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Early Flower Development in *Arabidopsis*

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The early development of the flower of *Arabidopsis thaliana* is described from initiation until the opening of the bud. The morphogenesis, growth rate, and surface structure of floral organs were recorded in detail using scanning electron microscopy. Flower development has been divided into 12 stages using a series of landmark events. Stage 1 begins with the initiation of a floral buttress on the flank of the apical meristem. Stage 2 commences when the flower primordium becomes separate from the meristem. Sepal primordia then arise (stage 3) and grow to overlie the primordium (stage 4). Petal and stamen primordia appear next (stage 5) and are soon enclosed by the sepals (stage 6). During stage 6, petal primordia grow slowly, whereas stamen primordia enlarge more rapidly. Stage 7 begins when the medial stamens become stalked. These soon develop locules (stage 8). A long stage 9 then commences with the petal primordia becoming stalked. During this stage all organs lengthen rapidly. This includes the gynoecium, which commences growth as an open-ended tube during stage 6. When the petals reach the length of the lateral stamens, stage 10 begins. Stigmatic papillae appear soon after (stage 11), and the petals rapidly reach the height of the medial stamens (stage 12). This final stage ends when the 1-millimeter-long bud opens. Under our growing conditions 1.9 buds were initiated per day on average, and they took 13.25 days to progress through the 12 stages from initiation until opening.

INTRODUCTION

The genetic control of flower development in *Arabidopsis* has been the focus of several recent studies including our own (Pruitt et al., 1987; Komaki et al., 1988; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989; Okada et al., 1989). In each case the effects of mutations that specifically disrupt flower morphogenesis have been characterized in detail. It is argued that such mutations are likely to occur in genes whose products control the regulatory decisions that underlie flower development. Ultimately, the cloning and molecular characterization of such genes are likely to allow their mechanism of action to be deduced (Meyerowitz et al., 1989). To understand better the aberrant development in mutant plants, we have characterized wild-type development in detail.

The mature flower of *Arabidopsis thaliana* has a simple structure typical of the Brassicaceae. It has a calyx of four free sepals and a corolla of four petals, whose positions alternate with those of the sepals. There are four medial and two lateral stamens, the former longer than the latter. The superior gynoecium has two carpels whose locules are separated by a false septum. Ovules arise on the parietal placentae on each side of the septum.

The structure and development of the *Arabidopsis* flower from the time of its opening to the release of mature seed from the fruit have already been characterized in detail (Müller, 1961). Changes in the apical meristem as it moves from vegetative to floral growth also have been described by several authors (Vaughan, 1955; Miksche and Brown, 1965). However, a detailed study of the intervening early stages of flower development in *A. thaliana* has not been reported.

In this paper we describe the structure of *Arabidopsis* buds by scanning electron microscopy from their first appearance as a buttress on the apical meristem until they open as fully developed flowers. By examining the shape, size, and surface features of developing floral organs, we have divided the continuous process into 12 stages defined by landmark events. The duration of each stage has also been estimated. This analysis provides a series of reference observations of importance for interpreting the mechanisms of action of genes that control flower development in this model species.

RESULTS

Description of Developmental Events

Flower initiation in *Arabidopsis* occurs continuously in an indeterminate spiral at each floral apex. Thus, flowers can be placed in order of age and developmental stage by their

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position on an inflorescence. In this study all flowers on the main apex of three plants in which the oldest flowers had just opened were analyzed in detail. One plant was 22 days old, the other two were 24 days old. Observations were made directly on primordia and buds at the earliest stages of development. At later stages the buds are enclosed by sepals. To reveal the organs developing underneath, one medial and the two lateral sepals were dissected from the bud. After detailed examination they were photographed from the side and the lengths of the remaining lateral sepal; one or two of the petals, one or two long stamens, a short stamen, and the gynoecium were recorded as the linear distance between the base and the tip of the organ in each case. (In some buds further organs had to be removed in turn to reveal previously hidden organs.) Detailed measurements of buds from one of the three inflorescences are shown graphically in Figure 1. The overall appearance of another inflorescence before dissection is shown in Figure 2A.

Based on observations and measurements from these inflorescences, we have divided the continuous process of flower development from initiation until the bud opens into 12 stages, as shown in Table 1. A summary of information on later stages has been added from the study of Müller (1961).

Stages 1 to 5

Flowers arise on the flank of the apical meristem, which is a dome of cells about 45 μm in diameter at this time (Figures 2B to 2D). Stage 1 begins when a floral buttress appears. This increases in size by lateral outgrowth from the apex. At the time it reaches a breadth of about 22 μm to 25 μm, a groove separates it from the main apex. This defines the beginning of stage 2. Growth of the hemispherical primordium continues almost at a right angle to the main apex, which is itself lengthening and widening at the point of attachment of the bud. Stage 3 begins when sepal primordia appear. By now the flower primordium is 30 μm to 35 μm in diameter and becoming stalked with an incipient pedicel. Its growth is also becoming more vertical. The abaxial sepal primordium arises first, followed soon after by the adaxial and the two laterals. The sepal primordia arise initially as ridges that lengthen and curve inward until they begin to overlie the dome-shaped flower primordium. Again, the abaxial sepal is the first to do this, at which time stage 4 begins. Pedicel elongation continues concurrent with an increase in the diameter of the developing bud to about 65 μm to 70 μm.

Stage 5 begins with the appearance of stamen and petal precursors (Figure 2C). Primordia of the four medial (long) stamens are first seen as wide outgrowths on the central dome of cells. Barely visible are the four petal primordia that arise between the sepals and close to their base. The two lateral (short) stamens develop from primordia that appear a little later in stage 5 (Figure 3A). [This definition of the beginning of stage 5 is slightly earlier than that in our earlier paper (Bowman et al., 1989), where it was defined as occurring when the lengthening medial sepals overlapped.] Stage 5 ends and stage 6 begins when the bud is fully enclosed by both medial and lateral sepals. The tip of the abaxial sepal overlies that of the adaxial, whereas the two shorter lateral sepals meet or overlap underneath (Figure 2A).

Stages 6 to 12

From the start of stage 6, sepals completely cover the bud. During stage 6, primordia of the four long stamens bulge out and become distinct from the central dome of cells (Figure 3B). The two lateral stamen primordia arise slightly lower on the dome and develop slightly later. The petal primordia increase in size somewhat, but are still relatively small. A rim around the central dome of the flower primordium now begins to grow upward to produce an oval, hollow tube that will be the gynoecium.

