EI and L type segregates were found to branch freely from the basal nodes, whereas DN segregates had a low tendency to form basal branches. In the same way, in *Lathyrus odoratus*, the late flowering photoperiodic types (genotypes $Dn^{h}$ and $Dn^{i}$) were reported to branch profusely whereas the DN phenotypes (genotype *dn*) consisted mainly

of a single shoot (Ross, 1983). The use of two lines nearly isogenic, except at the *Sn* locus, strongly indicates that the *Sn* flowering gene encourages the production of basal laterals as suggested by Murfet and Reid (1985). Ross (1983) reported that the alleles at the *dn* locus in *Lathyrus odoratus* influence branching independently of the effect that the apical bud exerts on axillary buds since the initial stimulation of lateral outgrowth induced by removal of the apex was similar in magnitude in both L and DN phenotypes. It was suggested by Ross, that the alleles at the *dn* locus may control lateral outgrowth by influencing assimilate partitioning. It seems very likely that a similar mechanism could control lateral growth in the related species *Pisum sativum,* especially since in *Pisum* stocks, the *Sn/sn* gene difference influences axillary bud outgrowth in a *Lathyrus* scion (Ross, 1983). Further more, as in *Lathyrus odoratus,* branching does not appear to be a consequence of the flowering behaviour (i.e., early or late initiation) as the early photoperiodic plants have the same tendency to branch as the late photoperiodic types (Murfet and Reid, 1985).

Thus it is possible that *Sn* activity by favouring vegetative growth at the apex and at the basal part of the plant, perhaps by diverting metabolites to these regions, deprives the growing fruits of assimilates. It would certainly be rewarding to follow the accumulation of labelled material in the different parts of *Sn* and *sn* plants in relation to the reproductive events, defined if possible, at a molecular level (e.g., DNA activity) to distinguish between cause and effect of the assimilate partitioning.

TABLE 5.1 Means for 3 parameters[1,2,3] characterizing the curve of elongation of the proximal pod growing at the first reproductive node for plants of line 53. Treatments are:- LD 24h, plants intact (T24); SD 10h, plants intact (T10); SD 10h, distal flower excised at NFP (T10.1); SD 10h, distal flower excised at all reproductive nodes (T10.2). Data from Experiment 5.1. (F: $P \leq 0.001$.)

| Treatment | MGR[1] | TMGR[2] | T95%ML[3] | n |
|---|---|---|---|---|
| T24 | $1.24^{c}$ | $4.94^{a}$ | $9.48^{a}$ | 14 |
| T10 | $0.89^{a}$ | $7.26^{b}$ | $13.98^{c}$ | 8 |
| T10.1 | $0.98^{ab}$ | $7.48^{b}$ | $14.10^{c}$ | 8 |
| T10.2 | $1.02^{b}$ | $6.84^{b}$ | $12.81^{b}$ | 9 |

[1] Maximum growth rate (cm $d^{-1}$)

[2] Time to reach maximum growth rate (day after first open flower)

[3] Time to reach 95% of the maximum length (day after first open flower)

*a,b,c* Means followed by the same letter within each column are not significantly different at the 0.05 probability level.

TABLE 5.2 Peduncle length (PL,cm), flower life span (FLS,days) and dry weight (mg) of the biggest seed per pod (SWmax, mg) for the proximal pod at the first reproductive node on line 53 plants. Treatments are:- LD 24h, plants intact (T24); SD 10h, plants intact (T10); SD 10h, distal flower excised at the first reproductive node (T10.1); SD 10h, distal flower excised at all reproductive nodes (T10.2). Data from Experiment 5.1. n = number of plants.

| Treatment | PL | | | FLS | | | SW max | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| T24 | 4.75 | 0.38 | 14 | 1.60 | 0.13 | 14 | 372 | 15 | 12 |
| T10 | 10.21 | 0.58 | 8 | 3.63 | 0.26 | 8 | 333 | 10 | 8 |
| T10.1 | 7.00 | 0.32 | 9 | 3.38 | 0.18 | 8 | 333 | 10 | 8 |
| T10.2 | 6.75 | 0.38 | 12 | 3.25 | 0.13 | 12 | 334 | 10 | 10 |

TABLE 5.3 Means for 4 parameters[1,2,3,4] characterizing the curve of elongation of the pod growing at the first reproductive node for plants of lines $299^+$ and $299^-$ intact ($299^+$I and $299^-$I) or decapitated ($299^+$D and $299^-$D). SD = 12h. Data from Experiment 5.2.

| Treatment | ML[1] | MGR[2] | TMGR[3] | T95%ML[4] | n |
|---|---|---|---|---|---|
| $299^+$ | $7.30^a$ | $1.05^a$ | $7.70^b$ | $12.97^c$ | 20 |
| $299^-$ | $9.08^b$ | $1.34^b$ | $5.73^a$ | $10.58^a$ | 18 |
| $299^+$D | $10.51^c$ | $1.65^c$ | $7.70^b$ | $12.39^c$ | 10 |
| $299^-$D | $10.77^c$ | $1.63^c$ | $5.71^a$ | $10.80^b$ | 13 |
| F ratio ($P \leq 0.001$) | 89.24 | 40.22 | 59.58 | 25.74 | |

[1] Maximum pod length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to maximum growth rate (days after first open flower)

[4] Time to reach 95% of maximum length (day after first open flower)

$^{a,b,c}$ Means followed by the same letter within each column are not significantly different at the 0.05 probability level.

TABLE 5.4 Flower life span (FLS,days), final dry weight per seed (SW,mg) and number of developing seed per pod (DS/P) for lines $299^+$ and $299^-$ either left intact ($299^+$I and $299^-$I) or decapitated ($299^+$D and $299^-$D). SD (12h). Data from Experiment 5.2. n = number of plants.

| Treatment | FLS | | | SW | | | DS/P | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| $299^+$I | $3.87^b$ | 0.14 | 18 | $220.5^a$ | 5.13 | 18 | $4.1^a$ | 0.29 | 18 |
| $299^-$I | $2.11^a$ | 0.11 | 18 | $292.2^b$ | 11.14 | 18 | $6.2^b$ | 0.32 | 18 |
| $299^+$D | $3.89^b$ | 0.19 | 10 | $309.8^b$ | 10.56 | 10 | $6.4^b$ | 0.49 | 10 |
| $299^-$D | $2.03^a$ | 0.13 | 14 | $379.1^c$ | 19.29 | 14 | $6.9^b$ | 0.40 | 14 |

$^{a,b,c}$ Means followed by the same letter within each column are not significantly different at the 0.05 probability level.

TABLE 5.5 Means for 4 parameters[1,2,3,4] characterizing the curve of elongation of the proximal pod growing at the first reproductive node for lines $299^+$ and $299^-$ grown in SD (12h). Treatments are: Plants intact, all laterals removed ($299^+$I and $299^-$I); plants decapitated, all laterals removed ($299^+$D and $299^-$D); plants decapitated and laterals allowed to grow after decapitation ($299^+$DLAT and $299^-$DLAT). Data from Experiment 5.3.

| Treatment | ML[1] | MGR[2] | TMGR[3] | T95%ML[4] | n |
|---|---|---|---|---|---|
| $299^+$I | $7.76^a$ | $1.35^a$ | $7.3^c$ | $11.65^b$ | 10 |
| $299^-$I | $9.50^{bc}$ | $1.58^b$ | $5.33^a$ | $9.80^a$ | 10 |
| $299^+$D | $9.82^{bc}$ | $1.66^b$ | $6.00^b$ | $10.40^a$ | 8 |
| $299^-$D | $11.14^d$ | $1.75^b$ | $5.37^a$ | $10.10^a$ | 11 |
| $299^+$DLAT | $9.04^b$ | $1.31^a$ | $6.30^b$ | $11.40^b$ | 9 |
| $299^-$DLAT | $10.28^c$ | $1.62^b$ | $5.30^a$ | $10.03^a$ | 10 |
| F Ratio ($P \leq 0.001$) | 17.22 | 4.75 | 22.49 | 9.23 | |

[1] Maximum pod length (cm)

[2] Maximum growth rate (cm $d^{-1}$)

[3] Time to maximum growth rate (days after first open flower)

[4] Time to reach 95% of maximum length (day after first open flower)

$^{a,b,c,d}$Means followed by the same letter within columns are not significantly different at the 0.05 probability level.

TABLE 5.6 Number of developing seeds per pod (DS/P) and final dry weight of the seed (SW,mg) for the first pod for lines $299^+$ and $299^-$ in SD (12h). For a description of treatment codes, see legend for Table 5.5. Data from Experiment 5.3. n = number of plants.

| | DS/P | | | SW | | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| $299^+$I | $4.27^a$ | 0.31 | 10 | $213.2^a$ | 13.88 | 10 |
| $299^-$I | $6.30^b$ | 0.26 | 10 | $253.98^{bc}$ | 12.18 | 10 |
| $299^+$D | $6.25^b$ | 0.67 | 8 | $271.87^{cd}$ | 17.01 | 8 |
| $299^-$D | $7.08^b$ | 0.50 | 12 | $311.15^d$ | 18.27 | 12 |
| $299^+$D Lat | $6.30^b$ | 0.41 | 11 | $222.88^{ab}$ | 10.66 | 11 |
| $299^-$D Lat | $6.50^b$ | 0.70 | 10 | $296.7^d$ | 13.78 | 10 |

$^{a,b,c,d}$ Means followed by the same letter within each column are not significantly different at the 0.05 probability level.

