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SHOOT AND FLORAL DEVELOPMENT IN CALLA PALUSTRIS (ARACEAE-CALLOIDEAE)

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Calla palustris shows annual production of one or two inflorescences on each ephemeral seasonal shoot. Shoots are sympodial; the terminal inflorescences are produced in the summer of the year before the mostly perfect flowers open. Overwintering terminal buds show limited preformation of foliage leaves on renewal shoots; most leaves are neoformed. The spadix produces a series of prominent flower primordia in up to eight irregular orthostichies. Each primordium develops no perianth but a series of indistinctly trimerous stamen initials in centripetal order. The gynoecium originates as a single dorsiventrally asymmetric collar-like outgrowth that becomes ovate and encloses the series of basal anatropous ovules, showing no evidence of a pseudomonomerous condition either in development or vasculature. Floral variation within a spadix is considerable, distal flowers are usually wholly male, basal flowers show reduced stamen number, but no strictly female flowers are produced. Vascular traces to the floral appendages are derived from an anastomosing system that obscures any uniform pattern. Morphogenetic considerations describe the vascular system better than any reference to imaginary evolutionary antecedents.

Introduction

Despite considerable floral diversity in Araceae, virtually no developmental study of aroid flowers has been carried out. Diversity of flowers is usually described, in evolutionary terms, as a modification of a dimerous or trimerous hermaphroditic flower, even though Araceae with perfect flowers have themselves considerable organizational diversity (Engler 1884). Developmental studies of flowers are essential to a precise understanding of morphology that can reveal common floral plans of systematic and phyletic significance (Leins et al. 1989). In this present study we show that the inflorescence axis (spadix) of *Calla* initiates lateral floral primordia that lack any subtending bracts. All floral appendages are initiated without reference to any consistent symmetry, in part because stamens are "interpolated" in an irregular centripetal pattern. The gynoecium shows no developmental evidence for a trimerous condition. The sequence of appendages conditions a vascular system without obvious regularity.

Calla palustris is a temperate herbaceous representative of the primarily tropical family Araceae, but with a wide circumboreal distribution. It persists by means of creeping horizontal axes, which are either permanently or temporarily submerged, or at the surface of swamps. Leaves are always emergent. The shoot units are ephemeral, lasting little more than a year, but populations persist by both regenerative and proliferative branching, in the terminology of Tomlinson

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(1974). Flowers open in the late spring, the fruits ripening in late summer of the same year.

In the classification of the Araceae developed by Engler and Krause (Engler 1877; Krause 1908), *Calla* is included in the subfamily Calloideae together with three other north temperate genera, *Lysichiton, Orontium,* and *Symplocarpus.* Grayum (1984, 1990) suggests, primarily on the basis of pollen morphology, that *Calla* is more isolated within the family and includes it as a monotypic subfamily, Calloideae, the remaining three genera being added to other groups. Barabé and Forget (1987) also suggest a position isolated from *Lysichiton, Orontium,* and *Symplocarpus* on the basis of cladistic analysis, although they still retain all four genera in the subfamily Calloideae.

Previous accounts of the shoot organization of *Calla* go back to Doll (1843) and Wydler (1856) and have been summarized by Braun (1859), Engler (1877), Krause (1908), and Dudley (1937) without the addition of significant new data. Dudley provided additional information on seed and seedling morphology and pollen development and clarified the cytology. More recently Barabé and Labrecque (1983) described the floral vasculature and concluded that this provided evidence for a pseudomonomerous condition, the gynoecium being interpreted as being made of three fused carpels because of the presence of three principal carpellary traces. This interpretation is contradicted by the observations on floral vasculature by Hotta (1971). The concept of pseudomonery in aroids apparently originated with Engler (1884) and was extensively elaborated upon by Eckardt (1937).

Material and methods

Populations of *Calla palustris* were studied from Black Gum Swamp, Tom Swamp, and Harvard Pond, Petersham, and Spectacle Pond, Athol, Massachusetts. Since no differences in patterns

of shoot and floral development were found among these collections they have been treated as one. Shoots were sampled at about biweekly intervals throughout the growing season (May–October 1990) and also in the spring of 1991, to a total of about 20 samples of over 150 shoots.

Material was either dissected fresh before fixation or after fixation in 70% F.A.A. For the study of shoot and floral ontogeny fixed spadices were dehydrated to 95% ethyl alcohol, stained overnight in 1% acid fuchsin in 95% alcohol, and photographed using epi-illumination techniques (Posluszny et al. 1980). For scanning electron microscopy (SEM) spadices were dehydrated through an ethanol series into absolute ethanol, critical-point dried in a SamDri PVT. 3 critical-point dryer and coated with gold palladium for 5 min in a Hummer III sputter coater. Spadices were viewed using an AMR 1000 SEM set at 10 kV at the Museum of Comparative Zoology of Harvard University. For the study of histology and vasculature at several stages of development, material was embedded in paraplast after routine dehydration in a tertiary butyl alcohol–ethanol series, serially sectioned at 7–10 μm in longitudinal and transverse planes and stained in safranin and alcian green. Serial sections of spadices and shoots were analyzed by recording individual sections on a Panasonic GX-4 model AG-1950 multifunction video cassette recorder using a Javelin Model JE3012 video camera attached to a Wild compound microscope. This method of analysis was complemented by drawings of sections made with a Wild drawing apparatus. Additional specimens for the study of vasculature were prepared by clearing thick sections of older spadices in 5% alcoholic sodium hydroxide, followed by lactic acid, after washing in water. These specimens were stained in 1% basic fuchsin, dehydrated, and cleared in xylol.

Results

SHOOT MORPHOLOGY

Shoots of *Calla* are sympodial, each unit ending in a terminal spadix (Sx) with an enveloping spathe (Se) (figs. 1, 2, 3C). The regenerative or renewal shoot (RS in fig. 3A, B) arises from the axil of the penultimate foliage leaf (Lp in figs. 1, 2). The renewal shoot may develop as an extended vegetative axis that either overwinters as a condensed shoot and grows out the following spring (in about 60% of examples) (figs. 1, 2A) or immediately proceeds to form a second spadix on an axis with only three foliage leaves (fig. 2B). An extended renewal shoot again arises in the axil of the penultimate foliage leaf of the first renewal shoot ($L'p$ in fig. 2B) and subsequently overwinters as above. The reproductive potential of a shoot may therefore be one or two spadices

per year, although the younger spadix in shoots with two spadices may sometimes abort. Phyllotaxis is distichous throughout, with no change in the plane of distichy at branching. Although no leaves are present in figure 3D the distichous phyllotaxis of the shoot is made obvious by the arrangement of axillary buds. In nature, the plane of distichy is horizontal so that plants spread over the surface of the substrate with all leaves similarly erected by unequal growth at the base of each petiole.

