

Evolutionary Transition from Resprouter to Seeder Life History in Two *Erica* (Ericaceae) Species: Insights from Seedling Axillary Buds

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Received: 2 September 2004 Returned for revision: 13 October 2004 Accepted: 15 November 2004

• **Background and Aims** The genus *Erica* represents the epitome of plant biodiversity in the South African Cape region. It includes seeder and resprouter species, but both species diversity and narrow endemism are tightly associated with the seeder habit. It also includes 'mixed' species, in which both seeder and resprouter life histories are found. This intraspecific variation in life history is genetically based.

• **Methods** The cotyledonary region and basal stem of seeder and resprouter seedlings of two 'mixed' species, *Erica calycina* and *E. coccinea*, were examined to detect morphological and anatomical differences in axillary bud development between regeneration forms.

• **Key Results** While at least some bud activity was observed for resprouter seedlings, none was detected for seeder seedlings. A closer examination allowed the detection of some axillary buds in seeder seedlings of the two species, but they appeared in an unequivocally atrophied state.

• **Conclusions** The seeder and resprouter life histories are two character states and the seeder one (i.e. loss of resprouting) is derived within these two *Erica* species. Results allow the hypothesis that the loss of resprouting in a fire-prone scenario such as the Cape fynbos has promoted high diversification rates in seeder *Erica* lineages.

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Key words: Axillary buds, Cape floristic region, cotyledonary region, fynbos, *Erica*, lignotuber, resprouter, rudimentary characters, seeder.

INTRODUCTION

Woody plants from fire-prone environments, such as Mediterranean ecosystems, have been traditionally classified as either 'seeder' or 'resprouter' based on the way they respond to fire (Keeley, 1986; Bond and van Wilgen, 1996). Most resprouter woody taxa survive fire and regenerate from a lignotuber, a swelling modification of the stem base which bears a supply of concealed buds ('bud-bank') on its surface (James, 1984) to allow copious post-disturbance shoot growth (Kummerow, 1989; Noble, 2001; Whittock *et al.*, 2003). Lignotubers in woody plants seem to be genetically determined (Verdaguer and Ojeda, 2002; Whittock *et al.*, 2003) and their ontogenetic formation has been described at great length in *Eucalyptus* (Carr *et al.*, 1984), *Quercus* (Molinas and Verdaguer, 1993a, b) and *Banksia* (Mibus and Sedgley, 2000) species. Initially, the lignotuber can be detected as a thickening of the 'cotyledonary region' (i.e. upper hypocotyl, cotyledonary node, and the first one or two leaf nodes; see also Whittock *et al.*, 2003) of seedlings in which numerous buds are found in the axils of the cotyledons and leaves of the first nodes (Carr *et al.*, 1984; Molinas and Verdaguer, 1993b; Mibus and Sedgley, 2000).

Seeder taxa, by contrast, lack a lignotuber, and adult seeder plants are killed by fire, with stands being re-established by a massive seedling recruitment from a pre-existing, soil-, or canopy-stored seedbank. Although

both seeder and resprouter life histories imply resilience to recurrent wildfires at the population level, their effects on the dynamics of populations are altogether different (Bond and van Wilgen, 1996). These differences in the population dynamics have crucial consequences in the long-term diversification patterns of seeder and resprouter lineages (Wells, 1969; Ojeda, 1998).

During the last three decades, it has been hypothesized that resprouting is ancestral in Mediterranean woody taxa (Wells, 1969; Axelrod, 1975; Keeley, 1986; Lloret *et al.*, 1999) and that recent, rapid speciation is associated with the loss of resprouting, i.e. with 'derived' seeder lineages (Wells, 1969; see also Cowling, 1987; Cowling and Lamont, 1998).