TABLE 5.7 Total length of laterals on decapitated plants of lines $299^+$ and $299^-$ measured three times after apex removal. Data from Experiment 5.3. n = number of replicates. SD (12h).

| Line | Day 7 | | | Day 14 | | | Pod Maturity | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| $299^+$ | 9.30 | 1.38 | 11 | 32.70 | 3.60 | 11 | 142.91 | 14.60 | 11 |
| $299^-$ | 9.73 | 2.25 | 9 | 26.60 | 2.70 | 10 | 45.60 | 3.22 | 10 |

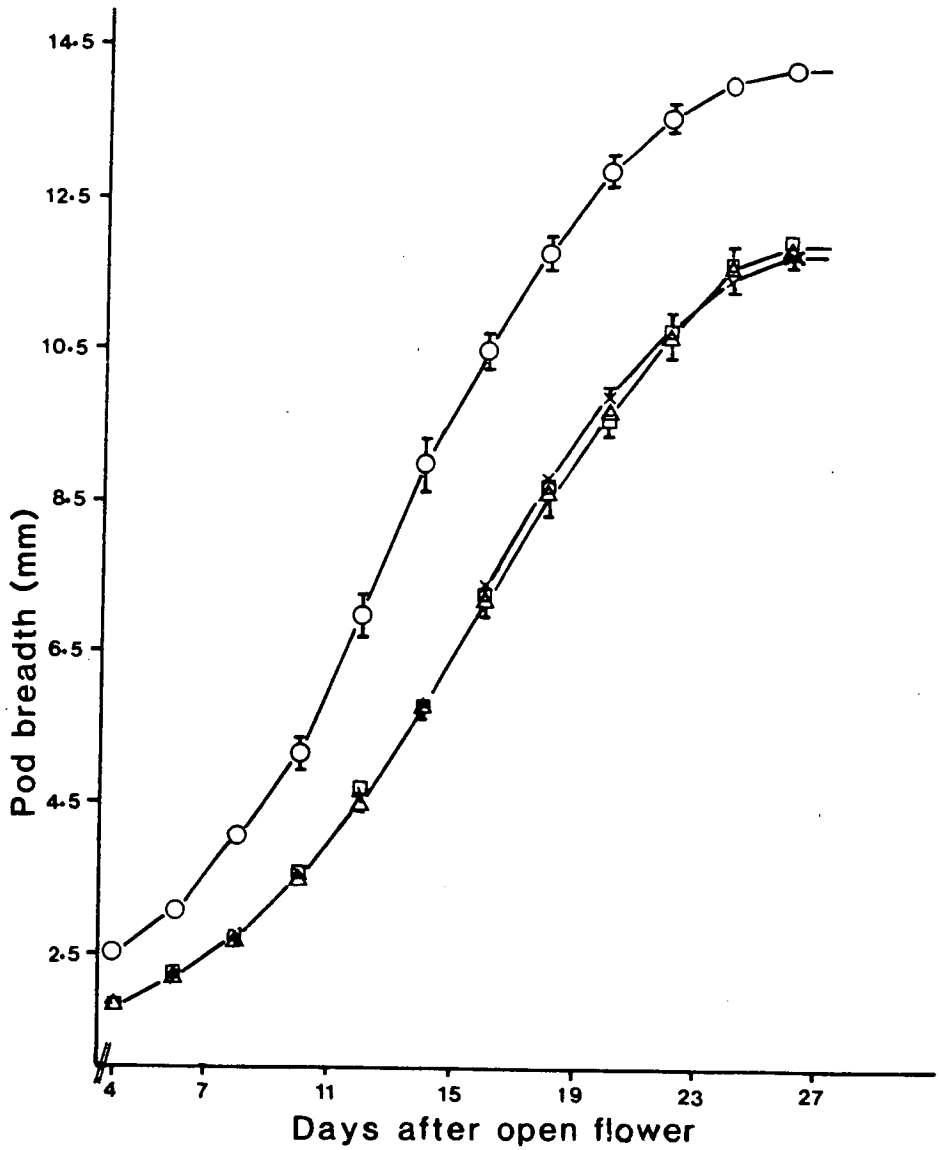



FIGURE 5.1 Changes in pod breadth over time for the proximal pod at the first reproductive node for line 53. Treatments are:- LD (24h), plant intact (○); SD (10h), plant intact (△); SD (10h), distal flower excised at NFP (□); SD (10h), distal flower excised at all reproductive nodes (×). SE>±0.2 mm are shown as vertical bars. (n≥8). Data from Experiment 5.1.

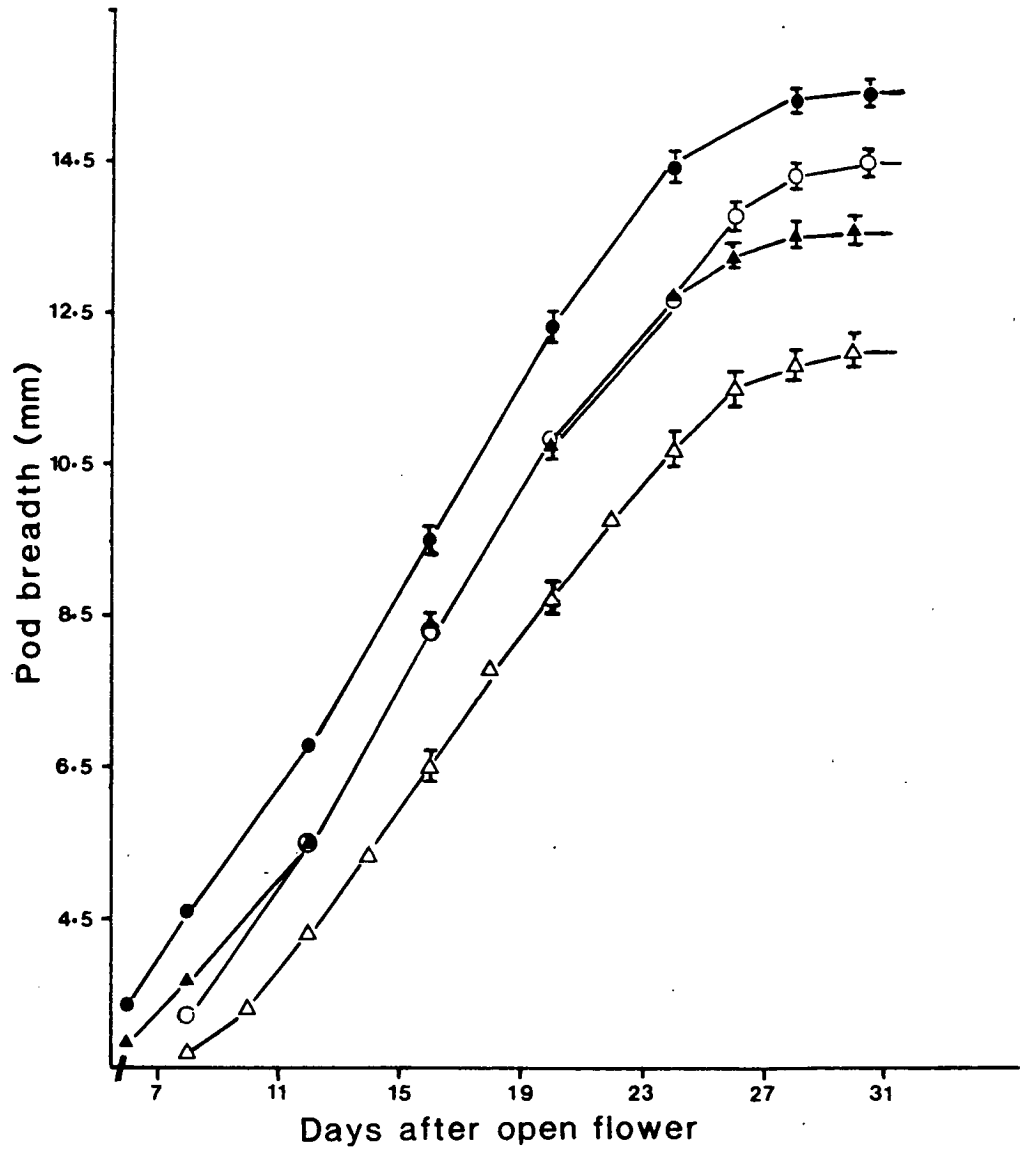



FIGURE 5.2 Changes in pod breadth over time for two near-isogenic lines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*) under SD (12h) either left intact (△ and ▲) or decapitated above the first pod-bearing node (○ and ●). SE>±0,2 mm are shown as vertical bars (n≥10). Data from Experiment 5.2.