The regenerative renewal shoot is developed by syllepsis (Hallé et al. 1978) and is adnate to the parent axis for about half the associated internode (dotted line in fig. 2). A bicarinate membranous prophyll is present in the adaxial position (P in figs. 1, 2, 3B). The first foliage leaf on the renewal shoot is inserted on the same side as the prophyll, not the opposite side, as would be expected with distichous phyllotaxis ($L'o$ in figs. 1, 2, 3B, 6A). This unusual arrangement also characterizes proliferative vegetative shoots, which arise in the axils of each foliage leaf from dormant buds by prolepsis (PS in fig. 3D). Only axillary buds farthest from the shoot tip grow out and form proliferative shoots with the degree of suppression of buds increasing markedly toward the shoot tip. The ultimate (Lu) and the unusually placed (Lo) foliage leaf usually lack axillary buds. Damage of the leader shoot brings about the release of previously dormant buds.

Individual foliage leaves show a regular symmetry, which influences overall shoot symmetry, described as antidromous by Engler (1877). Each leaf has a basal open sheath with overwrapping margins, an extended petiole, and a somewhat hastate blade with convolute vernation. The blade margin of each leaf overwraps in a direction opposite to that of its sheath; only the overwrapping of sheaths is shown in figure 1. Leaves may be designated as right handed or left handed as determined by direction of overwrap. Leaves on the same side of the axis always overwrap in the same direction (i.e., leaves in one orthostichy are always either right handed or left handed), whereas leaves in the opposite orthostichy always have contrasted handedness (fig. 1). There is thus a consistent contrast in leaf symmetry (antidromous) except that the renewal shoot can originate in the axil of either a left-handed or a right-handed leaf.

The mouth of the leaf sheath is prolonged as an extended ligule, which encloses all younger organs (*lig* in fig. 3A, B). The prophyll of the regenerative shoot (P in fig. 1) does not encircle the axis at its insertion; symmetry is determined distally by its overwrapping margins (dotted lines in fig. 1). Prophylls of proliferative shoots encircle the axis completely and overwrap in the manner of foliage leaves (Pp in fig. 1).
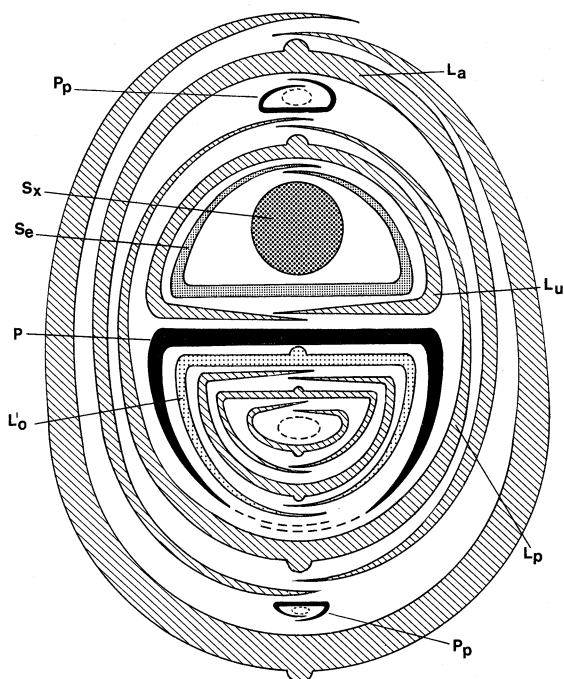
Fig. 1 *Calla palustris.* Diagrammatic transverse section of flowering shoot with renewal shoot in axil of its penultimate foliage leaf (*Lp*). Foliage leaves, hatched except *L'o*; prophylls (*P*) of regenerative shoot (*Pp*) of proliferative shoot, black; spathe (*Se*), stippled; spadix (*Sx*), heavily stippled; *La*, antipenultimate foliage leaf of flowering shoot; *Lu*, ultimate leaf of flowering shoot; *L'o*, adaxial foliage leaf of renewal shoot not alternate with prophyll and interrupting normal distichy.

SHOOT PHENOLOGY AND DEMOGRAPHY

The shoot system may be described as "polyphyllous," since there is no fixed number of foliage leaves on each renewal shoot; values ranged from 16 to 30 ($\bar{x}$ = 23.5, SD = 3.9; N = 31). Seasonality of growth is reflected in the long internodes produced during early summer extension and the short internodes during late summer and fall when dormancy is induced. This series of short internodes corresponds to the region of sympodial branching, which begins in the middle of August and is associated with production of spadices that will flower the following May as mapped in figures 1 and 2. In figure 3C and D the region of short internodes is to the right. In figure 3C growth was terminated with the spadix (*Sx*) now represented by the scar where the infructescence has abscised. The shoot shown in figure 3D did not flower in the previous year. Many proliferative shoots do not flower for several years until some critical size is attained.

The precise degree of preformation of foliage leaves on the renewal shoot within the winter bud varies considerably and is dependent on whether a shoot produces one or two spadices. Of 21 shoots examined in the months of September and October the mean number of preformed leaves on

dormant renewal shoots was 5.4 (SD = 1.2) although the range was from four to eight leaves. On shoots producing two spadices the first renewal shoot always terminated in a spadix after the production of three foliage leaves. The subsequently produced renewal shoot thus tended to have only four or five leaves compared with a maximum of eight found in shoots having only a single spadix. The two or three outermost leaves present in terminal shoots at the time of overwintering were always reduced, although they still possessed the morphology of foliage leaves. The series of parts illustrated in figures 1–3 can only be exposed by dissection of the overwintering renewal shoots. These observations indicate that the majority of leaves are formed in the season of growth, as the renewal shoot extends. Floral expansion on the preformed spadices occurs in early spring with first flowering occurring in May, although flowering can continue into late June as the second spadix present on some plants expands.

Each previous renewal shoot dies in late summer up to the region of short internodes, several internodes below the point of last year's flowering (i.e., below the node of origin of the renewal shoot; fig. 3C) so that any population is represented by plants that are a maximum of 2 yr old. Senescence of leaves in the fall months yields rhizomes of the appearance shown in figure 3C and D where no leaves except those reduced leaves of the terminal shoot are present on the axis in late fall and spring, and older parts of rhizome segments have collapsed and decayed.

Proliferative shoots diverge at 90° to the parent axis (*PS* in fig. 3D), growing through the back of the sheath of the subtending leaf. Because of the annual necrosis of basal parts of the shoot each daughter proliferative shoot becomes autonomous from the parent axis within one year of production. The clonal population therefore consists of shoots that migrate some 10–50 cm per annum, depending upon shoot vigor. The "perennial" nature of the plant is thus determined by shoot units that are intrinsically annual in their life cycle. Despite the common basic plan of shoot organization there is considerable plasticity in shoot size depending on the number of parts produced per year, the amount of internodal extension and the number of proliferative shoots produced.

SPADIX PHENOLOGY

Spadices are initiated in the July or August previous to the year that they will flower. Development occurs rapidly such that the majority of first spadices on shoots have flowers at the point of early gynoecial closure (fig. 4F) by the time dormancy sets in. Development of first spadices is well synchronized such that they are at

similar stages of development at the time of over-wintering. It is only on the second spadix of shoots producing two spadices that the early developmental stage of flowers can be found in the fall months. In these cases spadices were often just showing the beginning of gynoecial initiation (fig. 4*D*, *E*) when they went dormant.