With approx. 650 species and a high level of endemism, the genus *Erica* represents the epitome of plant biodiversity in the Cape region (Goldblatt and Manning, 2002). This genus shows a pattern of high species concentration in fynbos, a fire-prone, heathland-like vegetation (Goldblatt and Manning, 2002; Linder, 2003), and includes seeder and resprouter species (Ojeda, 1998), like most fynbos woody taxa (Schutte *et al.*, 1995). Resprouter plants in this genus have a conspicuous lignotuber and store large amounts of starch in their woody roots, whereas seeders lack any trace of lignotuber and have significantly lower amounts of root starch (Bell and Ojeda, 1999).

As in other speciose fynbos woody genera, species diversity and narrow endemism in *Erica* are strongly associated with the seeder life-form (Ojeda, 1998). Following Wells's

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hypothesis (Wells, 1969), the evolution of the seeder life history in *Erica* out of ancestral resprouter lineages might have favoured increased diversification rates in a fire-prone scenario by promoting nonoverlapping generations and increasing generation turnover (Ojeda, 1998; see also Cowling, 1987; Cowling and Lamont, 1998; Cowling and Pressey, 2001). However, recent phylogenetically informed analyses have failed to support the presumed ancestry of the resprouting life history in Mediterranean woody species (Bond and Midgley, 2003; see also Veski and Westoby, 2004) and, to date, it remains unclear how transitions between regeneration responses occur (Veski and Westoby, 2004).

Here is presented a morphological and anatomical analysis of the cotyledonary region and first leaf nodes of seeder and resprouter seedlings of *Erica coccinea* and *E. calycina*, two 'mixed' *Erica* species (*sensu* Ojeda, 1998) in which both seeder and resprouter life histories occur, often in disjunct populations. Seeder and resprouter forms within these 'mixed' species are easily authenticated in natural populations on the basis of the presence (resprouter) or absence (seeder) of a lignotuber (Bell and Ojeda, 1999; F. Ojeda, pers. obs.). In a previous study, it was shown that seeds collected in natural populations from seeder and resprouter plants of these two *Erica* species produced seeder and resprouter seedlings, respectively, when grown in the glasshouse under common garden conditions. Resprouter seedlings of both species presented a consistently more pronounced swelling of the upper root region and higher relative amounts of upper-root starch and upper-root storage tissue, as well as higher root-to-shoot allocation than their seeder counterparts (Verdaguer and Ojeda, 2002). It was then concluded that this within-species life-form variation must be genetically based.

The aim of this study was to detect early ontogenetic differences in axillary bud development between seeder and resprouter life histories. This morphological and anatomical comparative analysis of the basal stem portion, out of which the lignotuber would develop (see Whittock *et al.*, 2003), reveals the polarity of the transition between life histories within these two *Erica* species. Furthermore, such two-fold within-species comparison may help to gain a deeper understanding of the evolution of the speciose Cape genus *Erica*.

MATERIALS AND METHODS

Plant material

Mature seeds from randomly selected seeder and resprouter adult plants of *Erica calycina* and *E. coccinea* were sown and grown under common garden conditions (for further details on seed collecting and glasshouse cultivation, see Verdaguer and Ojeda, 2002). Harvesting of seedlings took place 9 months after sowing while many seedlings were still germinating (i.e. germination was not synchronous).

Harvested seedlings were classified into three age classes: young, intermediate and old (0.5–2.0, 2.0–3.5 and 3.5–12.0 cm, respectively), using shoot length as an approximate surrogate of seedling developmental stage. Cotyledons were present in all seedlings. These were clearly distinguished

from the first true leaves in both *Erica* species (Fig. 1A and B). In all seedlings, the first stem internode was very short (<0.1 mm), so the cotyledons and the first pair of leaves were very close and arranged perpendicularly to each other (Fig. 1A–D and I). Thus, from now on, the cotyledonary node and the first leaf node will be referred to as the 'cotyledonary region'. The other leaves typically appear in opposite and decussate position separated by internodes of approx. 1 mm in length (Fig. 1C and D).

Morphological analysis

After harvesting, the cotyledons and first pair of leaves were carefully removed under a stereomicroscope to search for axillary buds in the cotyledonary region. Photographs were taken with a digital camera (Olympus Optical Co. Ltd, Germany) connected to the stereomicroscope. A minimum of five seedlings per age class, regeneration form and species was examined, except for seeder *E. coccinea*, where seedlings within the old class were not available, and for resprouter *E. coccinea*, where only three seedlings within the old class could be analysed.