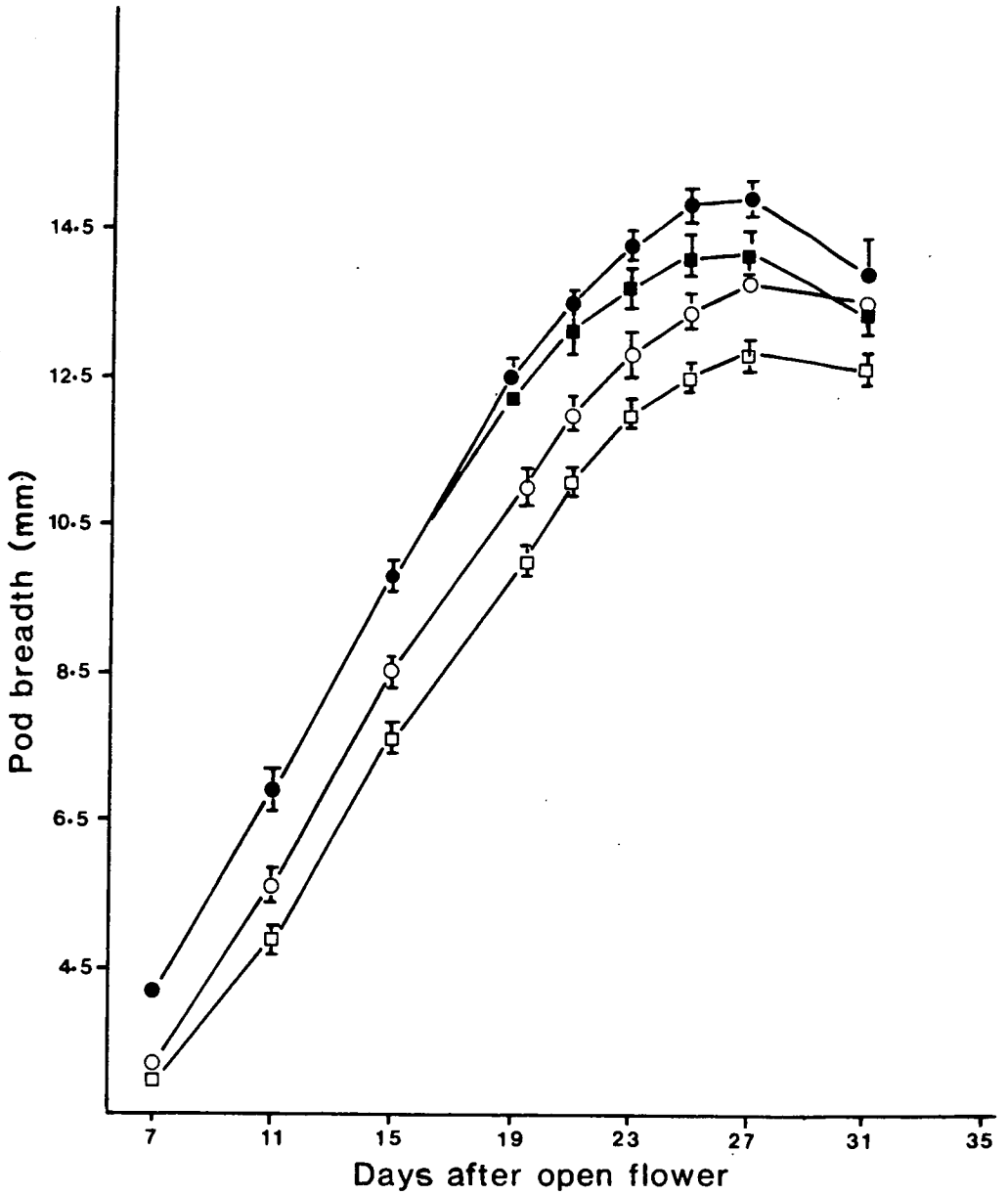



FIGURE 5.3 Changes in pod breadth over time for two near-isogenic lines $299^{+}$ (*Sn*) and $299^{-}$ (*sn*) decapitated above the first pod-bearing node and with laterals left to develop (□ and ■) or excised (○ and ●). SD (12h), SE>$\pm$0.2 mm are shown as vertical bars; n≥8. Data from Experiment 5.3.

# CHAPTER 6 EFFECT OF LACK OF POLLINATION AND/OR FERTILISATION ON FLOWER LIFE-SPAN AND POD DEVELOPMENT; RATE OF POLLEN TUBE GROWTH IN *Sn* AND *sn* PLANTS

## 6.1 Introduction

The flowering gene *Sn* has a pleiotropic effect on flower life-span (Chapters 4 and 5; Murfet, 1984) and the prolonged FLS in the photoperiodic line $299^{+}$ in SD was associated with a retardation in the onset of pod elongation and seed development (Chapter 4). Both FLS and the early retardation in pod growth were not affected by treatments which promoted the rate of pod growth (Experiments 5.1 and 5.2). The question arises as to whether the early delay in pod (seed) growth and the prolonged FLS are due to a delay in pollination and/or fertilisation. The stimulatory effect of pollination (fertilisation) on the onset of ovary growth has been observed in numerous species (see Nitsch, 1970). In peas, an increased level of hormones in the endosperm is concomitant with pericarp elongation (Eeuwens and Schwabe, 1975) and extracts of pea seeds restored normal growth of seedless pods (Eeuwens and Schwabe, 1975). These authors suggested that pollination and/or fertilisation could provide a stimulus for fruit growth by initiating the synthesis and degradation of hormones in the tissues of the ovules. On the other hand, fertilisation (and/or pollination) has been reported also to shorten FLS in orchids (see Goodwin, 1978) and carnations (Nichols, 1971).

In Experiment 6.1, the effect of lack of pollination (fertilisation) on FLS of pea flowers was examined using a progeny segregating for genetically fertile and male sterile phenotypes. In the latter plants, the flower does not produce any fertile pollen grains and fertilisation therefore does not occur. In this experiment the subsequent development of the fertile and male sterile flowers is also examined since the latter frequently give rise to small parthenocarpic pods.

The aim of the second experiment is two-fold. There is a pronounced and consistent deficiency of recessive plants in families segregating at the *Sn* locus (Murfet, 1971a). Previous studies attempted to establish whether the deficiency could be attributed to a differing growth rate between *Sn* and *sn* pollen tubes using *in vitro* culture (G. Rowberry, unpublished data). Rowberry found that *Sn* pollen tubes grew faster than *sn* pollen tubes during the first 3 hours but after 6 hours, the pollen

tube length of both genotypes did not differ. This early promotion of *Sn* pollen tube growth appears not to fit the hypothesis that a delay in fertilisation could account for the delay in onset of pod elongation and the prolonged FLS in *Sn* plants. However, pollen tubes growing *in vitro* are isolated from maternal influences, whereas the stylar tissue could influence the growth of the pollen tube as suggested by other authors (Wolf, 1973a,b; McLeod, 1975). Nitsch (1965) has also pointed out the unreliability of certain *in vitro* techniques for the study of pollen tube growth. Thus, an attempt was made to follow, *in situ*, the growth of the *sn* and *Sn* pollen tubes until they enter the micropyle, to see if gametophytic selection and the early lack of pod elongation could both be attributed to a difference in pollen tube growth (Experiment 6.2). The influence of the maternal tissue was studied using reciprocal crosses.

## 6.2 Materials and Methods

### Experiment 6.1

To investigate the effect of lack of pollination and fertilisation on FLS and pod development, several $F_4$ progenies segregating for fertile and male sterile plants were used. Male sterility was inherited as a single-gene recessive trait (see Chapter 2; Section 2.1). All plants in the progenies were homozygous for the major flowering genes *Lf*, *Sn*, *Dne*, and *hr* and they therefore behaved as members of the late phenotypic class (Murfet, 1971a; see Section 1.1). The use of genetically male sterile plants is preferable to mechanical removal of the young anthers from the floral bud as wounding of the keel and other floral parts may cause physiological changes which could affect FLS,e.g., release of ethylene (Hall, 1951).

Plants were grown two per pot and placed either in SD (8h of natural light) or in LD (24h). The plants were grown in Glasshouse A and details of this facility are given in Section 2.2.2. "Night" temperatures varied for the LD treatment with a mean of about 15°C but were held at 17±1°C for the SD treatment. Temperatures ranged from 20 to 26°C during the exposure to natural light.

Three times a day, the flower at NFD was checked for its opening stages as defined by Maurer *et al.* (1966). On the Maurer scale, the standard petal starts to open at 0.3 and at 0.9 the standard completely encloses the keel and shows signs of wilting.