Despite the advanced stage of development of first spadices on shoots in the fall months, these spadices were only one-half to one-third the size of spadices at anthesis the following spring, indicating that extensive expansion of spadices and flowers occurs in the spring months prior to flowering. This is illustrated well if one compares figure 5*A* (a spadix at time of dormancy) with figure 5*E* (flowers close to the time of anthesis). Both figures are the same approximate magnification.

FLORAL DIFFERENTIATION

The inflorescence axis extends above the primordium of the spathe as an elongated ellipse. Floral primordia are initiated as prominent outgrowths of the surface in such a way that an irregular, somewhat angular mound is produced (fig. 4*A*). There is no subtending bract that precedes floral initiation. Primordia are initiated in acropetal order, and the whole of the axis is ultimately consumed so that the last-formed primordium is pseudoterminal. The most obvious pattern is the series of oblique, irregular orthostichies, each with about five or six primordia; parastichies are equally irregular so that it is not clear if the series is spiral or whorled (fig. 4*B*). Primordia may or may not at first be in contact with each other early in development; there is considerable variability between inflorescences. Irregular contact later establishes the angular outline of the primordia, which is accentuated by the initiation of floral appendages. The irregular shape of the primordia is correlated with the subsequent irregularity in sequence of appearance of floral parts, so that a uniform acropetal and radially symmetrical arrangement of parts, e.g., stamens, is not possible. An ultimate extension of this is the development of "twin" primordia, which resemble two primordia fused laterally and always in the horizontal plane.

Description of development of flowers initially deals with those in the central region of the spadix, which are bisexual; those in distal and prox-
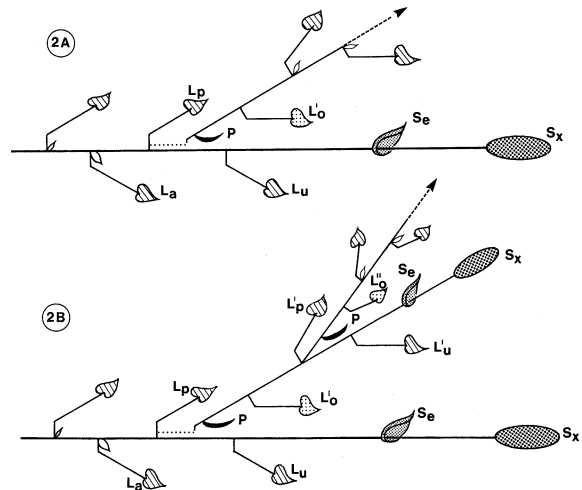

Fig. 2 *Calla palustris.* Diagram of shoot construction in region of flowering. *A*, Spadix with renewal shoot in axil of *Lp*. *B*, Spadix with first-order renewal shoot terminating in a second spadix after producing only four leaves ($P + L'o + L'p + L'u$); second-order vegetative renewal shoot (including $P + L''o$) from axil of $L'p$. Hatching and labeling as in fig. 1.

imal regions of the spadix are described later under gender variation.

No perianth is initiated. The first appendages are stamen primordia that arise at the periphery of the floral primordium, which becomes flattened. Stamen primordia originate as protuberances that are initially dome shaped, soon becoming dorsiventral and flattened and subsequently becoming bilobed. There is no obvious circumferential sequence to the initiation of stamens except for a strong tendency for the first-formed stamen to be in an abaxial position reminiscent of a subtending bract (*A* in fig. 4*A*). An indistinct trimery may be recognized, which is largely a consequence of the irregularly hexagonal shape that the primordium adopts when appendages are first evident. The abaxial stamen often develops precociously and remains larger than later-initiated stamens until late in development, particularly in upper flowers of the spadix. Later initiated stamens appear in approximated centripetal order (fig. 4*F*, *G*; stamen primordia next to gynoecium), but it is clear that new primordia can be inserted tangentially between existing primordia. As organogenesis progresses the increasing contact between adjacent

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Fig. 3 *Calla palustris.* Shoot construction. Bar = 300 μm, *A*. Bars = 2 cm, *B–D*. *A*, Epi-illumination photograph of partly dissected shoot apex; renewal shoot (*RS*) with enveloping prophyll, ultimate leaf of shoot (*Lu*) with inflated sheathing base enclosing spathe and spadix (cf. fig. 2*A*). *B*, Later stage of a shoot similar to *A* with developing renewal shoot (*RS*) and flowering shoot of previous sympodial unit. Bicarinate prophyll (*P*) and its immediately succeeding leaf (*L'o*) inserted on the same side of the axis. *Se*, spathe; *Lu*, ultimate foliage leaf. *C*, Sympodial unit with leaves removed, scar of spadix (*Sx*) of previous unit. *D*, Vegetative shoot of same age arising from a previous terminal bud; short internodes to right indicate position of shoot at time of previous year's dormancy. *PS*, proliferative shoots from previously dormant axillary buds.

A

B

C

D
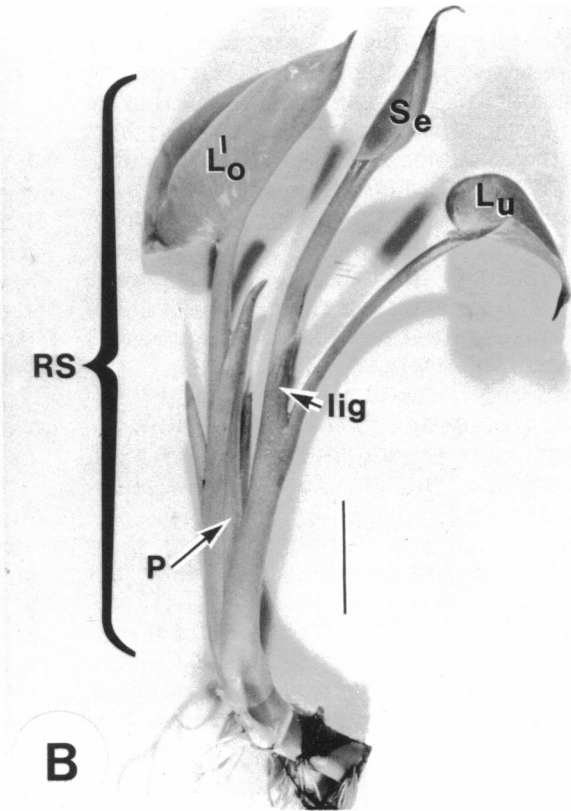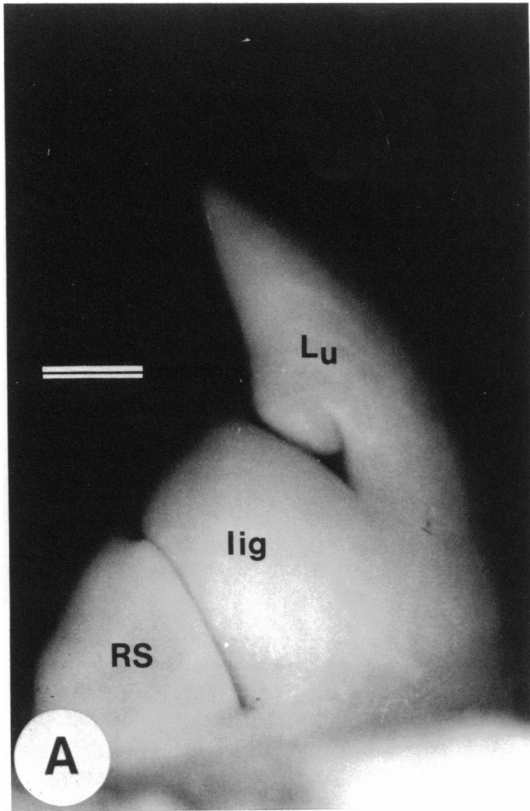
floral primordia appears to constrain both the shape of floral primordia and sites of stamen initiation and expansion (fig. 4C–G). The tendency for stamens to be initiated and to expand at locations of available space between adjacent floral primordia is visible in figure 4C in the flower immediately below the arrowhead.

