Bud and shoot structure may relate to the distribution area of South American Proteaceae tree species

Amaru Magnin [a,*], Javier Grosfeld [a], Daniel Barthélémy [b], Javier Puntieri [a,c]

[a] INIBIOMA, CONICET-UNCOMAHUE, Quintral 1250, Río Negro, Argentina
[b] INRA, UMR AMAP, TA A51/PS2, 34398 Montpellier, Cédex 5, France
[c] Universidad Nacional de Río Negro, Sede Andina, Argentina

ARTICLE INFO

ABSTRACT

The capacity of preformation and neoformation and the structure of winter buds are vegetative attributes that may vary between plant species and according to ontogenetic stages of the same species. The present study describes and evaluates these features for the four tree species of Proteaceae occurring in Patagonia. In particular, it analyzes the structure and development of the distal buds of the trunk and the preformed or neoformed nature of the organs involved. Two of the species, *Embothrium coccineum* and *Lomatia hirsuta*, have scaly buds, in which primordia of green leaves are covered by cataphylls. The shoots of both species may include neoformed organs, more frequently so in juvenile trees. *Lomatia ferruginea* and *Gevuina avellana* have naked buds with a low number of primordia; in juvenile and adult trees of both species trunk shoots are entirely preformed. The structure of buds and shoots suggests two different growth modalities of the axes, which would be related to ecological breadth (narrower in the species with naked buds than in those with scaly buds) and distribution area of these species. The considerable morphological differences between the two *Lomatia* species studied raise the question whether they ought to be included in the same genus.

© 2012 Elsevier GmbH. All rights reserved.

Introduction

Two main morphogenetic processes are involved in the length growth of the axes making up the aerial structure of vascular plants. In the first place, new metamers, i.e. nodes and internodes, are added by means of differentiation occurring at each axis' apical meristem. The second process is the extension of the differentiated organs (Barthélémy and Caraglio, 2007). In many plants, these two processes take place simultaneously, so that no single metamer remains in a state of dormancy before its extension. In other plants, temporal separation between differentiation and extension occurs, so that new metamer primordia, known as preformed organs, are built up in a bud for a variable period of time. In contrast, those metamers with simultaneous differentiation and extension are described as neoformed organs (Hallé et al., 1978). The extents of preformation and neoformation and bud structure have been found to exhibit within-species variation related to ontogenetic factors (Heuret et al., 2006; Sabatier et al., 2003a).

From an evolutionary perspective, it has been stated that variations in the way plant axes grow are the result of different selection pressures that acted on basic structural patterns (Rowe and Speck, 2005). One of the ideas derived from this perspective is that plant species from sites with a strongly seasonal climate rely more on the preformation of organs than those from sites with a non-seasonal climate (Bell, 1991; Diggle, 2002; Hallé and Martin, 1968; Hallé et al., 1978; Rawat and Gaur, 2004). This idea is supported by the observation that many tree species from temperate regions have conspicuous buds with preformed primordia covered with scaly leaves (Diggle, 2002). Knowledge regarding structural features of plant axes has markedly increased in the last decades, paralleling the number of studies on plant architecture (Barthélémy et al., 2009; Costes et al., 2003; Grosfeld and Barthélémy, 2004; Puntieri et al., 2007; Seleznyova et al., 2003). Nonetheless, only a few studies have dealt with variations in the preformed or neoformed nature of metamers integrating plant axes to compare plant species belonging to a particular evolutionary linage and match metamer structure with species distribution (Guédon et al., 2006).

The Proteaceae family is one of the most prominent families of the southern hemisphere, and consists of 79 genera and over 1700 species (Douglas, 1995). It has a main diversity center in Australia, and secondary centers in South Africa, New Caledonia, Southeast Asia, Madagascar, New Zealand, and South America (Weston and Crisp, 1996). This family, apparently of tropical origin (Mabberley, 2009), is characterized by a wide spectrum of variation in vegetative morphology (to which the name 'Proteaceae' refers, from the Greek god Proteus, who changed his shape; Heywood, 1993).

* Corresponding author.
  E-mail address: amagnin@comahue-conicet.gob.ar (A. Magnin).