Stage 7 begins when the growing primordia of the long stamens become stalked toward their base (Figure 3C). This region will give rise to the filament, the wider upper region to the anther. This occurs when the long stamen primordia are around 25 μm to 30 μm long overall. Vertical growth of the slotted gynoecial tube keeps pace with that

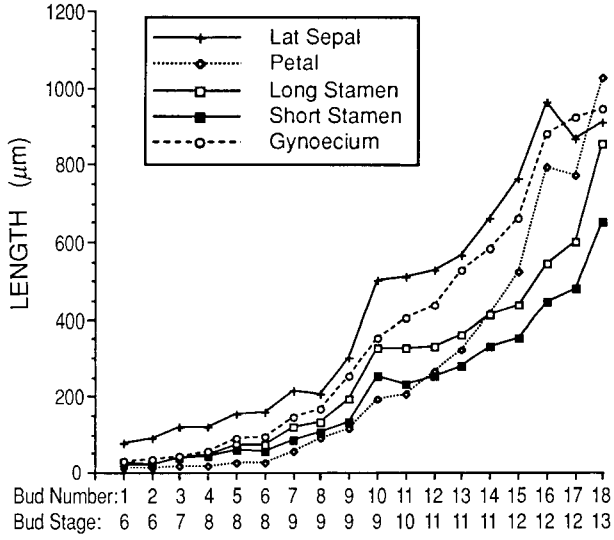

Figure 1. Length of Flower Organs in Buds on the Main Inflorescence Apex of a 22-Day-Old Plant.

The youngest bud scored (bud 1) was at stage 6 and the oldest (bud 18) had reached stage 13. The stage reached by each of the other buds is indicated.

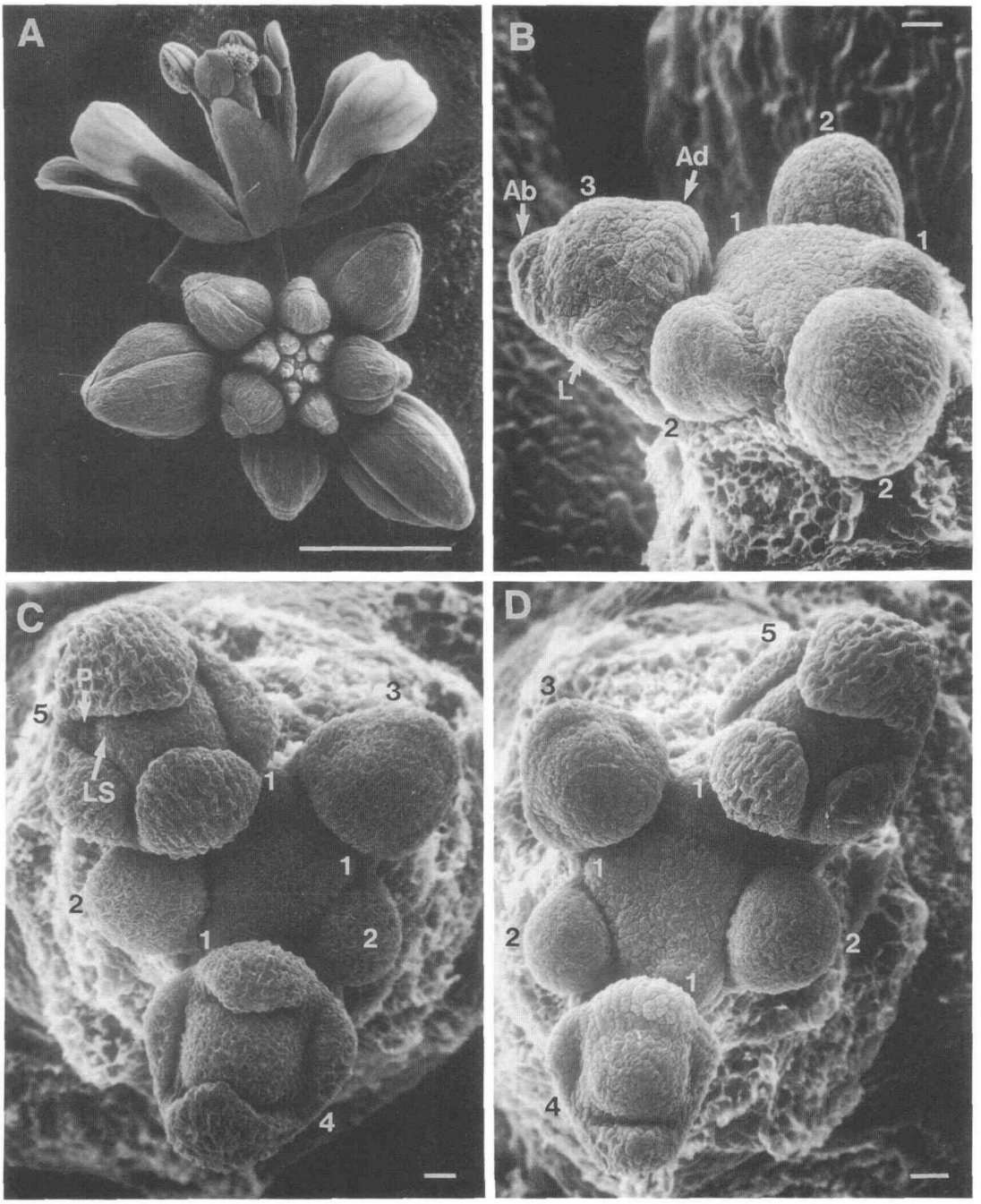

Figure 2. Scanning Electron Micrographs of the Main Flowering Apex of *Arabidopsis*.

(A) Vertical view of the apex of a 24-day-old plant in which the oldest flower has reached stage 14. (The sepals and petals of this flower have spread during preparation.) Bar = 1000 μm.

(B) Lateral view of the youngest buds on an inflorescence after the older buds have been removed. The stage reached by each bud is shown. The abaxial (Ab), adaxial (Ad), and lateral (L) sepal on the stage 3 bud are also indicated. Bar = 10 μm.

(C) and **(D)** Vertical views of two inflorescences in which buds are arising in the opposite spiral sense. In **(C)** younger buds appear in a counter-clockwise direction from the next older bud; in **(D)** the direction is clockwise. The stage of each bud is indicated. Petal (P) and long stamen (LS) primordia are just visible on the oldest, stage 5 buds. Bars = 10 μm.