Typical numbers of stamens are nine to 12 per flower, but this is difficult to establish precisely from surface morphology because of the absence of a limiting perianth and the close packing of flowers. Stamen number per flower is more easily determined from sections, since the xylem of stamen connective traces is oriented toward the center of the flower. Barabé and Labrecque report 10–12 stamens per flower on this basis. However, as reported below, stamen number per flower also varies according to position in the inflorescence. Sections show evidence of aborted stamens; these are always inner and presumed late-differentiating stamens.

Further stamen development involves broadening of the future connective as the filament becomes slightly bilobed (arrows in fig. 4F, G). The connective itself becomes bilobed as the separate microsporangia are formed. As development continues, the two lobes of each anther become delimited (fig. 5C) and are arranged in a divergent manner. The two halves of each stamen remain appressed along their inner margins (asterisks in fig. 5C, E) until anthesis when rapid filament elongation produces an evident bilobed stamen with the four microsporangia externally visible. At later stages of floral development, as the boundaries of individual flowers coalesce, it becomes increasingly difficult to distinguish the flower of origin for outer stamens. In this respect, it is of some help that the plane of dorsiventrality of the stamens remains somewhat tangential to the circle circumscribed by the ovary of each flower. Apart from late interpolation of stamens, further irregularity results because stamens may not complete development of functional microsporangia or are aborted, as mentioned above.

Gynoecium initiation is marked by the torus-like extension of a mound of tissue, immediately within the innermost series of stamen primordia. Initiation of the gynoecium always begins abaxially with gradual uplifting of the ovary wall in a bidirectional fashion until a complete ring primordium is formed. For a brief period the torus is U-shaped (1 in fig. 4E). A progressive series in

the development of the gynoecium is indicated by the numbered sequence in figure 4D and E. At no time during initiation of the gynoecium is there evidence of the trimerous development of structures interpretable as separate carpels. Development of the adaxial part of the gynoecium lags considerably behind the abaxial portion until later in ontogeny. The shape of the developing gynoecium varies considerably and is dependent upon space available for its enlargement as determined by continued stamen initiation and expansion. The gynoecium tends to be somewhat angular according to the original shape of the floral primordium. The center of the primordium within the torus remains flat as the future placenta (fig. 6B).

Further floral development involves enlargement of the receptacle, so that appendages are somewhat separated, together with continued upgrowth of the gynoecium wall (fig. 4F). As the gynoecium continues to develop, it becomes widely flask shaped, and the opening becomes progressively narrower (figs. 4F, G; 6B, C), obscuring the development of the basal ovules which can only be revealed by further dissection. Gynoecium closure is completed by its distal extension to form an abbreviated style (fig. 5E, arrow). The short hollow canal of the style opens directly onto the stigma which has no visible papillate structure (figs. 5E, 6C).

Ovule primordia are initiated on the flat, floor-like receptacle as closure of the gynoecium occurs. The ovary has free-central placentation. A number of placental lobes are initiated, corresponding to the number of ovules that will appear (e.g., six in fig. 5C). The number of ovules present varied widely. The first indication of ovule initiation is meristematic upgrowth of the flattened interior floor of the ovary (fig. 6B). As development continues, placental lobes become distinguishable as distinct protuberances (o in fig. 6C). The ovules develop as outgrowths from the top of these lobes with the inner and outer integuments successively enclosing the nucellus from the proximal end of the primordium (Io, Ii, in fig. 6D).

GENDER VARIATION

In all spadices examined the distal four to eight flowers are always male. All male flowers examined showed initiation of stamens in the usual fashion but no subsequent gynoecial initiation. Male flowers typically displayed a central flat-