Experiment 6.2

Pollen tubes were examined using the technique of fluorescence microscopy (see O'Brien and McCully, 1981). A fluorochrome (aniline blue in this case) confers a yellow fluorescence to the callose plugs formed by the pollen tubes while they are elongating and the fluorescent compound can then be viewed with a microscope equipped with a series of special filters allowing the transmission of the emitted fluorescence. The 2 near-isolines $299^{+}$ and $299^{-}$ described in Section 2.1 were used. The plants were grown in Glasshouse B under SD = 12h (10h of natural light + 2h of artificial light; for details see Section 2.2.2). Temperatures at the time of experimentation fluctuated between 20 and 25°C during the exposure to natural light and was 16±0.5°C in the "dark" chamber.

Preliminary observations showed that pollination (i.e., contact of pollen with the stigmatic surface, after release of the pollen by the anthers) occurred at a similar stage of flower bud development in both line $299^{+}$ and line $299^{-}$, i.e., between stage 0.1 and 0.2 on the Maurer scale when the buds measure about 10 mm. Emasculation of the flowers to be used as females was done at a very similar stage for *Sn* and *sn* flowers, between 0.1 and 0.2 on the Maurer scale, but slightly ahead of anther dehiscence. Pollen was taken from the donor when the flowers were between 0.3 and 0.5 on the Maurer scale. Four crosses (*Sn*♀ x *Sn*♂ and *sn*♂ and *sn*♀ x *Sn*♂ and *sn*♂) were carried out. It is essential to use flowers at a similar stage of development to limit variability among factors which could influence pollen tube growth, e.g., receptivity of the stigma, pollen vigour etc. However, not all the flowers to be used were ready at the same time. To allow for the fact that pollination had to be done at different times, the flowers for the 2, 4, 8 and 23 hour treatments were chosen at random amongst the pollinated flowers. After removal of a large part of the pericarp, the remaining gynoecium was fixed in an ethanol/acetic acid solution (3:1) for a minimum of 4h and transferred to a 70% ethanol solution for storage. Then the technique used by R.B. Knox (Melbourne University; N. Brown, personal communication) and Williams, Knox and Rouse (1982) was followed. The gynoecium was transferred to a 10% sodium sulfite solution for at least 24h to soften the tissue and rinsed in distilled water before being stained in decolorized aniline blue for 24h. Then, the gynoecium was mounted in the stain and the style was squashed beneath the coverslip to liberate the pollen tubes from the stylar canal.

Though softened, the styles were not easily squashed and only slides with well squashed styles were observed under the epifluorescence microscope. A total of 64 flowers were examined. The fluorochrome also became bound to the lignified walls of the vascular tissue; however, the latter was easily recognisable because of its lignification pattern and thus could not be confused with pollen tubes. The material was not treated to remove autofluorescence which gave a yellow colour (see Plate 6.1).

## 6.3 Results

In Experiment 6.1, FLS varied from 28 to 66 hours depending on the phenotype and the photoperiod (Table 6.1). FLS was longer for male sterile than fertile plants by 25h in LD and 13h in SD (Table 6.1). SD treatment also prolonged FLS regardless of pollination (fertilisation) since a significant photoperiod effect occurred in both fertile and male sterile flowers (Table 6.1).

The male sterile flowers developed into small, swollen, parthenocarpic pods about 4 cm long (Table 6.2). This represented 60% of the length of pods with seeds, which developed from the fertile flowers (Table 6.2). However, in SD, the male sterile flowers abscised a few days after withering at the first 7 reproductive nodes whereas in LD, they all developed into parthenocarpic pods (Table 6.2). In addition, the parthenocarpic pods of the male sterile plants were smaller in SD than in LD (3.15 and 4.15 cm, respectively; Table 6.2). No abortion occurred for the fertile plants so that NFI = NFP and pods had the same length in SD as in LD (Table 6.2).

Thus, the lack of pollination (fertilisation) prolonged FLS as did SD. However, the effect of SD is, partly at least, independent of the pollination (fertilisation) effect. The lack of pollination (fertilisation) also had a drastic effect on the node of the first developing pod and pod length which was accentuated in SD.

The germination and growth of pollen tubes as viewed under the fluorescence microscope is shown in Plate 6.1. The pollen grains entrapped in the stylar hairs germinated on the stigma tip (Plate 6.1a) and sent tubes down into the style (Plate 6.1b). The number of pollen tubes found in

the lower part of the style decreased dramatically compared to the number of pollen tubesvisible in the upper part. This is especially noticeable in hand-pollinated flowers as the self-pollinated flowers exhibited a larger number of pollen tubes all the way down to the ovule area. The pollen tubes continued to grow beneath the upper suture of the ovary and they finally reached the ovule micropyle (Plate 6.1c).

In spite of the difficulties sometimes encountered in following the tubes all the way down through the style, no apparent differences existed between the 4 crosses at any time. Two hours after pollination, germination of the pollen grains on the stigma was noticeable in all crosses but no pollen tubes were distinguishable along the style (Table 6.3). After 4h, no flower on the *sn* mother plants showed visible pollen tubes reaching two-thirds of the way down the style and only one flower out of 16 on *Sn* mother plants had pollen tubes reaching this region (Table 6.3). Eight hours after pollination, all flowers except one *Sn* flower showed pollen tubes in the area of the ovules and often it was possible to see a pollen tube close to the micropyle of an ovule (Table 6.3). The same situation occurred 23h after pollination. In this case also, pollen tubes were not visible in the ovary on one *Sn* flower (Table 6.3). However, in both cases where the pollen tube growth was apparently delayed very few pollen tubes were present in the upper part of the style. It was not always possible to see if the nearby pollen tube had or had not penetrated the micropyle of the ovules and thus the time of fertilisation cannot be determined, assuming that fertilisation occurs soon after the pollen tube enters the micropyle. Nevertheless, the fact that 8h after pollination all pollen tubes were seen in the micropyle area indicates very strongly that the 2 day difference in FLS and in TMGR generally observed between *Sn* and *sn* plants in SD cannot be accounted for by a difference in time of fertilisation.

It is not possible to conclude whether or not the rate of pollen tube growth differs between the crosses. However, the fact that, 4h after fertilisation, pollen tubes appeared to be at the same level in the style for all crosses (except for one flower) would suggest that there is no appreciable difference in the rate of pollen tube growth for *Sn* and *sn* pollen tubes on either *Sn* or *sn* maternal tissue. The difference, if any, is certainly too slight to be detected using this technique.

## 6.4 Discussion

The results show that, although the lack of pollination (fertilisation) has a prolonging effect on FLS, the longer FLS and the early delay in pod elongation observed in EI type plants in SD are not due to a delay in the time of fertilisation. In LD conditions, i.e., away from *Sn* activity, the flowers on male sterile plants stayed open 24h longer than their counterparts on fertile plants, which clearly indicates that the lack of germinating pollen and/or lack of fertilisation prolongs the life-span of the flower. However, the fact that FLS of the male sterile flowers was also delayed under SD compared to LD, suggests that, in these photoperiodic plants, FLS is controlled by an additional system, independent of pollination (fertilisation). This conclusion is supported by the fact that time of fertilisation does not appear to be involved in the difference in FLS between *Sn* and *sn* plants (Experiment 6.2), and that the setting of small parthenocarpic pods was delayed under SD (Experiment 6.1).

Ethylene is known to regulate, or at least to be associated with, flower senescence (Hall and Forsyth, 1967; Nichols, 1971). The senescence of pea flowers, considered as collapsing or wilting of the petals, appears also to be regulated by ethylene production (W. Bereznicki, unpublished). It is believed that pollination (fertilisation) stimulates flower senescence by increasing the level of auxin in the pistil of the flower (Hall and Forsyth, 1967), which in turn, stimulates autocatalytic ethylene production in the petals (Nichols, 1977). Though auxin and gibberellins have been found in the pollen of various species, pollen is believed to increase the hormonal activity by stimulating hormone synthesis rather than making a direct contribution (see Goodwin, 1978). When fertilisation does not occur, flower senescence is supposed to be due to autocatalytic ethylene production as the sensitivity to ethylene would increase with age (Nichols, 1977). The exogenous application of ethylene which causes an irreversible wilting of the flower petals of carnation also provokes the parthenocarpic development of the ovaries (Burg and Dijkman, 1967; Nichols, 1971). The difference between LD and SD treatments in FLS and parthenocarpic development of pod may be due to an initial difference in hormonal activity in the pistil. *Sn* activity could prevent or decrease the hormonal activity necessary to trigger flower senescence and pod development.

