Synchronization of Cell Division During Flower Initiation in Third-Order Buds of Silene

R. F. LYNDON

Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, Scotland, UK

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ABSTRACT

Plants of Silene coeli-rosa were induced to flower with seven long days and then returned to non-inductive short days. Third-order buds were formed more than three weeks after the beginning of induction and third-order flowers were initiated about one week later. Comparison of the mitotic index with the ratio of cells in the G2 and G1 phases of the cell cycle for each third-order apex provided evidence for synchronization of cell division just before flower initiation. It is suggested that this results from changes of competence of the apical cells to react to their internal environment rather than because of the arrival of a floral stimulus at the shoot apex.

Key words: Silene, cell division synchrony, flowering, evocation, mitotic index, cell cycle, competence.

INTRODUCTION

In plants induced to flower, successive peaks of mitotic index occur during evocation, and these have been interpreted as indicating some synchrony of cell division in the shoot apex (Bernier, 1971; Lyndon and Francis, 1984). More detailed observations have shown that in Sinapis and Silene, and also probably in Xanthium, the apical cells undergo one or more synchronous cell cycles during evocation or just before flower initiation (Bernier, Kinet and Bronchart, 1967; Francis and Lyndon, 1979, 1985; Jacqmard et al., 1976). Synchrony in Silene can be suppressed without suppressing flowering (Grose and Lyndon, 1984) and is therefore characteristic of normal flowering rather than essential to it. It may therefore occur in response to the arrival at the apex of some stimulus formed during induction and which has some essential effect in causing flower initiation, but at the same time promotes synchronization.

If synchrony were, in this way, an incidental effect of induction and evocation, it might be expected to occur in the apex only at this one time, before formation of the first flower. In this case the formation of much later flowers, after the plants have been returned to short days which are non-inductive but allow flowering to continue, should not be associated with synchronization of the apical cells, since there would be no reason to expect a new flowering stimulus arriving at the apex from the leaves.

Accordingly, the aim of this work was to examine the initiation of flower buds in Silene which are formed long after induction and evocation, and after the plants have been returned to a non-inductive environment, to see whether or not synchronization of cell division could be detected at the apex. The third-order buds were chosen for study because they are not yet formed when the plants are induced by seven long days. Only the second-order bud is present as an undifferentiated bud just below the developing first-order flower when it is initiating floral organs (Lyndon, 1978a), and the third-order bud has not been formed at all. The third-order flower is initiated well after the first-order flower has begun development and about one month after the beginning of induction, and therefore almost three weeks after evocation must have been completed. The exact time of initiation of the third-order flower buds, and the length of the cell cycle in them, was therefore measured and evidence was obtained that synchronization of cell division was indeed found in the third-order buds.

MATERIALS AND METHODS

Plants of Silene coeli-rosa (L.) Godron were grown from seed in short days (SD), after 28 d selected...
for developmental uniformity, subjected to 7 long
days (LD) and then returned to SD (Miller and
Lyndon, 1976). The day of selection and the
beginning of the 7 LD was designated day 0.

Squash preparations of Feulgen-stained shoot
apical meristems were prepared, and the mitotic
index (MI) and G2/G1 ratio (ratio of number cells
with > 3C amount of DNA to number of cells
with < 3C amount of DNA) were measured as
described and detailed previously (Francis and
Lyndon, 1978). For measurements of MI, all the
cells of an apical squash were examined. Only
metaphases, anaphases and telophases were
counted as mitotic figures, because prophase was
not always distinguishable from some interphase
nuclei. For G2/G1 ratios usually the first
measurable 150 cells encountered on a slide were
recorded. For some apices fewer cells, but never
less than 50, were measured.

The stage of development of an apex was
recorded after dissection and observation under a
dissection microscope. Volumes of longitudinal
median sections were measured from camera
lucida tracings and were proportional to total
volumes (Miller and Lyndon, 1976).

RESULTS

Formation of third-order buds and their rate of
growth

The inflorescence is a monochasial cyme. The
first-order flower is formed by transformation of
the terminal meristem of the shoot. Immediately
below the first-order flower an axillary bud grows
out, producing one (sometimes two or very rarely
three) pair(s) of leaves before forming the
second-order flower. This process is repeated by a
bud immediately below the second-order flower to
produce the third-order flower and so on as the
inflorescence grows.

The first-order flower is initiated in 50 per cent
of the plants by day 10-5, i.e. the 3rd sepal of the
first-order flower has been initiated, allowing it to
be unequivocally identified as a flower (Lyndon,
1979 and unpublished). At this time the second-
order bud is just an axillary bulge to one side of
the young first-order flower (Plate 1B, C in
Lyndon, 1978a). The third-order bud is not formed
until day 20-0 (Fig. 1), when just over 50 per cent
of the second-order buds have initiated flowers
(data not shown). The leaf pair on the third-order
bud is initiated by day 25-5 (Fig. 1) and the flower
by day 32-5.