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Fig. 4 *Calla palustris* SEM (A, B, F, G) and epi-illumination (C–E) photographs of developing spadix. Bars = 100 μm. A, B, Spadix from side (A) and above (B) to show acropetal inception of irregular flower primordia. A, Primordium of first stamen. C, D, E, Successive stages of inception of floral organs; numbers in D and E show successive stages in the asymmetric inception of the gynoecium. F, G, Later stages of stamen development, with irregular interpolation of central stamens after gynoecial development; arrows show bifurcation and enlargement of connective.
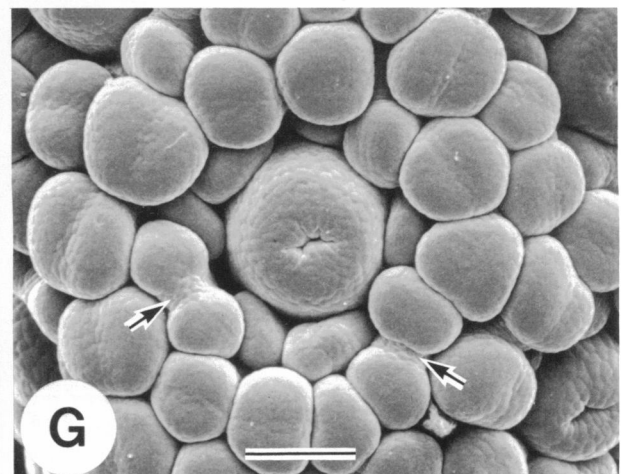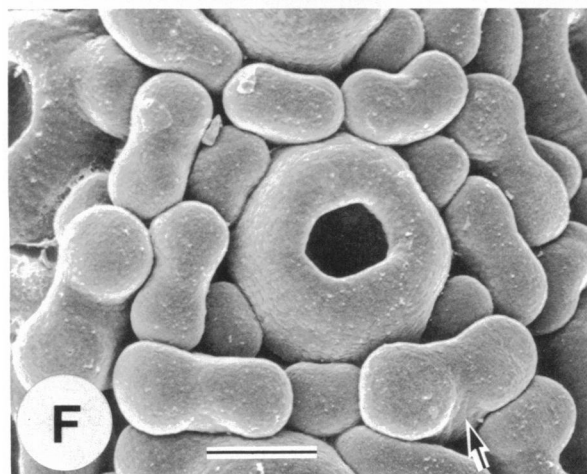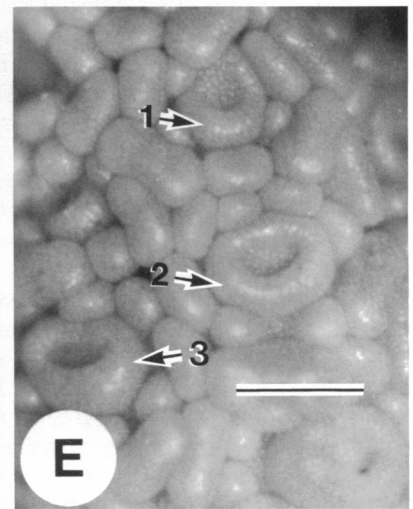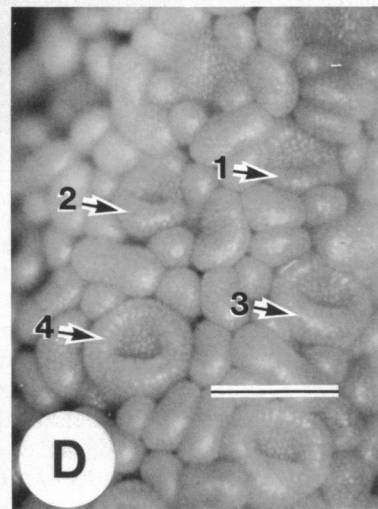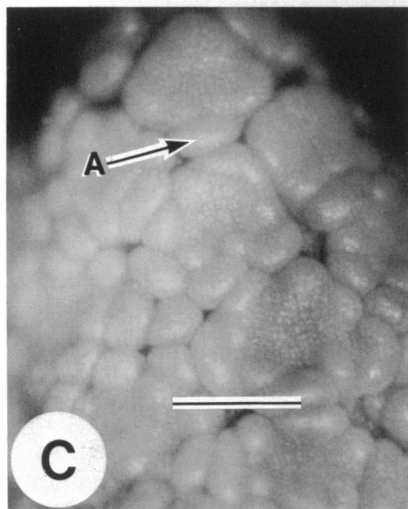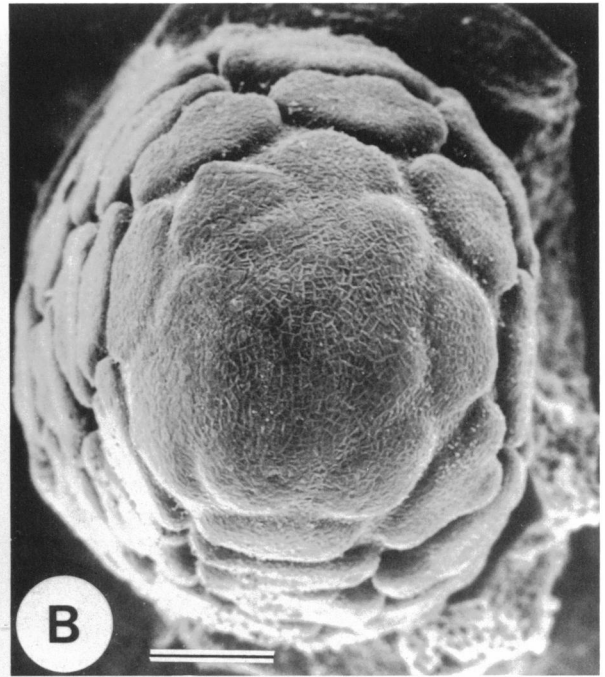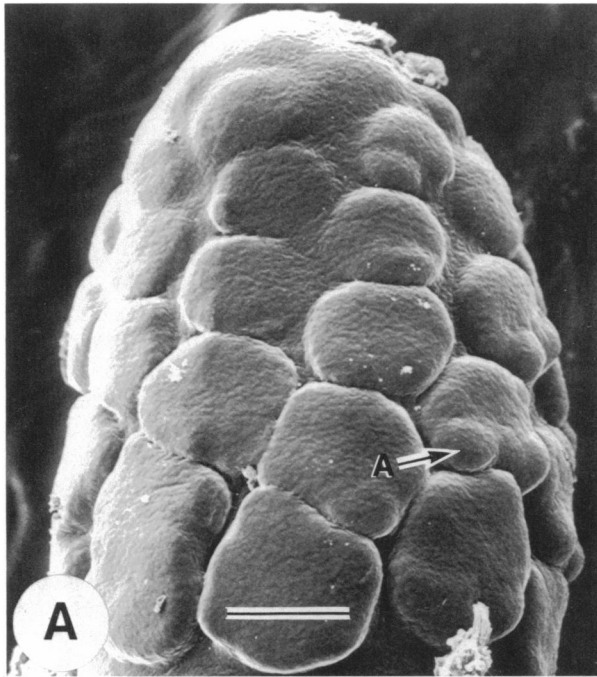
tened region in place of the site of gynoecial initiation (arrow in fig. 5A, D). Late in development further stamens may be initiated in this region.

In addition to the presence of distal male flowers in *Calla* there is a strong tendency for increasing femaleness toward the base of most spadices. Increasing femaleness is expressed through both decreased stamen number and increased gynoecial size and number of ovules (fig. 5B). Distal female flowers typically had two to three ovules, while basal flowers more often had six to eight ovules. The extent of the gradient in femaleness can be quite extreme (fig. 5B). Also compare the size of gynoecia in figure 5A with that in figure 5B where both figures are the same magnification. Although perfect flowers below the male flowers often had smaller gynoecia than proximal flowers, no flower with a visibly aborted gynoecium containing aborted or no ovules was observed. Perfect flowers are protogynous, as noted by Kunth (1892).

FLORAL VASCULATURE

The spadix includes a series of axial vascular bundles within a lacunose parenchyma (fig. 6E); peripheral bundles are somewhat more crowded. Anastomosing between bundles is extensive, not only in association with flowers but also between axial bundles. Figure 7D represents the main features of vasculature without showing all bundles of one flower. A conspicuous feature of mature flowers is a plexus of anastomosing strands immediately below the placenta (fig. 7D, p.p.). From this plexus single traces to the ovules (o.t.) are derived directly. The carpel wall includes bundles that also anastomose, converging apically and to a lesser extent basally. The carpel bundles at maturity are continuous with the margin of the placental plexus. Stamens each have a single trace (s.t.) that is derived either directly from the axial system (when its origin may be quite deep-seated, e.g., fig. 7A), or indirectly from a branch of the placental complex. Traces to stamens on the same radius sometimes diverge from a common strand (s.t. in fig. 7C). We saw no direct evidence of stamen traces to different flowers diverging from a common source; however, the extensive anastomosing of strands within the spadix before traces to specific organs can be recognized means that indirect connection must occur.

In development (fig. 7A–C), traces to the earliest-formed stamens are first recognized when the stamen primordium is only about 30 μm high (fig. 7A). The first-formed stamen traces are usually the most deep-seated but can become interconnected by extensive anastomoses before the placental plexus is conspicuous (fig. 7C). Differentiation of carpel traces within extending carpel walls occurs relatively late (cf. figs. 6B, 7B). Carpel traces at this and subsequent stages may appear basally discontinuous (e.g., fig. 7C) because the placental plexus with which they unite differentiates last in terms of recognizably discrete vascular strands. With the differentiation of the procambial strands of this plexus ovular traces are also evident and carpel traces become clearly continuous with it and, indirectly, with the axial system of the spadix via further peripheral anastomoses (fig. 7D). The system is without numerical regularity because of extensive anastomosing and variation from base to apex of the spadix. Carpel traces are at a maximum of 12 to 13 at the middle height of the mature gynoecium but are fewer in the smaller, distal flowers (cf. range of flower size in fig. 5B).