The parthenocarpic development of pods in Experiment 6.1 shows that although developing seeds are important for fruit development, they are not indispensable. There are numerous studies indicating that, after pollination, the leading role in controlling fruit development is taken up by the seeds (see Nitsch, 1970). Various fruit shapes can be obtained by removal of the seeds of strawberry (Nitsch, 1970) indicating that the achenes stimulate the growth of the surrounding receptacle. In pea, application of seed extract can restore the normal development of pods where seeds had been killed (but left *in situ*) (Eeuwens and Schwabe, 1975). In addition the pericarp swells only in the region adjacent to growing seeds (Rosenstand, 1978) indicating an essential role for seeds in the development of a nominal pod. Thus, although seeds appear essential, the pericarp may elongate and swell to a certain extent without the presence of developing fertilised ovules inside the pod, as shown by the results in Table 6.2. As exogenous application of $GA_3$ or $GA_{20}$, a native GA identified in seeds at a later stage of maturation, (Sponsel and McMillan, (1977)) can replace the effect of the developing seed on pericarp length and breadth (Eeuwens and Schwabe, 1975; Sponsel, 1982) it was suggested that developing seeds act on pod growth by producing GAs or by maintaining GA production in the pod (Sponsel, 1982). It is possible that the development of parthenocarpic pods is due to a low level of hormonal activity in the unfertilised ovules and/or in the pericarp (pistil) itself. The fact that SD delayed the setting of parthenocarpic pods and not of pods with seeds may indicate that a decrease in hormonal activity in the pistil, supposedly brought about by *Sn* activity, may be largely compensated for by pollination (fertilisation).

The fact that all pollen tubes were at a similar level in the style 2 and 4 hours after pollination indicates that the deficiency in recessive *snsn* plants in the $F_2$ is not likely to be due to a difference in pollen tube growth rate. However, only a slight precedence in pollen tube growth of *Sn* grains over *sn* grains could account for a higher percentage of ovules fertilised by *Sn* pollen. Such a slight difference might not be detectable with the technique used. On the other hand, it may be possible that the gametophytic selection operates through the potentiality of the pollen tubes to compete in a mixed population as *Sn* and *sn* grains germinate on the same stigma on the $F_1$ plants. It is also possible that the capacity for germination of the *Sn* and *sn* pollen grains produced in the $F_1$

varies. Those questions may be partly answered using *semi-vitro* culture (see Brewbaker and Majunder, 1961). Stigmata of the detached styles of *Sn* and *sn* flowers, cultured *in vitro,* could be pollinated with a determined amount of *Sn* and *sn* pollen grains. If the two genotypes were marked cytologically, it would be possible to check the germination and pollen tube growth of the *Sn* and *sn* tubes in a mixed population.

TABLE 6.1 The flower life span (hours) for an $F_4$ progeny segregating for male sterile and fertile plants grown either in LD (24h) or SD (8h). Data from Experiment 6.1. n = number of replicates.

| Phenotype | LD | | | SD | | | t-test |
|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | |
| sterile | 53.00 | 3.34 | 12 | 66.58 | 3.65 | 12 | *** |
| fertile | 28.00 | 2.16 | 13 | 53.92 | 2.92 | 13 | *** |
| t-test | | *** | | | ** | | |

**, ***: Means are significantly different at the 0.01 and 0.001 probability level, respectively.

TABLE 6.2 Node of first flower initiation (NFI), node of first pod (NFP) and pod final length (FL,cm) for an $F_4$ progeny segregating for male sterile and fertile plants grown either in LD (24h) or SD (8h). Data from Experiment 6.1. n = number of replicates.

| Phenotype | LD | | | | | | SD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NFI† | | | FL | | | NFI | | | NFP | | | FL | | |
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| Sterile | 15.40 | 0.18 | 8 | 4.17 | 0.07 | 12 | 22.90 | 0.34 | 12 | 29.00 | 1.06 | 11 | 3.15 | 0.12 | 12 |
| Fertile | 15.30 | 0.17 | 13 | 6.77 | 0.08 | 13 | 23.20 | 0.26 | 13 | 23.20 | 0.26 | 13 | 6.92 | 0.27 | 13 |

† in LD, NFI = NFP

TABLE 6.3 Distances reached by the pollen tubes of line $299^-$ (*sn*) and line $299^+$ (*Sn*) 2, 4, 8 and 23h after hand-pollination on the stigma of line $299^-$ and $299^+$ flowers. Data from Experiment 6.2.

| Cross (x) | Time | 2h | | | 4h | | | 8h | | | 23h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance† | 1/3 | 2/3 | Ov | 1/3 | 2/3 | Ov | 1/3 | 2/3 | Ov | 1/3 | 2/3 | Ov |
| *sn* x *sn* | | - | - | - | 4 | - | - | 4 | 4 | 4 | 4 | 4 | 4 |
| *sn* x *Sn* | | - | - | - | 4 | - | - | 4 | 4 | 4 | 4 | 4 | 4 |
| *Sn* x *Sn* | | - | - | - | 4 | - | - | 4 | 4 | 3 | 4 | 4 | 4 |
| *Sn* x *sn* | | - | - | - | 4 | 1 | - | 4 | 4 | 4 | 4 | 4 | 3 |

†the longest pollen tubes reached

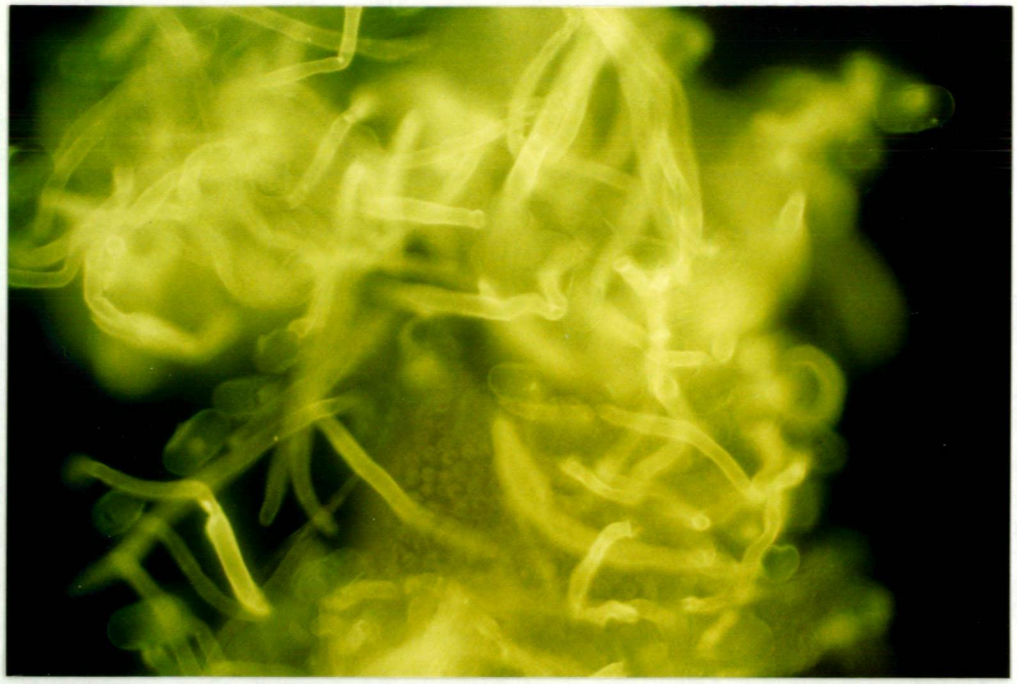
1/3 of the style
2/3 of the style
Ov the ovary area

PLATE 6.1 Fluorescence microscopy. Experiment 6.2.

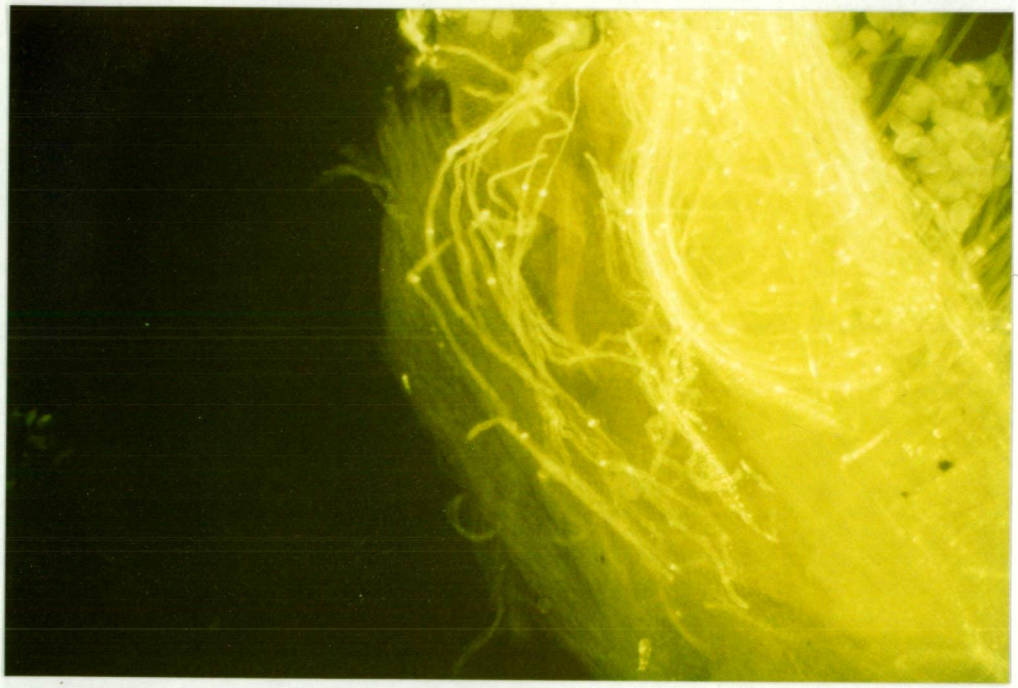
(a) Pollen grains germinating on the surface of the stigma. Photo from a cross of $299^-$ (♀) x $299^-$ (♂), 8 hours after hand-pollination (x128).