Two types of axillary buds were distinguished under the stereomicroscope: functional buds, and aborted or atrophied buds (thinner than functional buds, and blackish in colour). Within the functional buds, two basic stages were defined: first leaf primordium stage (buds showing an incipient first pair of leaf primordia), and activated stage (buds showing more than two leaf primordia).

A minimum of three seedlings per species and life history were also viewed under a scanning electron microscope. In this case, samples were fixed in 4 % formaldehyde in 0.1 mol L⁻¹ phosphate-buffered saline (pH 7.5) in a vacuum at room temperature for 48 h minimum. Then, they were dehydrated in a graded ethanol series, exchanged through amyl-acetate, and critical-point dried. The pieces were mounted on copper stubs and coated with gold. Specimens were observed using a Zeiss DSM 960A scanning electron microscope (Zeiss, Oberkochen, Germany) of the Electron Microscopy Service of the University of Girona.

To explore whether bud differences in the cotyledonary region between regeneration forms extend to leaf nodes further from the cotyledonary region, five seedlings of the intermediate class were randomly selected within each regeneration response for both species, and axillary buds were counted, not only in the cotyledonary region, but also in the next ten leaf nodes. The existence of differences between life histories in the overall amount of axillary buds in the leaf nodes further from the cotyledonary region (from the third to the tenth leaf node). Data were analysed using a mixed model two-way ANOVA, with the difference between the seeder and resprouter form treated as a fixed factor and species treated as a random factor.

Anatomical analysis

Sections including the cotyledonary region of a minimum of three intermediate and old seeder and resprouter seedlings of both species were examined under a light