Table 1. Summary of Stages of Flower Development in *A. thaliana* Listing the Landmark Events that Define the Beginning of Each Stage and Its Approximate Duration

Stage	Landmark Event at Beginning of Stage	Duration[a] (hr)	Age of Flower at End of Stage[a] (days)
1	Flower buttress arises	24	1
2	Flower primordium forms	30	2.25
3	Sepal primordia arise	18	3
4	Sepals overlie flower meristem	18	3.75
5	Petal and stamen primordia arise	6	4
6	Sepals enclose bud	30	5.25
7	Long stamen primordia stalked at base	24	6.25
8	Locules appear in long stamens	24	7.25
9	Petal primordia stalked at base	60	9.75
10	Petals level with short stamens	12	10.25
11	Stigmatic papillae appear	30	11.5
12	Petals level with long stamens	42	13.25
13[b]	Bud opens, petals visible, anthesis	6	0.5
14	Long anthers extend above stigma	18	1
15	Stigma extends above long anthers	24	2
16	Petals and sepals withering	12	2.5
17	All organs fall from green siliques	192	10.5
18	Siliques turn yellow	36	12
19	Valves separate from dry siliques	up to 24	13
20	Seeds fall		

[a] Estimated to nearest 6 hr.

[b] Results for stages 13 to 20 (after the flower opens) are summarized from Müller (1961) where they were named B3 to B10. Their timings are given separately because a different strain was grown under different conditions from those used in the present study.

of the long stamens (Figure 3D). Petal primordia are now hemispherical, although still relatively small (about 25 μm in diameter) (Figure 3C). The beginning of stage 8 is defined by another landmark of stamen development, when anther locules are visible as convex protrusions on the inner (adaxial) surface of the long stamens (Figures 3E and 3F). These stamens have a total length of 55 μm to 60 μm at this time, most of it made up by the developing anther. Locules also appear soon after in the short stamens.

Petal growth now accelerates, and when the petal primordia become stalked, stage 9 begins (Figure 4A). This stage involves a rapid lengthening of all organs, especially the tongue-shaped petals (Figure 1). These increase in length fourfold to fivefold, from about 45 μm up to 200 μm. The stamens also grow rapidly. By the end of stage 9, the medial stamens are around 300 μm long. Most of this growth occurs in the anther region, which still accounts for over 80% of their total length. The gynoecium continues to elongate as an open, oval tube with a somewhat tapered apex. The growth of sepals keeps pace so that the bud remains completely closed.

Stage 10 begins when the fast-growing petals reach the top of the short stamens (Figure 4B). Soon afterward, stage 11 begins when the upper surface of the gynoecium develops stigmatic papillae, although their outward growth is limited at first to regions not in contact with the overlapping sepals (Figure 4C). Once the petals reach the top of the long stamens, flowers move into the final bud stage, stage 12 (Figure 4D). [This corresponds to stage B2 of Müller (1961).] During stage 12 petals continue to lengthen relatively rapidly. The growth rate of lateral sepals continues to slacken while stamens and the gynoecium lengthen in concert. The anthers have almost reached their mature length of 350 μm to 400 μm for both the long and short stamens. Lengthening of the filaments now accelerates rapidly. The upper part of the gynoecium becomes differentiated into a short, 100-μm- to 120-μm-long style, which is slightly constricted toward its base. A sharp boundary separates it from a cap of stigmatic papillae (Figure 4D).

Bud stage 12 ends when the sepals open and stage B3 (Müller, 1961) (here renamed 13) begins (Table 1). The petals can be seen between the sepals and continue to elongate rapidly. The stigma is already receptive at this stage and anthesis occurs. Stamen filaments extend even faster, and the length of the long stamens outstrips that of the gynoecium at the beginning of stage 14 (Figure 2A).

Mature Flower

The mature floral organs show a range of different surface morphologies. The outer sepal surface has many stomata interspersed among irregularly shaped epidermal cells (Figure 5A). In addition, there are always several characteris-

tically elongated cells (up to about 300 μm long) extending longitudinally. Probable progenitors of these cells can be first recognized at stage 8 when the lateral sepals are only about 150 μm long (Figure 3E). The distal edges of the mature sepals are bordered by smaller epidermal cells that are pale green in the live plant (Figure 5A). The outer surface of the sepal may also have an occasional unbranched trichome (Figures 2A and 6C). These are lacking from the inner surface, as are stomata and the elongated cells. Both surfaces of the petal blade are covered with specialized, dome-shaped cells whose surface is finely ridged in a radial pattern (Figure 5B). Petals lack stomata.

The surface of the anthers consists of epidermal cells of uniform size (Figure 5C) that have already been formed by bud stage 10 (Figure 4B). The surface of the ovary in the mature flower is made up of vertical files of epidermal cells (Figure 5D). On the short style the cells are larger and are interspersed with stomata. The densely packed stigmatic papillae are 20 μm to 35 μm long when the bud opens at the end of stage 12. The development of the stigma is shown in Figures 5E to 5G. By the end of stage 9 there is not yet any differentiation at the tip of the gynoecium, which still has a slotted opening to the interior (Figure 5E). This opening is maintained through stage 10, although it becomes smaller. In addition, the eventual disc shape of the stigma surface is becoming apparent (Figure 5F). During stage 11, stigmatic papillae come to cover the entire stigmatic surface. The gynoecium closes over, and the short style begins to develop (Figure 5G).

Nectaries are present in mature flowers at the base of the stamens (Figures 5H to 5J). Lateral nectaries arise at the base of the short stamens toward the end of stage 9 (Figure 5H). They first appear as an outgrowth several cells thick. By stage 11 they have formed either a large ridge (Figure 5I), a single dome, or two domes of cells, usually with several stomata at the apex in each case (Figure 5J). One or the other of the lateral stamens was absent in one-quarter of the flowers we analyzed in detail (12 out of 47). In these the lateral nectary occupying the empty space was usually larger and more dome shaped. The occurrence of medial nectaries at the base of the long stamens was sporadic. If present, they were narrower than the lateral nectaries and arose later.

Commencement of Flower Development

Before flower development starts, the main shoot apex produces a limited number of rosette leaves. In a sample of 117 plants grown under our conditions, the mean number of rosette leaves was 7.25 (SE = 0.07, range = 5 to 9).

To trace the change from vegetative to floral development, a sample of plants was fixed every 2 days beginning 12 days after incubation at 25°C. Buds were first visible on 14-day plants, by which time some of the first-formed buds had reached stage 3 (Figure 6A). At 16 days several of the oldest buds on the main axis of some plants had developed beyond stage 5 (Figure 6B). Buds also arise on secondary floral apices; these occur in the axils of all cauline leaves on the main stem. In our 117 plants there were usually two such cauline leaves per main stem (mean = 2.05 ± 0.04, range 1 to 3). These leaves have clearly developed stipules at each side of their point of attachment to the main stem (Figures 6B and 6C). At 16 days these secondary apices were much less advanced than the main apex. The oldest buds, flanked in turn by secondary cauline leaves, had only reached stage 2 or 3.