Discussion

Accurate descriptions of shoot organization in *Calla palustris* follow a long historical precedent. The first description of shoots of *C. palustris* was that of Doll (1843), who noted the anomalous position of the first foliage leaf on the same side as the prophyll on each branch axis. Wydler (1856) observed and illustrated the leaf rolling characteristics, symmetry, and the same anomaly in phyllotaxis. He noted that the latter anomaly was absent from proliferative shoots, a point in disagreement with all subsequent authors, including ourselves.

Braun (1859) repeated much of Wydler's (1856) description and added details of shoot phenology including the observations that the history of shoots could be traced "for many years." This observation is contrary to our own that shoots are a maximum of 2 yr old and may represent a variable effect of habitat on shoot survivorship. Braun primarily discussed the occasional presence of spadices possessing two or three spathes. Engler (1877) and Warnstorf (1883) in turn repeated Braun's description and added details of

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Fig. 5 *Calla palustris*. SEM photographs of late stages of floral development. Bars = 500 μm, A, B, E. Bars = 100 μm, C, D. A, Entire spadix from the side with fully formed flowers; arrows indicate failure of gynoecium to develop, producing male flowers. B, Spadix with strong female tendency, the basal flowers with precocious gynoecia and few stamens; arrows indicate incomplete closure of gynoecial torus. C, Flower with top of gynoecium removed to show basal placentae (arrow), each of which will produce an ovule. D, Spadix from above to show apical wholly male flowers. E, Mature flowers in overwintering condition; arrow, stigma; asterisks, septum between pairs of anther sacs in individual stamens (connective obscured, cf. fig. 4G).
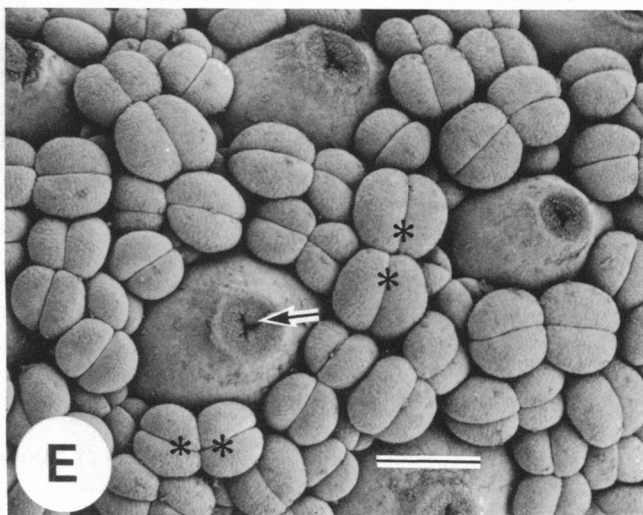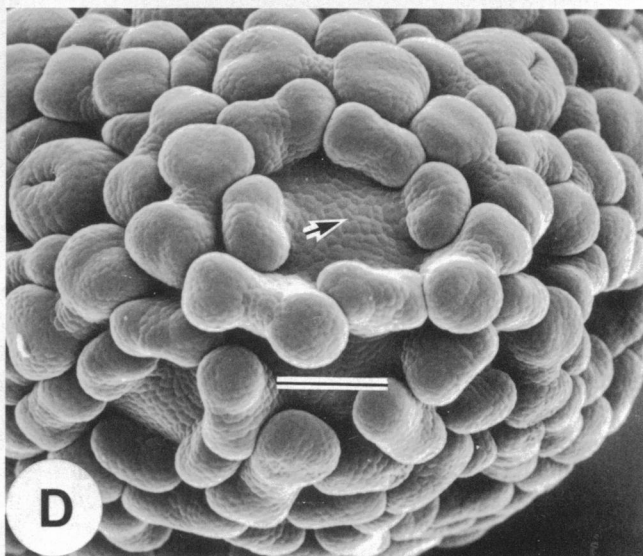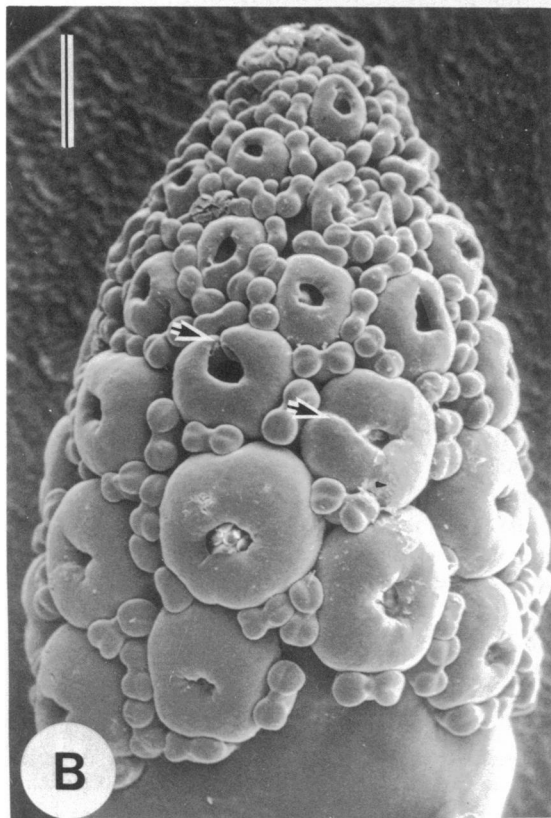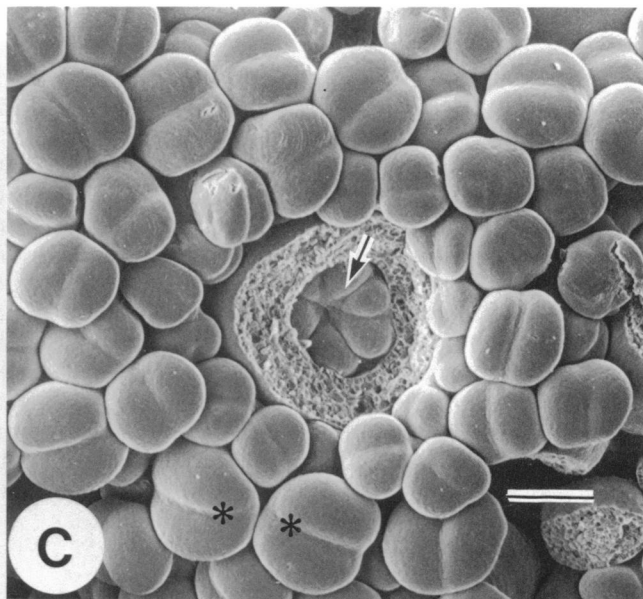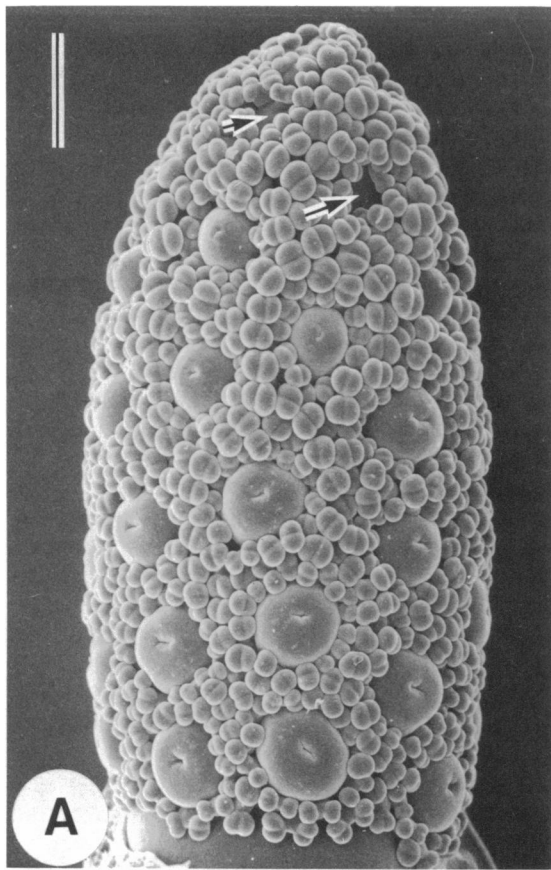
further irregularities including a second spadix in the axil of the ultimate foliage leaf.