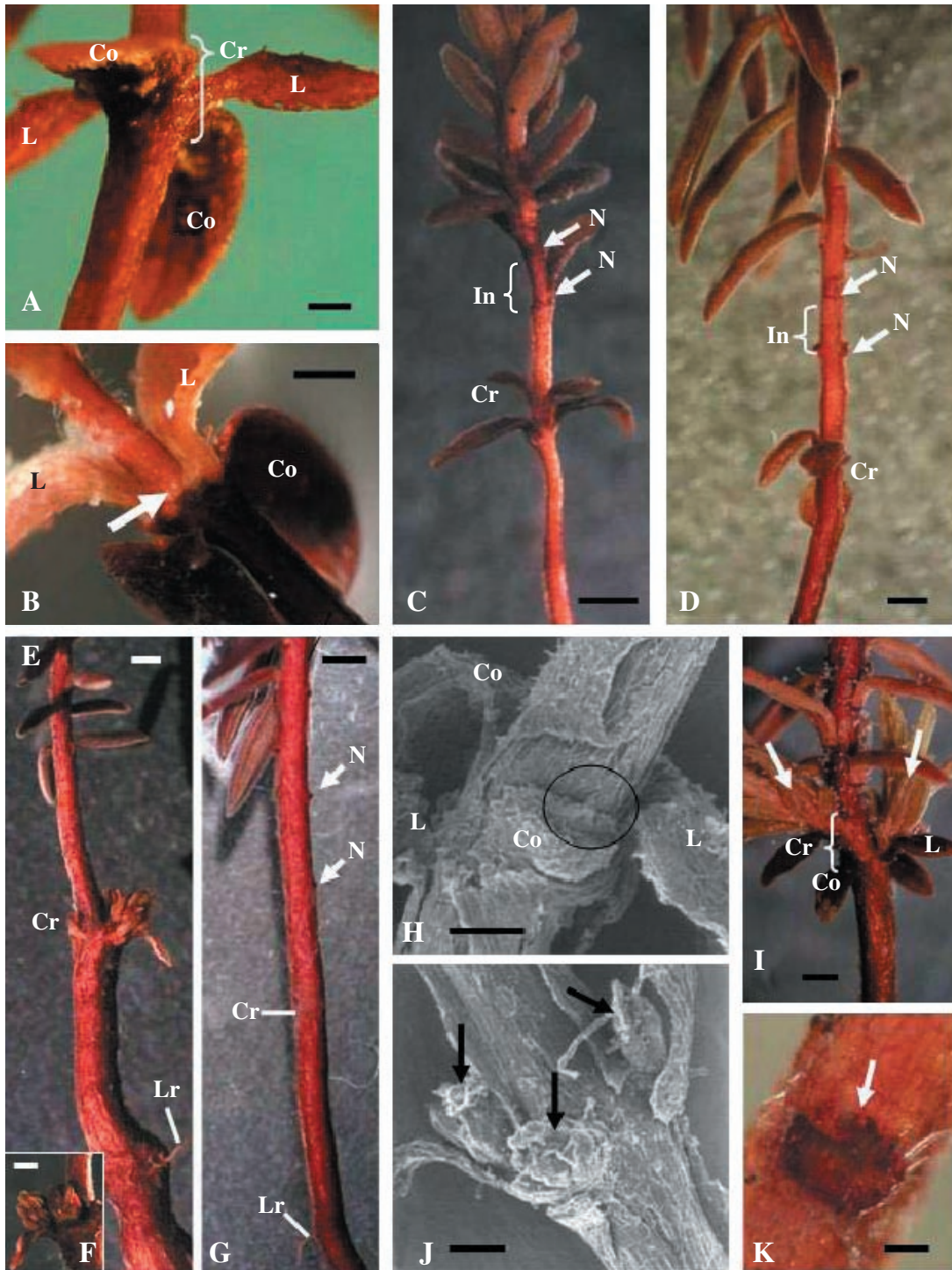


FIG. 1. (A) Cotyledonary region of a young resprouter *E. coccinea* seedling showing the cross-like arrangement of cotyledons and first pair of leaves. (B) Cotyledonary region of a young seeder *E. coccinea* seedling (arrow); note the close location of the cotyledons and first pair of leaves; cotyledons are broader and darker than leaves. (C) Young resprouter *E. calycina* seedling showing the opposite and decussate distribution of leaves (arrows) and the internodal length above the cotyledonary region. (D) Young seeder *E. calycina* seedling. (E) Intermediate resprouter *E. calycina* seedling showing two buds with more than two leaf primordia (activated buds) at the cotyledonary region; note the thickening of the upper root region. (F) Detail showing that buds originated from two different axils. (G) Old seeder *E. calycina* seedling showing no buds or lateral shoots at the cotyledonary region and the lowermost leaf nodes; note the similar width across the shoot–root transition. (H) Scanning electron micrograph of an old seeder *E. calycina* seedling showing a bud-like rudiment (circle) in the cotyledonary axil. (I) Old resprouter *E. coccinea* seedling in which axillary activated buds at the cotyledonary region have grown into small sprouts (arrows). (J) Scanning electron micrograph of an intermediate resprouter *E. coccinea* seedling showing developed axillary buds (arrows) in the cotyledonary region. (K) Close detail of a young seeder *E. coccinea* seedling with a black atrophied axillary bud at a lower leaf node (arrow). Scale bars: B–G and I = 1 mm; A, H and J = 0.2 mm; K = 0.1 mm. Abbreviations: Co, cotyledons; Cr, cotyledonary region; In, internode; L, leaves; Lr, lateral root; N, node.

microscope. They were dehydrated through an isopropyl alcohol series and embedded in glycol-methacrylate (Pascual *et al.*, 2002). Serial longitudinal and transverse sections, 3–5 μm thick, were cut on a rotary microtome Autocut 1150 (Reichert-Jung, Vienna, Austria) and mounted on glass slides. Routine staining was performed with thionin–HCl and toluidine blue to show up the cytoplasmic content (meristematic cells) and also tannins, and some sections were stained with periodic acid–Schiff (PAS) to highlight carbohydrates (Verdaguer and Molinas, 1997). Samples were viewed with an Olympus-Vanox light microscope (Olympus Optical, London, UK).