At around 18 days, the main inflorescence begins to elongate rapidly as the plant starts to bolt (Figure 6C). At this time between four and seven buds per plant were observed to be at stage 6 or beyond on the main apex. The secondary branches of the main inflorescence continue to develop, with buds now at stage 5. The cauline leaves that overlie these buds develop trichomes. Further inflorescences arise in the axils of the rosette leaves. At 18 days these have only just begun to develop, visible under a cover of their own cauline leaf primordia.

Duration of Stages 1 to 5

To estimate the relative numbers of each of the five early stages on the main axis, counts were made by light and scanning electron microscopy on 20 plants, four sampled on each of days 18, 20, 22, 24, and 26, as shown in Table 2. An average of 7.8 floral primordia were present per plant. There was no apparent change in the proportions of flower primordia in each of the five stages during this period. Overall, stages 1 and 2 were present most often with an average of more than two primordia per plant. Stages 3 and 4 were less frequent (1.40 and 1.35 per inflorescence), whereas there was only a 0.7 chance of seeing a stage-5 bud.

The absolute times spent in these stages can be assessed if an estimate of the rate of bud production is available. This was obtained by recording the numbers of flowers on 20 plants on seven consecutive days beginning on day 25, as shown in Table 3. Because of the difficulty in accurately scoring primordia at stages 1 to 5 on live plants, only buds with closed sepals (stage 6 onward) were recorded. Nevertheless, this allowed the number of buds per day that passed the stage 5/6 boundary to be deduced (Table 3). This was relatively constant from days 26 to 29, averaging 1.9 buds per day. [It fell significantly over the last 2 days (days 30 and 31), and these results have not been used in the following calculations.]

Because an average of 1.9 buds per day move *out of* stage 5 over days 25 to 29, it can be argued that this is also an estimate of the rate of movement of buds *into* stage 1 over the earlier period of days 18 to 26 because the number of buds in stages 1 to 5 is constant over this

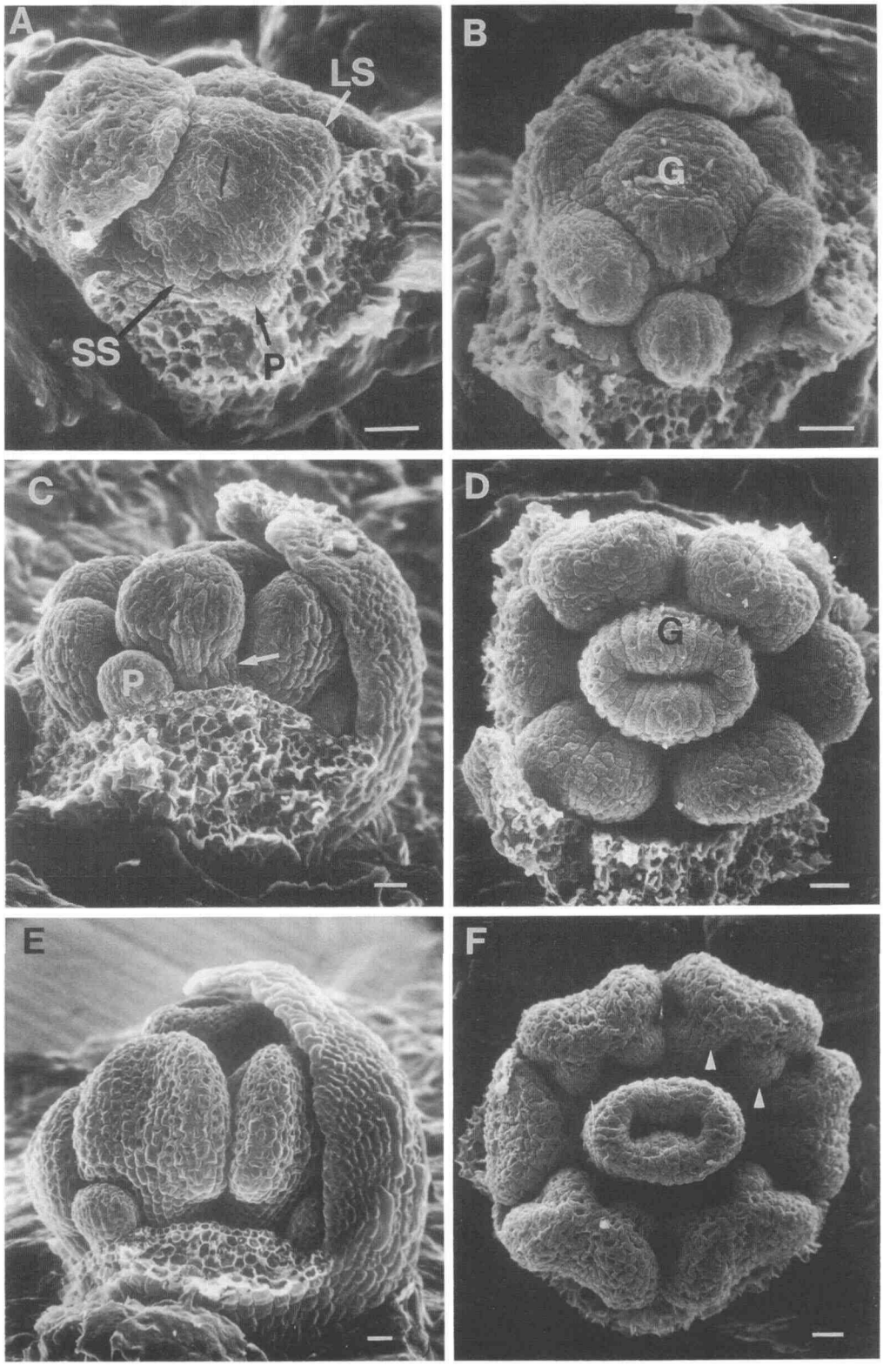

Figure 3. Individual Buds at Stages 5 to 8 with Sepals Dissected Away To Reveal Inner Organs.

period (Table 2). Under such circumstances, stages 1 to 5 occupy a total of 4.1 days on average (i.e., 7.8 buds per plant arising at the rate of 1.9 buds per day). Rounded off to the nearest 6 hr, stage 1 lasts for 24 hr, stage 2 for 30 hr, stages 3 and 4 are 18 hr each, and stage 5 occupies only 6 hr (Table 1).

Duration of Stages 6 to 12

The data in Table 3 also allow the total time in stages 6 to 12 to be estimated. The number of buds moving into stage 6 was in overall balance with the number moving out of stage 12 (into 13 and beyond). For days 25 to 29, the average number moving out was 1.7 buds per day, close to the 1.9 buds per day estimated earlier as moving into stage 6. Thus, the average flux of buds moving through this period is 1.8 buds per day. Given that there is an average of 16.4 buds per inflorescence in these stages (Table 3), a bud takes 9.1 days on average to pass through stages 6 to 12.