The time between the initiation of the first leaf
pair (day 25-5) and the subsequent pair of
primordia on the third-order bud (day 30-5),
whether they are another leaf pair (23 per cent of
the plants) or the sepals (77 per cent of the plants),
is 5-0 d (Fig. 1). The increase in volume of the
apical dome above the first pair of primordia when
just newly initiated (day 25-5) to that of the whole
meristem above this same pair when sepals have
just been initiated (day 30-5; Fig. 1) corresponded
to a volume doubling time of 45 h (Table 1). The
increase in volume of the whole 3rd-order bud
between the initiation of the first leaf pair and the
second pair of primordia corresponded to a

![Fig. 1. Initiation and growth of third-order buds of Silene. As indicated by the arrows, 50 per cent of plants have (a) a third-order bud (---) by day 20-0, (b) one leaf pair (---) by day 25-5, (c) two pairs of primordia (leaf pair, sepals, or two leaf pairs (---)) by day 30-5, (d) three sepals, i.e. unequivocally a flower (---) by day 32-5.](https://academic.oup.com/aob/article-abstract/59/1/67/145102)
doubling time of 52 h. From these two estimates it is therefore reasonable to assume a volume doubling time of 40–50 h and, assuming all the cells to be meristematic, a cell cycle time of 40–50 h.

The cell cycle in third-order buds

The simplest way to look for synchrony in third-order buds was to examine samples of buds on successive days and see if there were fluctuations in mitotic index (MI), as would be expected if there were synchrony and a cell cycle of about 2 d, or whether MI was more or less constant, which would be expected if the cells in the buds were asynchronous.

The MI varied considerably between apices, even on the same day, so that although there seemed to be variation in MI there was no evidence for synchrony (Fig. 2). However, apices were found on days 27 and 30, and one apex on day 29, in which there were no mitotic figures whatsoever. This was after careful examination of all the cells in the squash for each of these seven apices, a total of more than 18,000 cells altogether. Although the data in Fig. 2 therefore do not provide evidence for synchrony or asynchrony in division, there is a suggestion that there could perhaps be some synchrony in individual apices during the plastochron between the formation of the leaf pair on the third-order bud on day 25-5 and the formation of sepals on day 30-5. G2/G1 ratios were also measured, since if there were synchrony between, as well as within, apices this ratio should have varied with time. Like the MI, the variation on each day was too great to be sure that there was a real fluctuation (data not shown).

Since this evidence was unsatisfactory, perhaps

because of non-synchronous development of different apices, another approach was tried. Clearly if there were synchronization of the cell cycle within each apex in the plastochron before the formation of the sepals of the third-order bud, but different plants were only approximately or not at all synchronous with respect to each other, synchrony would not be demonstrated by taking replicated samples at intervals. However, certain predictions can be made about the relationship of G2/G1 ratios to MI, which should allow the detection of apices within each of which cell division is synchronous even though they may not be synchronous with each other.

In an asynchronously dividing population of cells the MI, the G2/G1 ratio, and the relationship

![Fig. 2. Changes in mean mitotic index (±s.e.) of third-order buds in Silene 25-34 d after the beginning of induction.](https://academic.oup.com/aob/article-abstract/59/1/67/145102)
between them should remain constant with time. However, if there is complete or almost complete synchrony of cell division and synchrony of the cell cycle within individual apices the MI and G2/G1 ratio should change as follows. When cells are in G1 the MI will be zero and G2/G1 will also be zero (A on Fig. 3). As the cells enter the DNA-synthesis phase of the cell cycle (S) the G2/G1 ratio should reach unity (B). As the cells emerge into G2 the G2/G1 ratio will rise to > 1, but when all cells reach G2 the G2/G1 ratio will become infinity (C). MI should remain zero throughout interphase. Then as the cells enter mitosis MI will rise. But as MI rises, some cells will progress through into G1 and so the G2/G1 ratio will fall and become measurable once more. Since at mitosis every cell leaving G2 gives rise to two cells in G1, when mitosis is maximal the G2/G1 ratio should reach 0-5, since at this point there are twice as many G1 cells as G2 cells (D in Fig. 3). Then as all the cells pass through mitosis and into G1, the MI will fall to zero at the same time as the G2/G1 ratio also falls to zero (D to A in Fig. 3). Although the progression along the graph from A, B, C, D to A represents the traverse through the cell cycle, time itself is not represented on this graph. Thus, if cell division and the cell cycle were synchronous within each apex of a population of sample, even though each apex were not synchronous with any other apex, the values for MI and G2/G1 ratio measured simultaneously in each apex should nevertheless fall on this graph, according to where the cells are in the cell cycle. If, on the other hand, the cells within each apex are asynchronous then MI and G2/G1 ratio should both tend to be constant and the values for all apices should cluster around a point, corresponding to mean MI and mean G2/G1 ratio.