RESULTS

Morphological analysis

Axillary buds were readily observed in the cotyledonary region of most resprouter seedlings (Fig. 1E, F, I and J), whereas in seeder seedlings buds in the cotyledonary region were either not visible (Fig. 1G) or appeared atrophied (Fig. 1H and K). This pattern was evident in both species (Table 1). Resprouter seedlings showed asynchronous maturation, since axillary buds at different stages of development were seen in the cotyledonary region of young and intermediate resprouter seedlings of the two species (Table 1). No accessory buds were observed in any seedling.

It is noteworthy that differences in axillary bud presence in the cotyledonary region were maintained in the two first leaf nodes of intermediate resprouter and seeder seedlings of the two species (Table 2). By contrast, such seeder *vs.* resprouter differences disappeared from the third leaf node onwards in the two *Erica* species, as revealed by the lack of significant differences in the two-way ANOVA (Table 3).

Anatomical analysis

Sections of the cotyledonary region of resprouter seedlings of both *E. calycina* and *E. coccinea* revealed that axillary buds have an endogenous origin, since vascular traces were continuous between the cortex and the inner vascular tissue of stems (Fig. 2A and B). They initiated as a flat shoot apical meristem (Fig. 2A) in which a pair of leaf buttresses appeared (Fig. 2D) and, whereas some grew readily (i.e. increase of leaf primordia and elongation of the shoot axis (Fig. 2C), others remained latent or had a slow growth (Fig. 2D).

Close examination of transverse and longitudinal sections under the light microscope confirmed the atrophied state of axillary buds of the cotyledonary region of seeder seedlings of *E. calycina* and *E. coccinea* (Fig. 2E–K). It also revealed the presence of such atrophied buds in axils where no buds were previously found under the stereomicroscope, both in seeder and resprouter seedlings. Figure 2D and E illustrate differences between functional first leaf primordium and atrophied buds. Atrophied buds appeared as round or first leaf primordia bud-like protuberances formed by distorted cells with a high tannin content (Fig. 2E–K). Careful examination of serial sections failed to ascertain the presence of true meristematic cells in these atrophied buds.

TABLE 1. Mean (standard deviation) number of first leaf (FL) and activated (AB) axillary buds in the cotyledonary region of resprouter and seeder seedlings of *Erica calycina* and *E. coccinea* at the three different size classes

	Resprouter		Seeder	
	FL	AB	FL	AB
<i>Erica calycina</i>				
Young ($n = 5$)	1.6 (2.2)	0.4 (0.9)	0.0 (0.0)	0.0 (0.0)
Intermediate ($n = 12$)	0.5 (1.2)	1.4 (1.5)	0.0 (0.0)	0.0 (0.0)
Old ($n = 6$)	0.0 (0.0)	2.8 (0.8)	0.0 (0.0)	0.0 (0.0)
<i>Erica coccinea</i>				
Young ($n = 5$)	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Intermediate ($n = 12$)	0.3 (0.7)	2.2 (1.4)	0.0 (0.0)	0.0 (0.0)
Old ($n = 3$)	1.0 (1.7)	2.3 (1.5)	–	–

TABLE 2. Mean (standard deviation) number of functional (first leaf and activated axillary buds) and atrophied axillary buds in the cotyledonary region, first, second, and third to tenth leaf nodes of resprouter and seeder intermediate seedlings of *Erica calycina* and *E. coccinea*

	Resprouter ($n = 5$)		Seeder ($n = 5$)	
	Functional	Atrophied	Functional	Atrophied
<i>Erica calycina</i>				
Cotyledonary region	2.0 (1.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
First leaf node	1.2 (0.8)	0.0 (0.0)	0.0 (0.0)	0.2 (0.4)
Second leaf node	1.4 (0.9)	0.0 (0.0)	0.0 (0.0)	1.0 (1.0)
Third to tenth leaf nodes	11.0 (6.0)	0.0 (0.0)	8.6 (3.5)	1.2 (1.3)
<i>Erica coccinea</i>				
Cotyledonary region	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)	0.8 (0.8)
First leaf node	0.3 (0.7)	2.2 (1.4)	0.0 (0.0)	0.2 (0.5)
Second leaf node	1.0 (1.7)	2.3 (1.5)	0.0 (0.0)	0.2 (0.5)
Third to tenth leaf nodes	12.0 (3.9)	0.0 (0.0)	10.6 (6.2)	0.2 (0.5)