Finally, an estimate of the times spent in each of stages 6 to 12 depends on counts of the numbers of bud per plant at each stage. This requires the removal of sepals and was done only for the three inflorescences used to define stages. The number of buds at each stage for one of these is given in Figure 1. (Note that there was an additional bud at stage 6 on this inflorescence that was too small to measure accurately.) In the second inflorescence there were two buds at stage 6, two at 7, two at 8, five at 9, one at 10, two at 11, four at 12, and one at stage 14. In the third inflorescence there were two buds at stage 6, three at 7, one at 8, four at 9, one at 10, two at 11, three at 12, and one at stage 14. Because of these limited data, stages have been estimated only to the nearest 6 hr (Table 1). The longest stage is stage 9 which, with 4.3 buds per inflorescence on average, lasts 2.5 days. Stage 10 is the shortest—about 12 hr.

Direction of Inflorescence Spiral

Buds arise acropetally on an apex, with each new bud arising around the apex at an angle of between 130° to 150° to the earlier bud (Figures 2C and 2D). The direction a new bud may take can be counter-clockwise (Figure 2C) or clockwise (Figure 2D) from the previous bud, as viewed from the top. This direction is maintained by later buds on an axis giving, respectively, a righthanded or a lefthanded helix.

One hundred seventeen mature plants were scored for the direction of spiralling of their main axis. The numbers were close to equality—60 spiralled clockwise, 57 counter-clockwise. Other axes arise on each plant as secondary and tertiary branches of the main stem and as rosette inflorescences. These do not necessarily adopt the same helical sense as the primary apex. Indeed, there is some tendency for them to spiral the other way. On the 60 plants whose main apex spiralled clockwise, 136 further apices spiralled clockwise but there were 183 spiralling counter-clockwise. On the 57 counter-clockwise plants, equivalent figures were 120 counter-clockwise but 135 clockwise. Overall, 256 apices adopted the same spiral sense as their main apex, whereas 318 were different, a significant departure from equality ($\chi^2 = 6.70$ with 1 *df*, $P < 0.01$).

DISCUSSION

Overall, the pattern of development of the *Arabidopsis* flower is similar to that described for other species in the family Brassicaceae, such as the wallflower *Cheiranthus cheiri* (Payer, 1857; Sattler, 1973) and oil seed rape *Brassica napus* (Polowick and Sawhney, 1986), in spite of the fact that *Arabidopsis* flowers are much smaller throughout all stages of development. For example, in *B. napus*, the early flower primordium is about 170 μm in diameter when sepals first appear (30 μm to 35 μm in *Arabidopsis*), and the mature bud is 5 mm long (1 mm in *Arabidopsis*). It will be of interest to determine whether this is a consequence of differences in the number of cells, their size, or both.

Organogenesis

There has been controversy about the relative time of appearance of floral organs and their location in whorls in

Figure 3. Individual Buds at Stages 5 to 8 with Sepals Dissected Away To Reveal Inner Organs.

(A) A bud at stage 5 in which a medial and lateral sepal have been removed. One of the small petal primordia (P) is indicated. The primordia of the long stamens (LS) are larger than that of the short stamen (SS).

(B) Lateral view of a bud at stage 6 in which the sepals had fully enclosed the bud. The stamen primordia are now dome shaped, whereas the petal primordia are still relatively small. The gynoecium (G) will arise from the central dome of cells.

(C) Medial view of a bud at stage 7 showing that the long stamen primordia are now constricted toward their base (arrow). The petal primordia (P) have become dome shaped.

(D) Vertical view of a stage-7 bud showing that the stamens do not yet show locule ridges on their adaxial surface. The gynoecium (G) is growing vertically as a slotted tube.

(E) A bud at stage 8 in which the stamen primordia have increased markedly in size, especially in relation to the petal primordia.

(F) Vertical view of a stage-8 bud in which locules (arrows) are now clearly visible in the stamens.

Bars = 10 μm.