We have never observed the production of a second spadix in the position discussed above. Rather, our observations indicate that when second spadices are produced they occur with a high degree of regularity, as illustrated in figure 2*B*. None of the earlier authors discuss the presence of spadices in this position.

Our observations confirm on the basis of developmental evidence the absence of a perianth in *Calla,* as reported by all earlier authors. Engler (1884) mentions that no perianth was visible at any stage of development but does not indicate how young were the flowers he examined. Developmental information also establishes the limited radial symmetry in floral organization, since at no stage can a precise trimerous condition be recognized, either in the sequence of stamen initiation or in the differentiation of the gynoecium. At most there can be an irregular hexagonal outline to the flower, seemingly the result of close-packing as the separate floral primordia impinge on each other. Bilateral symmetry may be claimed by the initiation of the first stamen in an abaxial position and the U-shaped torus of the young gynoecium.

On the basis of floral vasculature and comparison with *Orontium* and *Lysichiton,* Barabé and Labrecque (1983) suggest that the flower is trimerous in its organization and that six of the 12 stamens of *Calla* correspond to the six tepals of *Orontium.* Furthermore, they suggest that the gynoecium is pseudomonomerous and is derived from three carpels, primarily because they identify three principal carpel traces amid the 10 bundles that run in the carpel wall. These principal bundles were recognized because they were said each to share a common origin with one of the stamen traces and were the sole suppliers of the single circumferential bundle that becomes the supply to the series of basal ovules. Additional bundles of the carpel wall are derivatives of the principal bundles; they are described as "secondary bundles" and are not indicated in their interpretative diagram (fig. 8 of Barabé and Labrecque 1983). It was suggested that the principal bundles of the gynoecium correspond to the dorsal bundle of three separate carpels. Two of the derivative bundles are then considered to be ventral. However, we have shown that in ontogeny there is no evidence for a trimerous condition.

Furthermore, our observation of floral vasculature differs considerably from that reported by Barabé and Labrecque (1983) but agrees in principle with the brief description by Hotta (1971). We find no evidence for a consistent number of primary carpel traces; the placental plexus is not represented by a single girdling trace, and sta-

mens may make contact with carpel traces only indirectly via bundles of the placental plexus. The diagram by Barabé and Labrecque (their fig. 8) seems purely imaginary, although it is the basis for their recognition of a pseudomonomerous gynoecium. Hence, the tricarpellate ancestral model is used to generate supporting data in a purely circular way. In their cladistic analysis Barabé and Forget (1987) conclude that *Calla* should not be included in the Calloideae but suggest no alternative placement. A comparison between it and the other members of the Calloideae of Krause (1908) may not be appropriate. One could therefore choose other outgroups to create an alternate typology because numerous other members of the family have unilocular ovaries. The implication that a trimerous gynoecium is a necessary precursor to all such types seems unfounded. Eckardt (1937) used the criterion of three lobes to the stylar apex as evidence of pseudomonomery. Our figures 4*D–G* and 5*A, B* show that the closure of the stylar canal is irregular even though figure 5*E* (arrow) might be uniquely selected to show a three-lobed condition.

In addition, Barabé and Labrecque (1983) interpret the outer stamens of *Calla* as modified tepals, largely on the basis again of comparison with putative relatives, especially *Orontium,* that has six stamens. No developmental evidence for this interpretation is forthcoming, since stamens are all initiated in a like manner and the absence of precise symmetry does not allow any recognition of successive whorls of floral parts on the basis of position.

The desire to see structures in terms of antecedent evolutionary "types" that provide the basis for an interpretative "Bauplan" is also reflected in the work of Eyde et al. (1967) on floral vasculature in aroids. Certain flowers are referred to as structurally "aberrant" and are avoided in vascular analysis by choosing only those at the center of a group of flowers from the mid-level of a spadix or the center of a group of unisexual flowers. Such rejected flowers, of course, are only aberrant in relation to some hypothetical ancestral type to which all are expected to conform. It should be emphasized that aroid flowers do not function independently, since the spadix is the unit in reproduction. Some appreciation should be shown for meristic variation within a spadix, especially in those aroids that possess some perfect flowers, since deviations from a hypothetical hermaphroditic ground plan may be the raw material upon which natural selection could work. The developmental variation within and between aroid flowers needs to be surveyed more completely.