TABLE 3. Mixed model two-way ANOVA of the effects of regeneration form (seeder and resprouter, fixed effect) and species (*E. calycina* and *E. coccinea*, random effect) on the overall number of axillary buds from the third to the tenth leaf node

Source of variation	d.f.	MS	F	P-value
Regeneration	1	18.05	14.4	0.16
Species	1	11.25	9.00	0.21
Regeneration \times species	1	1.25	0.05	0.83
Error	16	25.53		

DISCUSSION

In lignotuberous woody species, the proliferation of dormant accessory buds concealed in the cotyledonary region from early seedling stages is a common trait (Carr *et al.*, 1984; Molinas and Verdaguer, 1993b; Mibus and Sedgley, 2000). In both *Erica* species, the lignotuber of resprouter

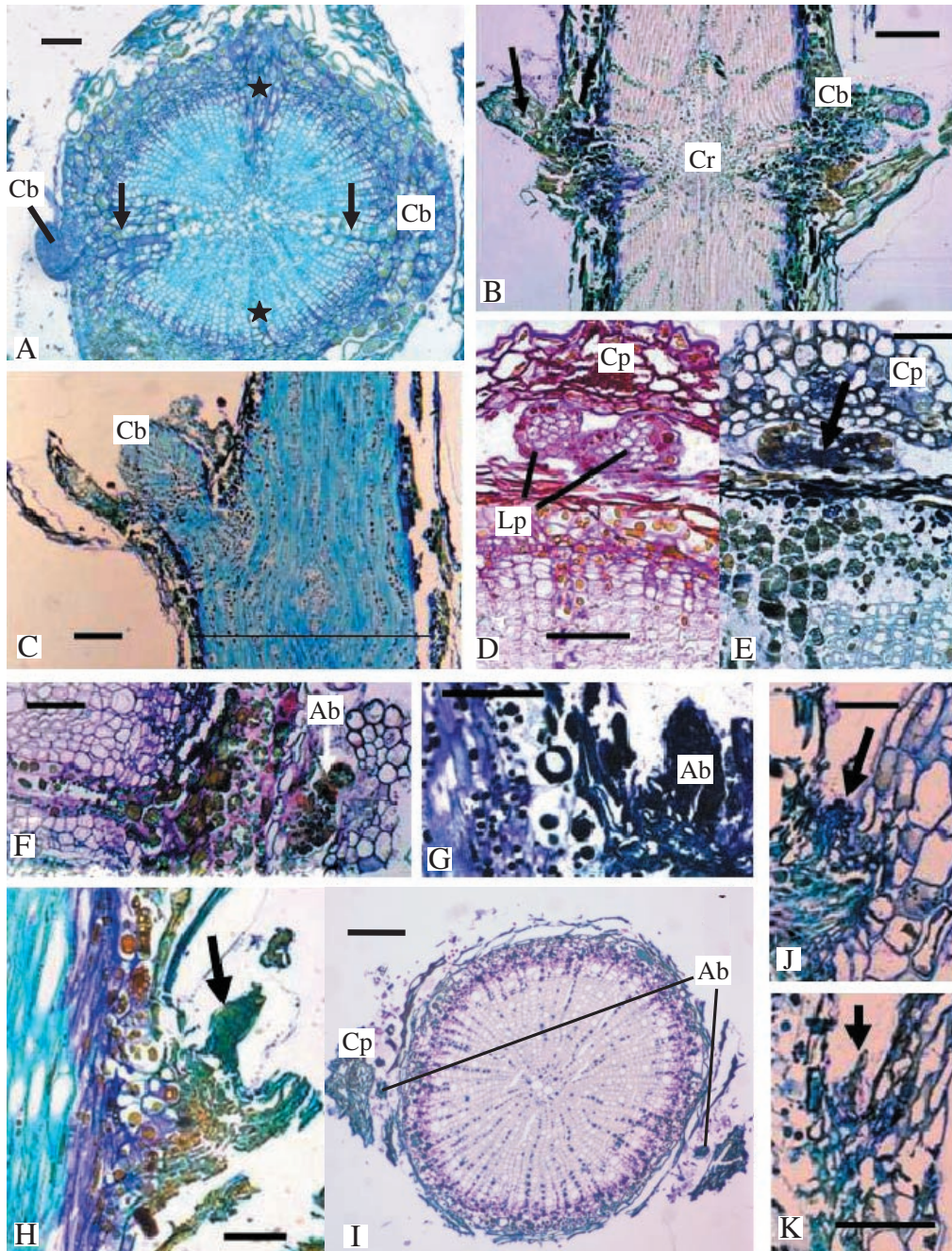


FIG. 2. (A) Transverse section at the cotyledonary region of an intermediate resprouter *E. coccinea* seedling showing buds in the axil of cotyledons with its vascular connection arising from the centre of the root (arrows) and also the incipient vascular traces of axillary buds of the first pair of leaves (asterisk). (B) Longitudinal section at the cotyledonary region of an old resprouter *E. coccinea* seedling showing a functional cotyledonary bud in first leaf primordium stage and, on the opposite site, tissue remains of the opposite cotyledonary bud (arrow); note also the continuous presence of vascular traces from the centre of the cotyledonary node of the bud tissue. (C) Longitudinal section of an old resprouter *E. calycina* seedling showing a functional cotyledonary bud and a thicker diameter of the upper root region than that of the basal shoot. (D) Transverse section of a functional bud in first leaf primordium stage of an old resprouter *E. calycina* seedling; note the presence of turgid cells with a meristematic appearance. (E) Transverse section of an atrophied bud in first leaf primordium stage (arrow) of an old resprouter *E. calycina* seedling, in which cells are completely filled with tannins. (F) Nearly medial