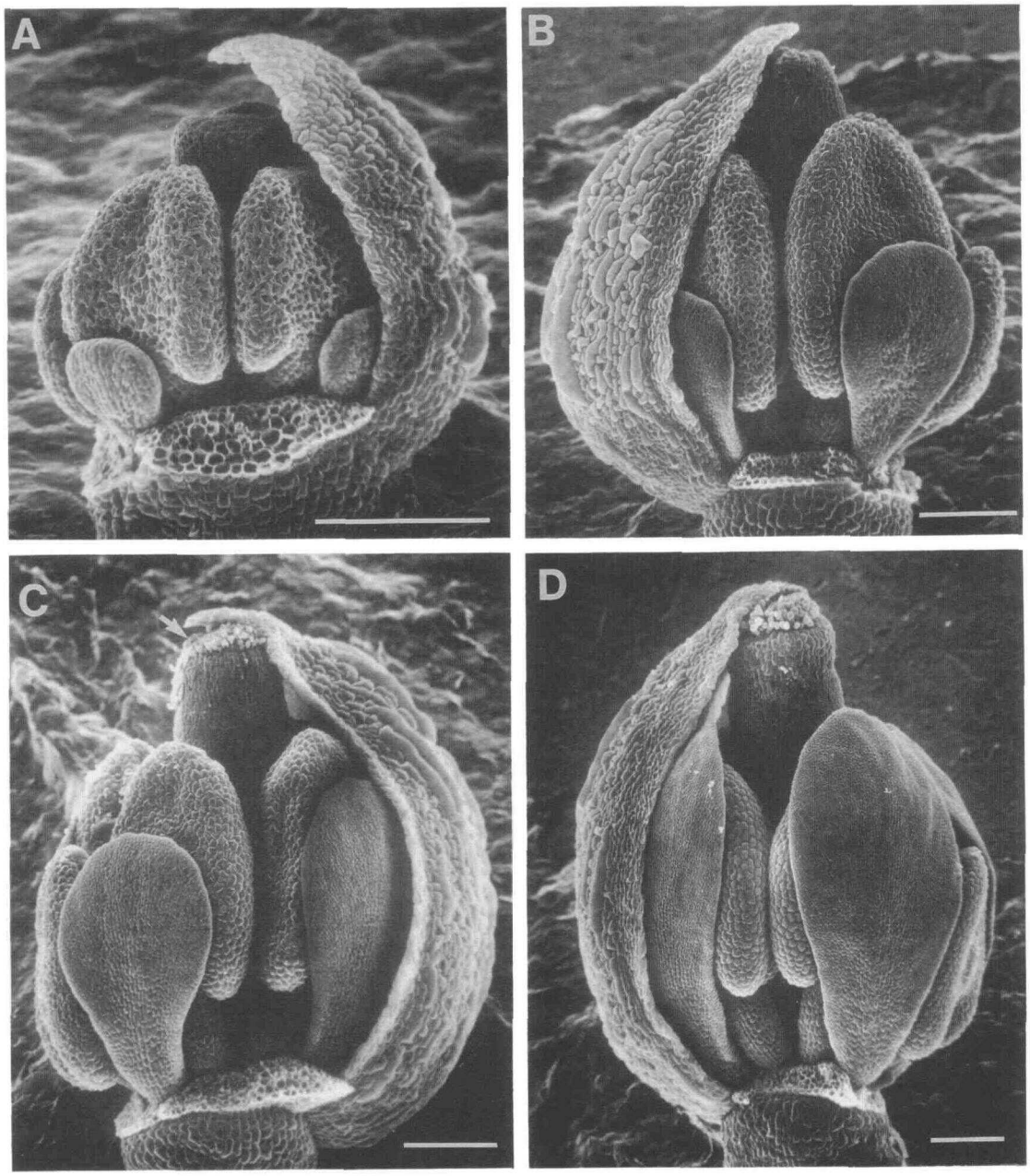

Figure 4. Lateral Views of Buds at Stages 9 to 12.

(A) A bud early in stage 9 showing that the petal primordia have become wider toward the top as they start growing rapidly.
(B) A bud in which the petals have just reached the height of the lateral (short) stamens, marking the beginning of stage 10. Buds more than double in size during the lengthy stage 9 [c.f. **(A)**].
(C) Stigmatic papillae (arrow) appear on the top of the gynoecium at the start of stage 11.
(D) Stage 12 is the final stage before the bud opens. It commences when the petals reach the height of the long stamens.
Bars = 100 μm.

the Brassicaceae flower. Whether all four sepals occupy one whorl or the two laterals occur in a separate whorl outside that of the abaxial and adaxial sepals has been in question. The order in which sepals arise does not help settle the matter. It is clear in all species examined that the abaxial sepal is the first to appear. In *Arabidopsis* we agree with Hill and Lord (1989) that the lateral sepals arise at around the same time as the adaxial sepal, although they are reported to arise earlier in *Cheiranthus* (Payer, 1857; Sattler, 1973) and *B. napus* (Polowick and Sawhney, 1986). What is established is that the laterals arise lower on the floral primordium (e.g., Figure 2B), and that their vasculature in the mature flower detaches below that of the medial sepals (Arber, 1931a).

Petals arise in one whorl in the Brassicaceae flower. Because their early development is relatively slow, there has been some doubt about whether they arise before or after the stamens. The petals have been reported to arise before the stamens in *C. cheiri* (Payer, 1857; Sattler, 1973) and after the stamens in *B. napus* (Polowick and Sawhney, 1986). In *Arabidopsis* we could not confidently separate their origin in time from that of the long stamens (see also Hill and Lord, 1989).

In *A. thaliana* and *B. napus* (Polowick and Sawhney, 1986) the four inner stamens arise a little earlier than the two outer ones, whereas in *Cheiranthus* the order reportedly is reversed (Payer, 1857; Sattler, 1973). In *Arabidopsis* we found that one of the outer stamens was absent in about one-quarter of the flowers examined. This has also been reported in three other races in which many flowers had only four stamens (Müller, 1961). The pattern of gynoecium development seems to be similar in *Arabidopsis* (the present study; Hill and Lord, 1989; Okada et al., 1989), *Cheiranthus* (Payer, 1857; Sattler, 1973), and *B. napus* (Polowick and Sawhney, 1986). The major difference lies in the stigma, which is markedly bilobed only in the latter two species.

Finally, our observations on nectaries, which concur with those of Müller (1961), show that they are variable in presence, size, and shape. They arise late in flower development (stage 9), and it may be that their growth is limited by available space and nutrients.

Origin of Flowers

The apical meristem of *Arabidopsis* changes from slightly convex to distinctly dome shaped during the transition from vegetative to floral growth (Vaughan, 1955; Miksche and Brown, 1965). Flower primordia then arise on the flank of the apical meristem by periclinal divisions in cells beneath the two-layered tunica. Under continuous light at 25°C, stage-3 buds were observed here after 14 days' incubation. Thus, the first bud is likely to have arisen at around 12 days in our plants, several days later than that observed by Miksche and Brown (1965) on Estland plants in soil-less culture. We estimated that an average of 1.9 buds arose per day on the main apex of 20 plants in this study. The only other report from *Arabidopsis* is for three plants of race Dijon grown at 20°C that produced buds at a slightly faster rate—2.3 buds per day (Müller, 1961). It is likely that these rates are influenced to a large degree by environmental factors.

The presence of stipules at the base of young cauline leaves in *Arabidopsis* (Figures 6B and 6C) is of interest. Payer (1857) was unable to see stipules in any species of the Brassicaceae, but Arber (1931b) reported well-developed stipules on young leaves of *Nasturtium officinale*. We have reported earlier that the presence of stipules on outer-whorl organs of *apetala2-1* flowers was evidence of their leaf-like structure (Bowman et al., 1989). Arber (1931a, 1931b) also commented on the presence of filamentous outgrowths at the base of some leaves and pedicels in a range of Brassicaceae species. She called these "squamules," and distinguished them from stipules by their smaller diameter and their presence at the base of pedicels as well as leaves. We were unable to see such organs on any leaves or buds of wild-type *Arabidopsis*, although we have seen them in various mutants, including *apetala1*. They may well be the "filamentous sepals" that Kunst et al. (1989) record between the outer floral organs of *apetala2-7* mutants and that Komaki et al. (1988) report in mutant FI-54.

The direction of flower production on any one main apex of *Arabidopsis* is apparently decided at random and is maintained unchanged. In this regard it resembles the pattern of phyllotaxis in many species including, for example, *Pharbitis nil* (Imai, 1927), *Nicotiana tabacum*, and *N. rustica* (Allard, 1946). It does seem in *A. thaliana* that the spiral direction taken by secondary and tertiary branches is somewhat influenced by the spiral pattern of the primary stem. We do not know whether this is the case in other plant species.