(b) Pollen tubes growing down the style. The callose plug may be seen at regular intervals along the pollen tube. Unpollinated pollen grains can be seen, trapped in the stylar hairs. The cross is of the type $299^-$ (♀) x $299^-$ (♂), 4 hours after hand-pollination (x50.4).

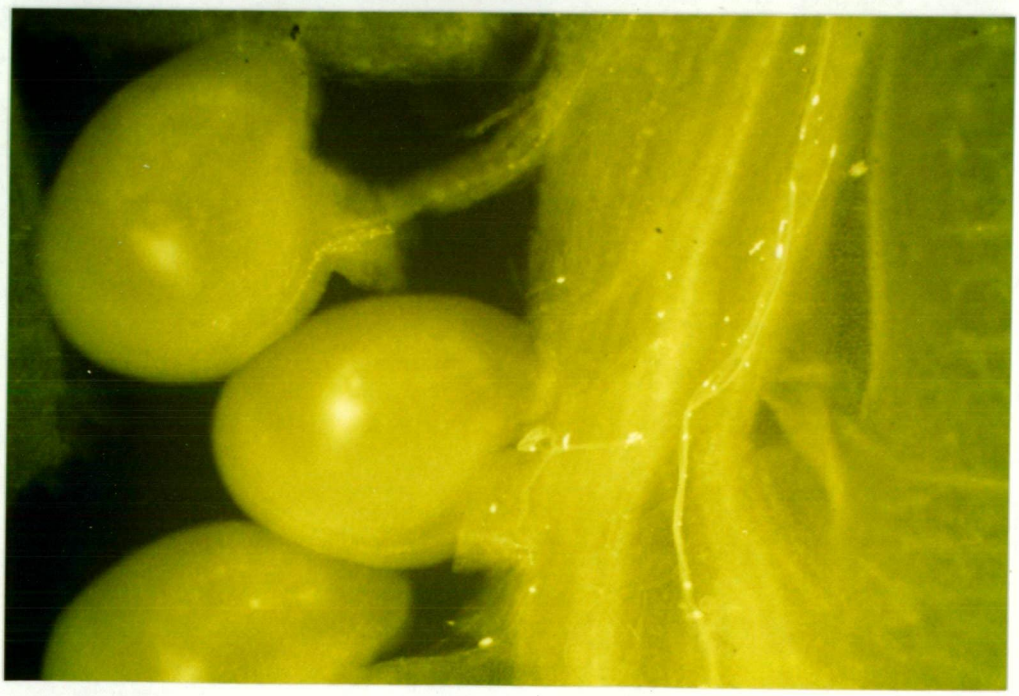
(c) Pollen tubes reach the ovule area. A pollen tube fragment can be seen near the micropyle of the ovule. Photo from a cross of $299^+$ (♀) x $299^+$ (♂), 23 hours after hand-pollination (x50.4).



a



b



c

# CHAPTER 7 EFFECT OF THE *Hr/hr* GENE DIFFERENCE ON NODE OF FLOWER INITIATION ON AN $Lf^d$ *sn Dne* BACKGROUND

## 7.1 Introduction

The six major flowering loci *Lf, E, Sn, Dne, Hr* and *Veg* (see Chapter 1) allow in all 128 pure flowering genotypes since *Lf* has 4 alleles. Several combinations have yet to be studied and in this chapter the genotype $Lf^d$ *sn Dne Hr Veg* is examined for the first time.

The heterozygous genotype *Lf1f snsn DneDne Hrhr* has been examined previously (Murfet, 1978). No conclusive evidence emerged that *Hr* had an effect on NFI on this background. However, the effect of *Hr* was not examined in homozygous *Lf sn Dne* plants. It is conceivable that, if *sn* is a leaky mutant (Murfet, 1971a; Reid, 1979b), some effect of segregation for *Hr/hr* might be detectable in *sn Dne* plants, homozygous for *Lf* or especially for $Lf^d$. Therefore a cross between lines 94 (*a 1f sn Dne Hr M*) and 89 (*A* $Lf^d$ *sn Dne hr m*) was made to test the effect on NFI of segregation for *Hr/hr* on an $Lf^d$ *sn Dne* background. (This cross was also used in Chapter 4 to examine the effect of segregation for $Lf^d/1f$ on several other reproductive characters.) The allele at the *E* locus is not known for line 89. However, since $Lf^d$ and *sn* are both epistatic to *E* (Murfet, 1971b, 1975a), segregation for *E/e* should not affect the result. The *Lf* locus is fairly closely linked to *A*, the basic locus for anthocyanin production (White 1917; Murfet, 1971b, 1975a) and *Hr* is tightly linked with the *M* locus which controls marbling of the testa (Murfet, 1973a). Cross 94 x 89 is set up in coupling so that markers *A* (reddish-purple flowers) and *M* (brown marbling on the testa on an *A* background) will give a very good indication of whether a plant carries $Lf^d$ or *Hr*, respectively. Because of epistasis, it was not possible to follow the *M/m* segregation in white flowered (*aa*) plants but this did not constitute a problem since interest centred on the $Lf^d$ segregates which mostly carried A.

## 7.2 Materials and Methods

Lines 94 and 89 (see Section 2.1) were crossed and the parents and $F_1$ were grown, one plant per pot, under SD = 10h and LD = 24h, in Glasshouse B (for details, see Section 2.2.2). Night temperature was 16±0.5°C. Day

temperatures ranged from 20 to 26°C. In a second planting, the parents, $F_1$ and $F_2$ were grown, one plant per pot, under LD = 24h and SD = 8h in Glasshouse A (for details, see Section 2.2.2). "Night" temperatures were 17±1°C in SD and 14 to 17°C in LD. Temperatures ranged from 18 to 23°C during exposure to natural light. In a third planting, $F_3$ plants were grown, 2 plants per pot, on the apron of the Glasshouse A, where they received 24h of light. Temperatures ranged from 14 to 17°C at "night" and from 19 to 26°C during exposure to natural light. To obtain the $F_3$, 4 to 12 seeds per $F_2$ plant were sown. When $F_2$ plants produced as little as 4 seeds, data were taken into account for the $F_3$ only if the given family showed a clear segregation, i.e., if at least one plant presented an NFI between 9 and 11. Data on NFI were recorded only from the main shoot. Laterals were regularly excised except for the $F_3$.

## 7.3 Results

The white flowered (*aa*) plants of line 94 flowered at nodes 9 to 11, independently of the photoperiod, whereas plants of line 89 (with red flowers *AA*) flowered at nodes 19 to 25 and they were slightly delayed in SD (Table 7.1). $F_1$ plants flowered between the two parents and they also exhibited a slight photoperiod response (Table 7.1) as NFI varied from 14 to 19 in LD and from 16 to 20 in SD.

The NFI distribution of the $F_2$ was multimodal and there were 3 regions of low or zero frequency in LD (at nodes 12, 18 and 22-24) and 2 regions of low or zero frequency in SD (at nodes 12 and 17-19). In addition, in both photoperiods, the $F_2$ contained a group of plants which did not flower on the main stem (6 plants in LD and 13 plants in SD; Table 7.1). Plants of this type, which appeared in the parental line 89 in other experiments, produced in excess of 25 vegetative nodes on the main stem. Even without taking these plants into account (as their NFI is not known), the NFI of the latest $F_2$ plants transgressed the upper limit of parental line 89 plants. For both photoperiods, there is strong evidence that plants with an NFI of 10 and 11 were homozygous *lf1f*. First, they were all white flowered apart from 3 plants. Second, the *lf1f* parent, line 94, flowered in this region (Table 7.1). Third, there was no evidence of a photoperiod response in these early segregates (Table 7.1). Cutting the $F_2$ distribution between NFI 11 and 12 gave segregation data for $lf/Lf^d$ in excellent accord with medelian expectation (Table 7.2). The flowering locus *lf* and the

marker gene *a* showed a close linkage of 4.3% (Table 7.2) which again is fairly consistent with the value of 8-10% usually obtained (Murfet, 1971b, 1975a).