Measurements were made for each of the 44 apices represented in Fig. 2, MI and G2/G1 ratio both being measured from the same squash preparation. The results are shown in Fig. 4, and correspond reasonably to the distribution as in Fig. 3 which would be expected for apices in which synchronization occurs. Note particularly that the highest MIs correspond to a G2/G1 ratio of about 0-5, as predicted from Fig. 3. Figure 4 differs from Fig. 3 in showing no values of a G2/G1 ratio of > 0-3 which correspond to MI of zero, nor any of infinity, but this would be consistent with a relatively long G1, where the G2/G1 ratio is < 0-5, and a relatively short G2, and incomplete synchrony. The data of Fig. 4 agree much more closely with the distribution expected of apices showing synchrony than of apices with completely asynchronous cell cycles, when the values would cluster around the point representing the mean MI of 0-75 and mean G2/G1 ratio of 0-51. The lack of values with MI < 0-65 in combination with G2/G1 ratios between 0-35 and 0-95 again corresponds better with Fig. 3 than with a random clustering. The maximum MI of 2-25 per cent corresponds to about 4-5 per cent if prophase had been included, and is therefore about half that

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**Fig. 3.** Theoretical relationships between mitotic index and G2/G1 ratio during progress through the cell cycle in populations of cells in which cell division is synchronized. Cells in (A) G1, (B) S, (C) G2, (D) mitosis. See text for further explanation.

**Fig. 4.** Relationship between mitotic index and G2/G1 ratio in third-order buds of *Silene* in the plastochron before sepal initiation.
observed during synchronization in first-order buds (Francis and Lyndon, 1979; Grose and Lyndon, 1984).

The conclusion to be drawn, therefore, is that loose synchronization of cell division and of the cell cycle does occur in third-order buds in the plastochron before the formation of sepals, but that it tends to occur independently in each apex, i.e. the apices are not synchronous with each other.

An observation of cytological interest is that in those apices in which MI was zero the structure of the nuclei was particularly granular, with distinct masses of heterochromatin, similar to the appearance described for articulate nuclei in G1 or G2 (Barlow, 1977).

DISCUSSION

The occurrence of loose synchronization of cell division and of the cell cycle in third-order buds poses the problem of what causes it. It is unlikely to be a specific stimulus produced as a result of induction since the third-order flowers form 32 d after the beginning of induction (Fig. 1) and 25 d after the plants have been returned to non-inductive SD. Also the formation of flowers on other axillary branches, and successive flowers on the same part of the inflorescence, is not synchronous with the formation of the third-order flowers. Synchrony in the third-order buds could perhaps result from some stimulus coming from the pair of leaves on the third-order branch immediately below the third-order flower. If so, this would imply that the young developing leaves become secondarily induced as they develop and then begin producing a stimulus which results in synchronization of the cells in the third-order bud.

The logical conclusion would then be that synchronization in the first-order bud is also produced by a stimulus from the leaf pair below it, and since these (the 9th pair) are formed only on the 6th day of induction (Miller and Lyndon, 1976) these presumably too could have been secondarily induced. Moreover, if synchronization is the result of a stimulus from the most recently formed pair of leaves it is not a direct event or effect of evocation, or else evocation occurs each time a pair of young leaves is formed at the apex of second-, third-, etc., order buds. The simplest conclusion is that synchronization is not an event of evocation but occurs as part of the process of the initiation of each flower in Silene.

A reduction of the size of the primordia at initiation presumably occurs each time a flower is formed, because of the distinctive change in primordial arrangement which accompanies flower formation (Lyndon and Battey, 1985). It also occurs in the third-order flowers (R. F. Lyndon, unpublished data). Since it occurs in many or all flowers, including those plants which require no special environmental stimulus to flower, it is presumably the result of a change in competence of the apex so that it can grow in a floral rather than a vegetative mode. If the occurrence of synchrony in the meristem just before the initiation of the sepals is also regarded as a result of change in competence of the cells there is no need to postulate a specific stimulus for these events. It is only necessary to assume that the apical cells undergo some change which allows them, just before the flower is formed, to react in a different way to floral stimuli already present in the plant so that the cells become synchronized.

The commitment to flower brought about by evocation is a change in competence of the cells. The change in competence to flower is at the cellular level, since it can be transmitted through tissue culture (Chailakhyan et al., 1975). Competent cells can, however, only form flowers when they are exposed to the right environment (Cousson and Tran Thanh Van, 1981). Similarly, the synchronization of cell division and of the cell cycle in the apex of Silene is expressed only when the cells are exposed to the appropriate environmental conditions (Grose and Lyndon, 1984). The reduction in the size of the primordia at initiation is progressive as the flower develops (Lyndon, 1978b) and occurs after synchronization of cell division (Grose and Lyndon, 1984). If synchronization and reduction in primordial size both result from a change in the competence of the cells we must conclude either that competence for different stages in development is achieved at different times or that the internal environment changes and thus allows the expression of different aspects of competence. This latter would be consistent with the hypothesis of differentiation-dependent development (Sachs, 1978), which implies that the first steps in differentiation modify the system in such a way that the pathway of development is subsequently affected. If the primary effect of the floral stimulus is to induce a change in competence of the apical cells only the first event of evocation can be unambiguously attributed to the direct effect of the floral stimulus. Although subsequent evocational events might be the result of the arrival of more floral stimulus, or new components of it, at the apex, it seems equally plausible that they may result from changes at the cellular level in the apex which have altered its competence to react to...
existing metabolities and ultimately to form flowers.

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LITERATURE CITED