Conclusion

Our definition of 12 stages in early flower development in *Arabidopsis* will be useful in interpreting the action of genes that control this process. Already we have indirect evidence that the product of the *APETALA2* gene is active during stages 2 to 4 (Bowman et al., 1989). Sepals, which are affected by the *apetala2-1* mutation, arise during this time. In the case of *APETALA3*, the gene product is apparently active later when petals and stamens, the organs affected by mutations in this gene, are differentiating (Bowman et al., 1989). The *pistillata* mutation also affects petals and stamens, and it is clear that in this case "petal" primordia arise as normal, but they differentiate with sepal-like properties under a petal timetable (Bowman et al.,

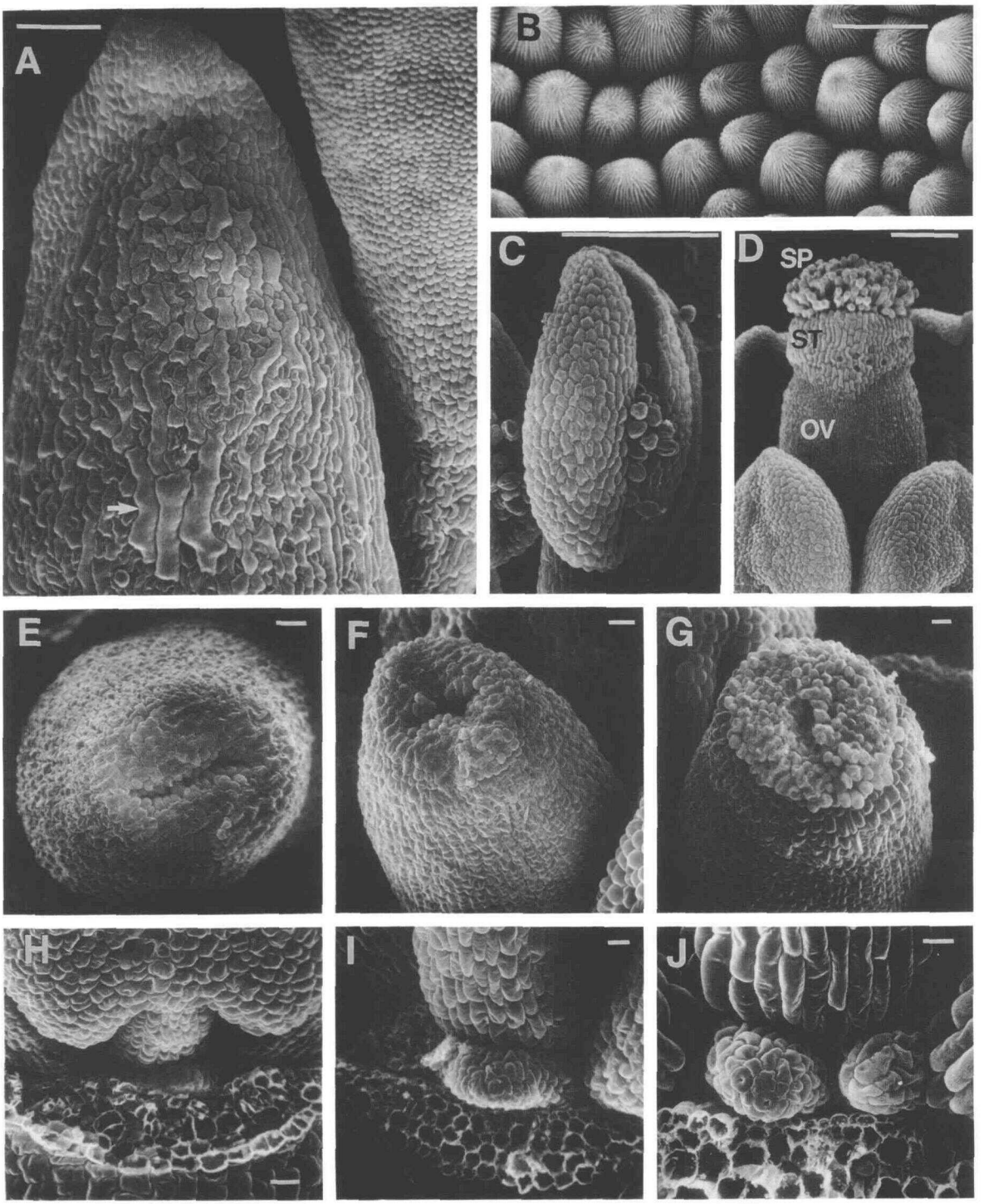

Figure 5. Surface Morphology of Mature and Developing Floral Organs.

(A) Outer surface of a mature sepal (left) showing several long epidermal cells (arrow), stomata, and a fringe of smaller cells. The lower part of the adjacent petal (right) shows a regular transition from longer cells in the claw to more ovoid cells in the blade. Bar = 100 μm.
(B) Higher magnification of the ridged cells that cover the surface of the blade of mature petals. Bar = 10 μm.
(C) A mature stamen after dehiscence showing epidermal cells of uniform size and pollen grains. Bar = 100 μm.