transverse detail of an atrophied cotyledonary bud of the bud tissue. (G) Medial longitudinal section detail of the cotyledonary region of an old seeder *E. calycina* seedling showing an atrophied bud with a high quantity of tannins. (H) Medial longitudinal section detail of the cotyledonary region of an intermediate seeder *E. calycina* seedling showing an atrophied bud with a high quantity of tannins (arrow). (I) Transverse section of the cotyledonary region of an old seeder *E. coccinea* seedling showing two opposite atrophied buds. (J) Atrophied cotyledonary bud (arrow) of an intermediate seeder *E. coccinea* seedling; note that cells have lost turgor and are filled with tannins. (K) Atrophied cotyledonary bud (arrow) of an intermediate seeder *E. coccinea* seedling shown as a protuberance formed by distorted cells. Scale bars: C, I = 100 µm; A, D–H, J and K = 50 µm; B = 125 µm. Stains: A, B, H and I, toluidine blue; C, E, G, J and K, thionin; D, PA Schiff; F, PA Schiff–toluidine blue. Abbreviations: Ab, atrophied bud; Cb, cotyledonary bud; Co, cotyledons; Cp, cotyledonary petiole; Cr, cotyledonary region; Lp, leaf primordium.

forms has been clearly identified at the shoot–root transition region of mature plants (Bell and Ojeda, 1999). However, neither clusters of accessory buds nor buds in a dormant state were found in the cotyledonary region and/or the lowermost leaf nodes of resprouter seedlings. Instead, axillary buds of this region seemed to stay active and develop into new shoots. Although more studies are needed to document lignotuber development in resprouter *Erica* plants, it is suggested that basal branches coming from those functional buds will bear many axillary leaf buds themselves. As growth proceeds, these close basal branches would fuse together and with the upper-root portion, which becomes thicker in old seedlings (Verdaguer and Ojeda, 2002; see also Fig. 2C), and would then form a conspicuous lignotuber with a high meristematic potential.