A morphogenetic interpretation of the vascular system of the *Calla* flower seems a realistic al-
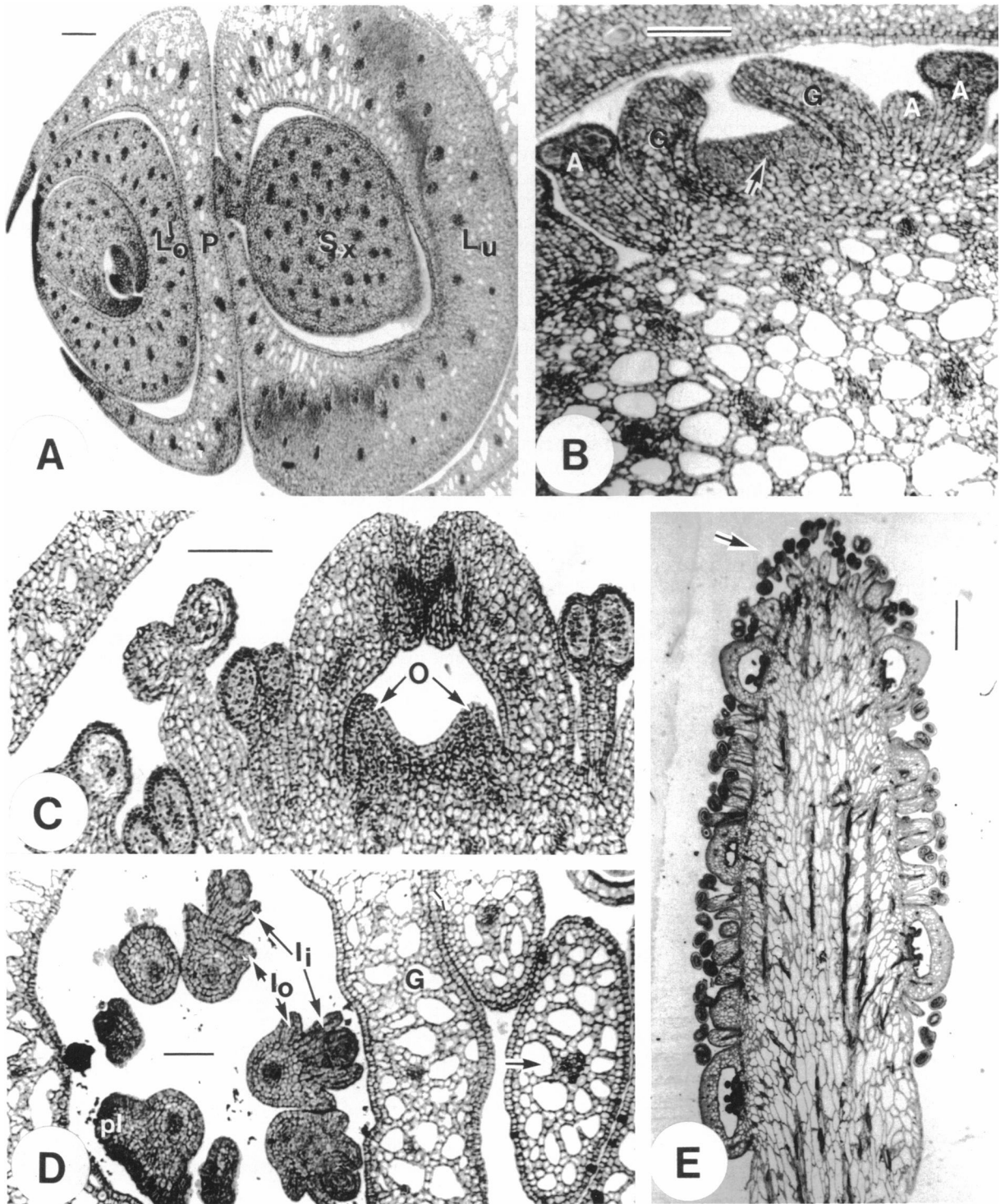
Fig. 6 *Calla palustris.* Spadix anatomy. Bars = 100 μm, *A–D.* Bar = 1 mm, *E. A,* Transverse section of shoot in region of renewal shoot; *Sx,* peduncle of spadix (below spathe, which does not appear at this level); *Lu,* ultimate foliage leaf of sympodial unit; *P,* prophyll; *L'o,* first foliage leaf of renewal shoot inserted on same side as *P. B,* Transverse section of spadix with developing flower; arrow, floor of ovary as future placental region; *A,* anther; *G,* carpel walls. *C,* Later stages with flower in longitudinal section, the gynoecium closed; *o,* young ovules. *D,* Flower in transverse section, placentae with developing ovules, each with outer (*Io*) and inner (*Ii*) integuments developing; *G,* gynoecium wall; arrow, filament of stamen with a single vascular bundle. *E,* Longitudinal section of immature spadix to show lacunose construction and terminal portion with wholly male flowers (arrow).

ternative to evolutionary hypotheses. Anasto-moses between inflorescence traces are part of the axial vascular system before floral differentiation begins. The first visible traces to the flower are those of the first stamens, each of which becomes a morphogenetic pole that becomes connected by procambial development to the axial system, by dedifferentiation of intervening parenchyma. Carpel traces within the gynoecium originate later and may appear to be discontinuous basally. They may correspond to the bundles of the "demand" type in the interpretation of monocotyledonous branch vascular attachment (Zimmermann and Tomlinson 1972). Carpel traces connect either with each other within the gynoecium or with the developing placental complex below the flower, which comes to serve as the dominant morpho-genetic pole in later stages of floral development since this region retains its meristematic capacity longest (cf. fig. 6*B*, *E*). Traces to stamens may also make the connection and so give the im-pression that a stamen and carpel bundle have a common origin. Developmentally this is not pos-sible; one cannot invoke a vascular system dom-inated by carpel traces that clearly appear late in floral ontogeny. Anastomosing between morpho-genetic poles accounts for the lack of regularity in the system because the flower is not very sym-metrical and the gynoecium is never obviously trimerous. Consequently we are forced to reject the notion of pseudomonomery in *Calla* since there is no structural or developmental evidence for its existence.

Our own observations on vascular anatomy make the point that despite the discreteness of the floral primordium, the vascular supply to a single flower does not have unitary organization. Appendages of a single flower are irrigated in-directly from several different axial bundles of the spadix because of the anastomosing nature of the vascular supply.
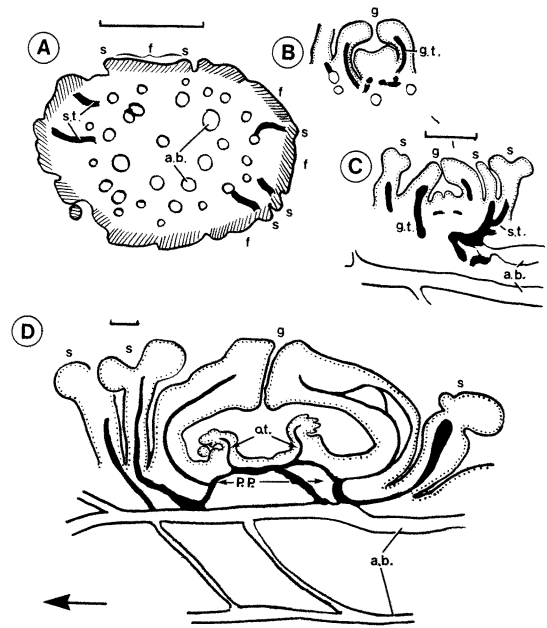

Fig. 7   *Calla palustris.* Diagrams of floral vasculature drawn from several adjacent sections superimposed on one structural outline; transverse sections in *A* and *B*, R.L.S. in *C* and *D*. Bars = 200 μm; *a.b.*, axial bundle; *f*, floral primordium; *g*, gynoecium; *g.t.*, carpel trace within gynoecium; *o.t.*, ovule trace; *p.p.*, placental complex; *s*, stamen; *s.t.*, stamen trace. *A*, Whole spadix, first stamen bundles just differentiated; meri-stematic region of floral primordia, cross-hatched. *B*, Young flower with gynoecium almost closed; carpel traces present in gynoecium wall. *C*, Older flower to show extensive anasto-mosing of stamen traces, still independent of placental plexus. *D*, Fully developed flower, with well-developed placental plexus, continuous via anastomoses with carpel and stamen traces.

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