There was no clear distinction between the homozygous $Lf^dLf^d$ and the heterozygous $Lf^dlf$ segregates. In LD, when the plants which did not flower are excluded and when the $F_2$ is cut between node 11 and 12 and at node 18, the observed numbers are in good agreement with a 1:2:1 ratio ($X_2^2 = 1.14$; $0.5<P<0.7$) suggesting partial dominance. However, it would also be possible to cut the distribution between node 11 and 12 and at node 22 and include the 6 plants which did not flower in the last group. In fact, in SD, the NFI of the $F_2$ heterozygotes identified by growing the $F_3$, varied from node 13 to node 24, which indicated that the low frequency region occurring between node 16 and 19 (under both LD and SD and in both $F_1$ and $F_2$) is not a consequence of the $Lf^d/lf$ segregation (Table 7.1). In SD, the NFI of $Lf^dLf^d$ plants varied from 24 to 26. The observed numbers of 14 *lflf*, 30 $Lf^dlf$ and 8 $Lf^dLf^d$ are in agreement with a 1:2:1 ratio ($X_2^2 = 2.53$; $0.2<P<0.3$). The genotype of a few plants flowering at node 20 to 24 has not been determined conclusively and these plants have not been included in the above analysis. Little dominance occurred between the two alleles (in SD, the degree of dominance was 0.05 in favour of *lf* over $Lf^d$).

On an $Lf^d$— background, gene *Hr* does not seem to have an influence NFI either under SD or LD. This is indicated by the fact that, in either photoperiod, segregation for *M/m* (*Hr/hr*) did not affect the mean NFI in $Lf^d$ segregates (Table 7.3). Again, when the NFI distribution is considered in SD, the $Lf^dlf$ plants varied from node 14 to node 23 on an *mm* (*hrhr*) background and similarly from node 13 to node 24 on a *M*— (*Hr*—) background (Table 7.1). There were too few $Lf^dLf^d$ plants to draw any meaningful conclusions. The $Lf^dlf$ *mm* (*hrhr*) $F_2$ plants gave, in the $F_3$, 10% of plants which did not flower on the main stem, whereas $Lf^dlf$ *M*— (*Hr*—) plants gave 14.8%. By contrast the $Lf^dLf^d$ plants with *mm* and *M*— backgrounds gave the high values of 55% and 40%, respectively (Table 7.1). Thus, if the plants do not flower on the main stem as a result of a genetic effect, this effect is unlikely to be brought about by the *Hr* gene.

## 7.4 Discussion

There is no evidence in the results to suggest that segregation for *Hr/hr* has any effect on NFI in $Lf^{d}-$ plants with background *sn Dne* in either SD or LD. Line 89 is considered to be a member of the DN phenotypic class since it has genotype *sn*. Nevertheless, it shows a small increase in NFI in SD. Moreover, the $Lf^{d}-$ segregates in the $F_2$ show the same pattern. This very small response to photoperiod may be a consequence of the leaky nature of allele *sn*. However, the response does not appear to be enhanced by the presence of *Hr*, whereas in true photoperiodic types with the genotype *Sn Dne, Hr* greatly increases the capacity to respond to photoperiod (Murfet, 1973a, 1977, 1984).

In the $F_1$, as in the $F_2$, there is a clear gap around node 17 and 18 which has been noticed in other crosses (Murfet, 1971b, 1973a, 1975a) and which is independent of segregation for *Hr/hr* and of homozygosity or heterozygosity for allele $Lf^{d}$. As the flower initiation at nodes 15-17 occurs at about the time the cotyledons are depleted (Murfet, 1977) the disturbance noticeable in this region could be due to the passage of the plant to the autotrophic stage (Murfet, personal communication).

During other experiments conducted with line 89, plants appeared which had no flower visible microscopically on the main stem and which produced up to 50 nodes on the main stem before dying. The apex decreased in size above about node 18 and it appears to have become too "weak" to produce any flower primordia. All such plants showed profuse branching from many nodes and in most cases flowers and fruits formed on at least some of the lateral branches. However, in extreme cases, these plants failed to produce any flowers and they eventually died after a greatly extended life span. Application of $GA_3$ to these plants and grafting of lateral buds to *Pisum* stocks known to promote flowering (e.g. line 58; Murfet, 1971c) failed to cause flower initiation (checked at the macroscopic and microscopic levels). Such phenotypes appeared in the parent line 89 (*hr*) before occurring in the $F_2$ and segregation for *Hr/hr* did not seem to influence the frequency of their occurrence. This suggests that it is a tendency related to the genotype $Lf^{d}$. Phenotypically, these non-flowering plants closely resemble the non-flowering plants of genotype *vegveg* which likewise exhibit profuse branching, thin and short upper internodes, small leaves and

prolonged life span (Reid and Murfet, 1984). However, genes *veg* and $Lf^d$ have been shown to be non-allelic (Reid and Murfet, 1984). The alleles at the *Lf* locus are reputed to operate at the shoot apex to determine the threshold level of the flowering signal which is necessary to induce flowering (Murfet, 1971c). It would appear that in extreme cases, the high threshold imposed by allele $Lf^d$ is sufficient to completely prevent flower initiation, at least in DN types.

As flowers may form on some of the lateral branches and not on the main shoot, this suggests that apical and axillary meristems have different sensitivity to the flowering factor(s) or the level of the factor differs between the main and lateral apices. For the reasons exposed in Chapter 3 and above, it would be of particular interest to study floral evocation in such lines, at a cellular and molecular level.

TABLE 7.1 Distribution of flowering node (NFI) for line 94 (*a 1f sn Dne Hr M*), line 89 (*A Lf$^d$ sn Dne hr m*) and the $F_1$, $F_2$ and $F_3$ of cross 94 x 89. Plants entered in the column headed ∞ did not flower on the main stem.

| Generation | Photoperiod | Node of First Flower | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | ∞ |
| 94 | 24 | | 2 | 1 | | | | | | | | | | | | | | | | | |
| 94 | 10 | | 2 | 1 | | | | | | | | | | | | | | | | | |
| 89 | 24 | | | | | | | | | | | | 2 | 1 | 2 | 1 | | | | | |
| 89 | 10 | | | | | | | | | | | | | 1 | 1 | 0 | 4 | | | | |
| $F_1$ | 24 | | | | | | 2 | 1 | 0 | 0 | 2 | 1 | | | | | | | | | |
| $F_1$ | 10 | | | | | | | | 1 | 0 | 0 | 3 | 1 | | | | | | | | |
| 94 | 24 | | 4 | | | | | | | | | | | | | | | | | | |
| 94 | 8 | 1 | 2 | | | | | | | | | | | | | | | | | | |
| 89 | 24 | | | | | | | | | | | 1 | 1 | 0 | 3 | | | | | | |
| 89 | 8 | | | | | | | | | | | | | | 2 | 1 | 3 | 1 | | | |
| $F_1$ | 24 | | | | | | | 1 | | | | | | | | | | | | | |
| $F_2$ | 24 | | 11 | 5 | 1 | 4 | 15 | 9 | 1 | 1 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 3 | 1 | 1 | 6 |
| $F_2$ | 8 | | 10 | 4 | 0 | 1 | 12 | 6 | 1 | 0 | 1 | 0 | 2 | 7 | 2 | 2 | 3 | 3 | 3 | | 13 |
| $F_2$ *Lf$^d$1f mm* | 8 | | | | | | 3 | 2 | | | | | | 2 | 1 | 1 | | | | | |
| | | | | | | | 2 | | | | | | | 2 | 1 | | | | | | |
| $F_2$ *Lf$^d$Lf$^d$ mm* | 8 | | | | | | | | | | | | | | | | 1 | | | | |
| | | | | | | | | | | | | | | | | | 1 | | | | |
| $F_2$ *Lf$^d$1f M—* | 8 | | | | | 1 | 9 | 4 | 1 | | 1 | | 2 | 5 | 1 | 1 | 1 | | | | |
| | | | | | | | 9 | 4 | 1 | | 1 | | 1 | 5 | 1 | 1 | | | | | |
| $F_2$ *Lf$^d$Lf$^d$ M—* | 8 | | | | | | | | | | | | | | | | 1 | 3 | 3 | | |
| | | | | | | | | | | | | | | | | | 1 | 3 | 2 | | |
| $F_3$ | 24 | 1 | 6 | 1 | 0 | 1 | 2 | 7 | 3 | 0 | 2 | 4 | 3 | 0 | 2 | 4 | | | | | 4 |
| $F_3$ | 24 | | | | | | | | | | | 1 | 0 | 1 | 2 | | | | | | 5 |
| $F_3$ | 24 | 7 | 30 | 3 | 0 | 8 | 18 | 10 | 6 | 2 | 3 | 7 | 11 | 13 | 11 | 5 | 2 | 2 | | | 24 |
| $F_3$ | 24 | | | | | | | | | | 1 | 2 | 2 | 13 | 7 | 6 | 1 | 5 | | | 25 |

TABLE 7.2 Segregation and linkage data for the flowering locus *1f* and marker gene *a* obtained from the $F_2$ of cross 94 (*a 1f*) x 89 (*A* $Lf^d$). The $F_2$ distribution was cut between nodes 11 and 12 to separate the *1f1f* and $Lf^d$– genotypes.