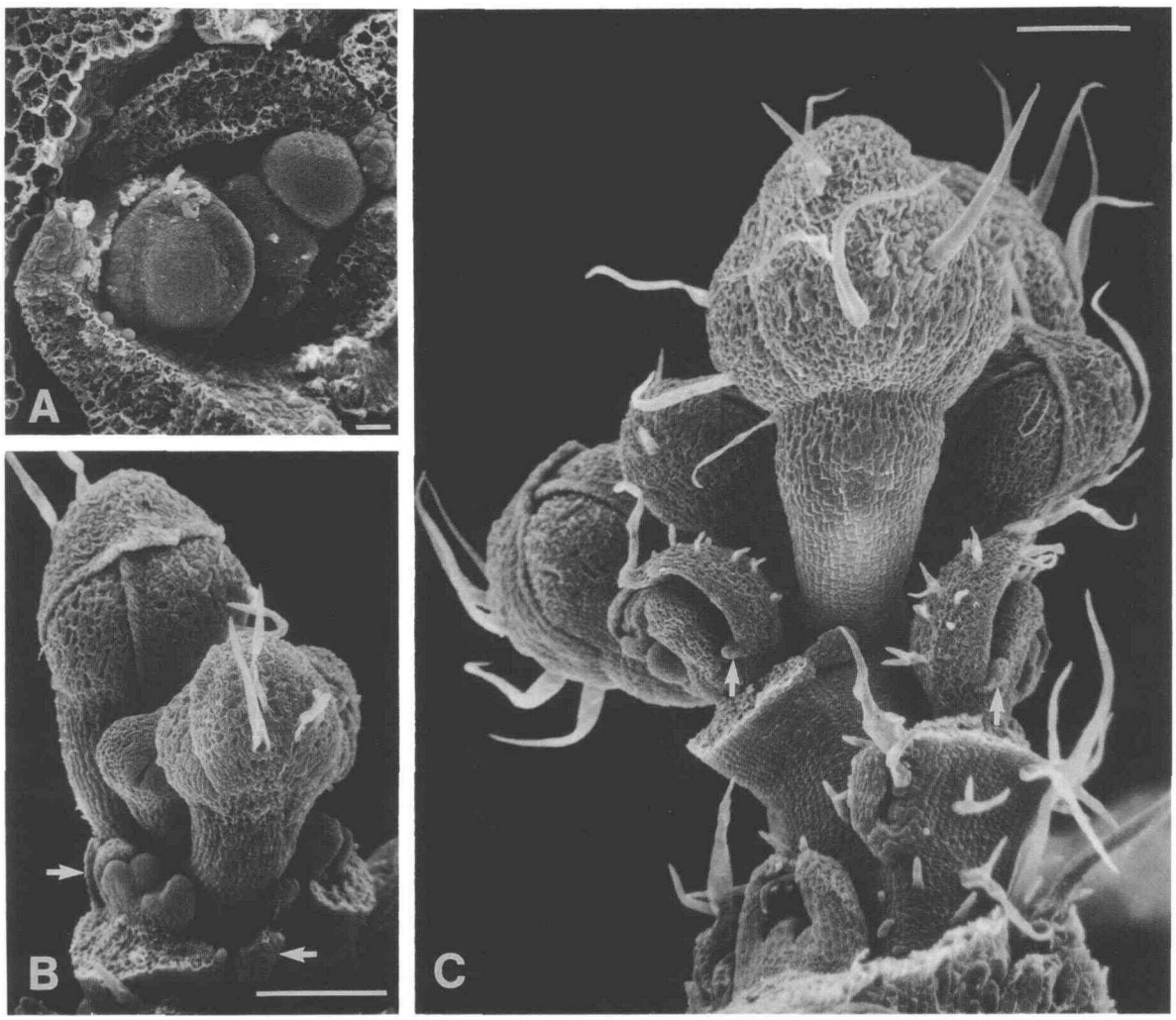

Figure 6. Appearance of Immature Inflorescences on Plants at the Commencement of Flowering.

Rosette and cauline leaves have been removed.
(A) Vertical view of the main apex of a 14-day-old plant showing that the oldest bud has advanced to stage 3. Bar = 10 μm.
(B) A 16-day-old-plant with four buds beyond stage 5 (sepals closed) on the main apex. Two cauline leaves have been removed from the main stem although their stipules remain (arrows). Secondary apices within these cauline leaves have also initiated flower development. These apices are flanked in turn by younger cauline leaves, and their oldest flower primordium is only at stage 2. Bar = 100 μm.
(C) Lateral view of an 18-day-old plant just beginning to bolt. The two secondary apices visible have advanced in concert, with buds reaching stage 5 on each. Their cauline leaves show developing trichomes and stipules (arrows). A new apex is just visible in the axis of one of the dissected rosette leaves (lower left) although flower development is not yet detectable under its cauline leaf primordia. Bar = 100 μm.

Figure 5. (continued).

(D) The upper parts of a gynoecium in a stage-12 bud showing stigmatic papillae (SP), large cells and stomata on the short style (ST), and smaller epidermal cells on the surface of the ovaries (OV). Bar = 100 μm.
(E) to **(G)** Structure of the developing stigma. The surface of the gynoecium is smooth and slotted at the end of stage 9 **(E)**. A cap becomes apparent at stage 10 when the surface is still not closed **(F)**. The appearance of papillae covering the stigmatic surface defines the start of stage 11 **(G)**. Bars = 10 μm.
(H) to **(J)** Structure of developing nectaries (lateral sepal removed). These first appear at the base of the lateral stamens in stage 9 **(H)** and grow outward during stages 10 and 11 **(I)**. They reach maturity when the flower opens and reaches stage 14 **(J)**. Stomata often occur at their apex. The righthand nectary in **(J)** has shrunken, probably because it has already released its nectar. Bars = 10 μm.

1989; Hill and Lord, 1989). The description of the surface characteristics of normal organs (the present study; Bowman et al., 1989; Hill and Lord, 1989; Kunst et al., 1989) also allows the effects of mutational changes, including the nature of mosaic structures, to be inferred.

When these and other genes are cloned, the site and time of their expression can be determined directly. In this way we may ultimately understand how gene activity directs flower development.

METHODS

All plants were of the Landsberg ecotype and were homozygous for the *erecta* mutation, which reduces plant height. Seeds were planted on the surface of a mixture of 3 volumes of commercial potting mix, 3 volumes of peat, and one of sand, spaced at about 4 cm^2 per plant. They were treated at 4°C for 2 days and then transferred to a growth chamber. The age of a plant was recorded from the time of this transfer. Conditions were held at 25°C and 70% relative humidity, and plants were grown under continuous light provided by cool-white fluorescent tubes.

For scanning electron microscopy, whole inflorescences were fixed in 3% glutaraldehyde in 0.025 M sodium phosphate buffer (pH 6.8) at 4°C for at least 12 hr. They were then rinsed in the buffer and further fixed in 1% OsO_4 in 0.05 M sodium cacodylate buffer (pH 7.0) for several hours before dehydration through a graded ethanol series. Inflorescences were then critical point dried using CO_2. Whole inflorescences were mounted on stubs and shadowed with gold and palladium (4:1) before viewing with an ETEC Autoscan scanning electron microscope. In many cases individual buds were then dissected from the inflorescence, outer organs removed using glass needles, and the buds examined again after further shadowing. Photographs were taken on Kodak type 4127 film.

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Table 2. Numbers of Flower Primordia at Stages 1 to 5 on the Main Apex of Four Inflorescences. Four Plants Were Sampled Every Other Day from 18 to 26 Days after Incubation at 25°C

	No. of Flower Primordia at Stages Shown							
Age (days)	1	2	3	4	5	*Total* (1–5)	6–12	13–15
18	9	9	5	5	3	31	24	0
20	8	10	6	6	1	31	42	0
22	8	11	6	5	4	34	67	1
24	9	8	5	5	3	30	68	2
26	7	8	6	6	3	30	61	10
Total	41	46	28	27	14	156		
Mean/apex	2.05	2.30	1.40	1.35	0.70	7.80		

Table 3. Cumulative Totals of Flowers per Plant on 20 Plants Scored Each Day from 25 to 31 Days after Incubation at 25°C

	Mean No. of Flowers per Plant at Stages Shown			Mean No. of Flowers Advancing	
Age (days)	6–12	13–17	Total	Into Stage 6	Out of Stage 12
25	15.9	0.5	16.4	–	–
26	16.0	2.2	18.1	1.7	1.7
27	16.7	3.8	20.5	2.4	1.6
28	16.9	5.3	22.2	1.7	1.5
29	16.7	7.4	24.1	1.9	2.1
30	16.2	8.9	25.1	1.0	1.5
31	15.6	10.6	26.2	1.1	1.7

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