By contrast, no evidence of functional buds in the cotyledonary or in the first two leaf nodes of seeder seedlings of the two species was detected. All axillary buds found in this basal region of seeder seedlings were in an unequivocally atrophied state. Such conspicuous differences between seeders and resprouters in functional bud presence within the basal stem region virtually disappeared when the upper stem regions were compared (see Table 3). Although the process leading to bud atrophy and subsequent shedding deserves further investigation, it is worth noting that the cotyledonary region and first two leaf nodes corresponds to the basal stem region out of which the lignotuber occurs in mature resprouter forms of both *Erica* species (Bell and Ojeda, 1999; Verdaguer and Ojeda, 2002) and species of other lignotuberous woody genera (Mibus and Sedgley, 2000; Whittock *et al.*, 2003).

Besides, seeder seedlings of these two ‘mixed’ *Erica* species presented significantly lower levels of root starch and upper-root storage tissue than their resprouter counterparts (Verdaguer and Ojeda, 2002). Thus, traits related to the resprouting life history—storage of starch reserves and bud-bank—are withered in seeder seedlings of these two *Erica* species, this difference having a genetic basis (see also Verdaguer and Ojeda, 2002). It can therefore be stated that seeder seedlings in these two species present ‘lignotuber rudiments’ or an ‘atrophied lignotuber’.

Darwin (1859) referred to rudimentary, atrophied and aborted organs as ‘the record of a former state of things’, and highlighted their relevance in tracing [structural] homologies and inferring ancestry between ‘different members of the same class’. However, apart from giving examples, such as the existence of teeth in the upper jaw of baleen whale calves (Darwin, 1859) or severely reduced hind limbs in python snakes (Cohn and Tickle, 1999), there are not many examples reported in which the existence of rudiments inform about the transition between life history character states. This study constitutes the first report, as far as is known, in which the existence of rudiments do give information about the polarity of a transition between life histories in plants. Thus, it can be stated that resprouting is ancestral in these two species, and that the seeder life history is the derived character state.

This two-fold intraspecific comparison constitutes a powerful tool (see Ackerly and Donoghue, 1995; Ricklefs, 1996) for suggesting that the seeder life history might be a

derived character state in the genus *Erica*. Thus, taking into account that species diversity and narrow endemism are markedly associated with the seeder habit (Ojeda, 1998), the genus *Erica* might stand as a compelling argument against dismissing Wells’s hypothesis (Wells, 1969) (see Bond and Midgley, 2003). It is believed that this hypothesis still provides a benchmark for explaining the massive, recent diversification of this genus in the fire-prone Cape fynbos; speciose, seeder lineages in *Erica* might ‘stem from their unique abandonment of the conservative, crown sprouting’, as Wells (1969) had proposed for speciose genera *Arctostaphylos* (Ericaceae) and *Ceanothus* (Rhamnaceae) from the fire-prone Californian flora. Nevertheless, until a resolved phylogeny of the genus *Erica* is produced, upon which the evolution of the seeder and resprouter life histories can be reconstructed, this hypothesis remains to be tested.

ACKNOWLEDGEMENTS

We thank A. García and R. W. Marriner for their help in collecting seed material, J. V. García and J. M. Higuera for their assistance in the greenhouse, A. Hitchcock for providing the mycorrhizal medium for growing Cape ericas, G. Pascual for her suggestions and expertise in histological analysis, and N. Niell for her assistance in the laboratory. J. R. Pannell, M. Verdú, J. Arroyo, W. J. Bond and R. M. Cowling made valuable comments on a former version of the manuscript. Field work was supported by a travel grant to F.O. from the Consejería de Educación y Ciencia (Junta de Andalucía). This study has been supported by a research grant attached to a ‘Ramón y Cajal’ contract (MCYT-UCA) to F.O. and the MCYT project AGL 2002/02136 AGR/FOR to D.V.

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