| Photoperiod | Phenotypes and Observed Numbers | | | | | Chi-squared | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *A* $Lf^d$ | *A 1f* | *a* $Lf^d$ | *a 1f* | Total | Seg *A/a* | Seg $Lf^d$/*1f* | Joint Seg† | Cr0% | SE |
| 24h | 40 | 0 | 2 | 16 | 58 | 1.13 | 0.21 | 60.69 | | |
| 8h | 43 | 3 | 0 | 11 | 57 | 0.99 | 0.01 | 34.48 | | |
| Total | 83 | 3 | 2 | 27 | 115 | 0.003 | 0.07 | 93.45 | 4.3 | 1.9 |

† Heterogeneity test on Joint segregation data: $X_1^2 = 1.72$; $0.2 > P > 0.1$

TABLE 7.3 Mean and SE of the mean for NFI for *M–(Hr–)* and *mm(hrhr)* segregates flowering at node 12 onwards (i.e., supposedly on a $Lf^d$– background) in SD (8h) and LD (24h) in the $F_2$ of cross 94 (*a lf Hr M*) x 89 (*A* $Lf^d$ *hr m*).

| Photoperiod | *M–(Hr–)* | | | *mm(hrhr)* | | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | ±SE | n | $\bar{x}$ | ±SE | n |
| SD | 18.68 | 0.80 | 33 | 18.30 | 1.33 | 10 |
| LD | 16.51 | 0.70 | 31 | 16.33 | 1.76 | 9 |

# CHAPTER 8 CONCLUDING DISCUSSION

The present study shows that, in pea, genes at the *Sn* and *Lf* loci control not only the transition between the vegetative and the reproductive phase but they also influence subsequent steps in reproductive development, from the flower primordium to the mature pod (Chapters 3 and 4). An effect of the "flowering" genes on the later stages of reproductive growth has also been reported for other species, e.g., for *Sorghum* (Quinby, 1972; Sorrells and Myers, 1982). The dominant alleles at both the *Sn* and *Lf* loci act to delay flower initiation. However, they have contrasting effects on the later stages of reproductive growth. Gene *Sn* is associated in SD with a delay in flower initiation, flower bud development, flower life-span (Chapter 3) and pod growth (Chapter 4). By contrast, for the ascending sequence $lf^a$, *lf*, *Lf* and $Lf^d$, flower initiation is delayed and may even be prevented in some $Lf^d$ plants (Chapter 7) but the subsequent developmental stages tend to be promoted (Chapters 3 and 4). The *Sn* gene also influences various other morphological traits such as peduncle length, branching, number of pods per raceme (Chapter 5; Murfet, 1984; Murfet and Reid, 1985) and the number of developing seeds per pod (Chapter 5). As discussed earlier, the use of the two near-isolines differing at the *Sn* locus and placed under the same photoperiod brings convincing evidence that differences observed previously for photoperiodic lines between LD and SD (e.g., pod/seed development in line G2; Ingram and Browning, 1979; Gianfagna and Davies, 1981) are true photoperiodic responses brought about by the *Sn* gene (Reid, 1979b).

For photoperiodic plants in SD (e.g., lines $299^+$ and 53), the above-mentioned phenotypic differences, together with the results previously reported on pod setting (Reid, 1979b, 1980) and apical senescence (Murfet, 1971a,b; Reid, 1979b, 1980), indicate that, after floral initiation has taken place, priority is still given to vegetative rather than reproductive growth. Alternatively, we may consider that priority is given to reproductive growth rather than vegetative growth in DN lines or in photoperiodic lines in LD. This is shown by the fact that, in SD, for EI (e.g., line $299^+$) or L lines (e.g., line 53), the rate of flower, pod and seed development is slower, the flower life-span is longer, and the number of developing seeds per pod is decreased whereas peduncle length, branching and the delay in apical arrest are promoted compared to the same lines in LD or DN lines.

The delay in the onset of pod and seed growth and the increase in FLS are unlikely to be due to a delay in the time of fertilisation (Chapter 6). Likewise, the smaller number of developing seeds per pod in *Sn* lines in SD does not seem to be due to a lack of fertilisation (Chapter 5). The delay in pod growth is not a direct consequence of the increase in the number of flowers (thus pods) per raceme since removal of the distal flower did not remove the difference between *Sn* and *sn* plants (Chapter 5). In contrast, pod growth appeared to be inhibited in a correlative manner by the apical bud and growth of laterals (Experiments 5.2 and 5.3). As decapitation removed partly the effect of the *Sn/sn* gene difference on pod growth it is suggested that *Sn* activity acts, at least partly, through an effect in the apical bud. *Sn* activity may create a situation within the plant which causes diversion of metabolites towards vegetative growth of the main apical bud and basal axillary buds, thus depriving reproductive growth. Such an action of *Sn* activity on assimilate partitioning has been put forward in *Pisum* (Murfet, 1984; Reid and Murfet, 1984) and in *Lathyrus* (Ross, 1983).

The hypothesis that the actively growing shoot constitutes a powerful sink depriving reproductive growth has been suggested by various authors for pea (Carbonell and Garcia-Martinez, 1980) and for other species (e.g., tomato - Tsé *et al.*, 1974; Kinet, 1977; Leonard *et al.*, 1983; orchids - Goh, 1977). However, the question remains as to whether the diversion of the metabolite flow towards the apex is a cause or a consequence of the metabolic activity of the apex (Leonard *et al.*, 1983). Alternatively, the results presented do not exclude the possibility that the apex may act as a source of inhibitor as suggested by Carbonell and Garcia-Martinez (1980) and as put forward for the inhibition of the axillary bud outgrowth (see Phillips, 1975). Indeed it has been suggested that the mechanism of competition between fruits and vegetative parts could be similar to the mechanism of correlative inhibition between buds of a single vegetative shoot (Nitsch, 1965).

Contrary to the results reported by Berry and Aitken (1979), the interval between initiation and open flower is not necessarily increased in late-flowering cultivars as shown by the decreasing FT-TPI value with the increasing NFI in DN type plants (Chapter 3). This difference in results is of fundamental importance in understanding the action of the *Lf* allelic series. The rate of flower bud development tended to increase with the sequence $lf^a$, *lf*, *Lf* and $Lf^d$ (i.e., with increasing node number),

pod development was slightly promoted in $Lf^d$ plants (Chapter 4) and internode length and leaf size decreased drastically before the first flower appeared in $Lf^d$ plants (Murfet, 1984). In addition, in $Lf^d$ plants several other characteristics indicate that the apical meristem loses dominance with the passage of time, e.g., the precocious inception of vegetative axillary buds (Chapter 3), the active growth of aerial laterals and the fact that yield may be confined to these laterals in some plants (see Chapter 7; Murfet and Reid, 1985). All these facts support the hypothesis that the underlying metabolism is strongly geared towards vegetative growth early in the life of the plant but autonomous metabolic changes occur with time such that the metabolism becomes geared in favour of reproductive growth (Murfet, 1984; Reid and Murfet, 1984). Thus, in the case of the *Lf* allelic series, the reproductive growth would be promoted with the ascending sequence $lf^a$, *lf*, *Lf* and $Lf^d$ as a consequence of the delaying effect of the higher order alleles on the time of flower initiation. In line 69, $lf^a$ confers a high threshold sensitivity to the flowering factor; hence, flower initiation occurs very early in the life of the plant when the metabolism is still geared towards vegetative growth and the latter would then compete, as discussed earlier, with the development of the reproductive parts.

In contrast, to the *Lf* series, gene *Sn* appears to influence reproductive growth in a more direct manner. It is proposed that *Sn* acts by delaying the time at which changes occur in the underlying metabolism. That is, the retardation in reproductive growth follows as a consequence of the influence of *Sn* activity, may be on the partitioning of assimilates within the plants. In addition, *Sn* activity may have some direct action in the flower and/or fruit. The fact that the effect of the *Sn/sn* gene difference on certain characteristics, e.g., final seed weight, was not fully removed after decapitation (Experiments 5.2 and 5.3) could suggest that *Sn* may also partly act in the seeds to prevent assimilation of extra nutrients. For example, the genotype of the embryo and testa could impose a limit on the final seed growth as suggested by Hedley and Ambrose (1980). This would explain the lack of response with respect to pod growth after removal of the distal flower (pod) in Experiment 5.1. With the hypothesis of a competition for metabolites between vegetative and reproductive growth, it would have been reasonable to expect a substantial effect to result from the removal of a major sink which may compete for the same source. A direct effect of gene *Sn* on pod growth, i.e., an effect independent of

vegetative growth has also been suggested by Ingram (1980). Seed growth for the early DN cultivar Alaska was unaffected by treatments which promoted (vegetative) apical growth, suggesting that the retardation in seed growth for the photoperiodic lines is probably not just an indirect result of the nutrient diversion to the shoot at the expense of the seed.

The use of a range of genotypically and phenotypically different lines which respond differently to environmental conditions, allowed further insight into the action of genes such as *Sn* and *Lf*. Both genes are of considerable interest from an agronomic point of view. However, only by understanding the mechanisms involved at the physiological, tissue and cellular levels will we be able to fully exploit the potential of the species. Such integrated studies using natural and induced genetic variation are suggested to provide a better understanding of the events essential for floral evocation (Chapters 3 and 7) and seed growth (Chapter 4).

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