However, the classification of genera in this family is based, as traditional in vascular plants, on the structure of flowers and fruits (Weston and Crisp, 1996). According to the latest revision of this family (Weston and Barker, 2006), eight genera of Proteaceae can be found in South America, all within the subfamily Grevilleoideae Engl.; six of them are endemic to South America and the other two occur also in Australia and Tasmania. The present study concerns all four tree species of Proteaceae native in southern Chile and Argentina (Patagonia): *Embothrium coccineum* J.R. Forst. & G. Forst. (Tribe Embothrieae), *Lomatia hirsuta* (Lam.) Diels ex J.F. Macbr. (Tribe Embothrieae), *Lomatia ferruginea* (Cav.) R. Br., and *Gevuina avellana* Molina (Tribe Macadamieae). Despite the ecological, evolutionary and potentially economic importance of these species, and of the family in general, few studies have dealt with their morpho-architectural traits (Johnson and Briggs, 1975). *Embothrium coccineum* and *L. hirsuta* inhabit both the western Patagonian rainforests (dominated by *Nothofagus dombeyi*, *Laureliopsis philippiana*, *Fitzroya cupressoides*, among other species) and the dry Patagonian forests, along the eastern side of the Andes (dominated by *Austrocedrus chilensis*, *Nothofagus antarctica*, *Araucaria araucana*, *Maytenus boaria* and *Lomatia hirsuta*; Donoso, 2006). *Lomatia ferruginea* and *G. avellana* are considered typical of the Patagonian rainforests (Cabrera and Willink, 1980). In this study we analyze the extents of preformation and neoformation, as well as bud structure for different ontogenetic stages of these species, so as to achieve a better understanding of their evolution and distribution.

Materials and methods

Study species

Embothrium coccineum is a small tree, up to 15 m tall, with alternate, simple leaves. It has been described as an evergreen tree (Parodi, 1959) or as a facultative deciduous tree (Souto et al., 2009), although the capacity of a tree to retain its leaves in winter seems to depend on the age of the tree (A. Magnin, unpublished). It occurs in Chile and Argentina, from about 35°S to 56°S in Tierra del Fuego (Cabrera and Willink, 1980; Sleumer, 1984), forming mixed populations in valley bottoms and low slopes (Donoso, 2006). *Embothrium coccineum* has been considered to be adapted to severe environmental changes due to its ecological plasticity; this species plays a significant role in the regeneration and restoration of degraded environments (Escobar et al., 2006).

Lomatia hirsuta is a tree with alternate, simple and perennial leaves. It may exceed 15 m in height and inhabits frequently disturbed sites, where it is important as a colonizer (Donoso and Escobar, 2006). The distribution of *L. hirsuta* ranges from central Ecuador to northern Peru, reappearing at about 30°S (central Chile), and reaching to approximately 43°S in both Chile and Argentina (Donoso, 2006; Sleumer, 1984).

Lomatia ferruginea is a small tree with opposite, compound and perennial leaves, and may reach 10 m in height. It thrives from about 37°S to 53°S in Chile. Small populations of *L. ferruginea* are found in Argentina between 39°S and 48°S (Donoso and Utreras,

2006). This species develops under the canopy of the evergreen forest in cool and wet places (Donoso, 2006; Sleumer, 1984).

Gevuina avellana is a tree with alternate, compound and perennial leaves, and can reach 20 m in height. It is distributed from 35°S to 45°S in Chile (Donoso, 2006); in Argentina it is found only in the hydrographical basin of Lago Puelo (42°00′S–42°20′S; Sleumer, 1984).

Study sites and data collection

In this study the following ontogenetic stages were defined for each species. (1) Sapling: this growth phase includes plants from the first year of life until the initiation of the first branches. (2) Juvenile: from the establishment of all categories of axes until the first flower production. (3) Adult: from the beginning of flower development and beyond. Ranges of diameters and heights were determined for each species-stage combination (Table 1).

For each ontogenetic stage of each species we selected a site in Argentina with a population of no less than 50 individuals at that stage, except in the case of the sapling stage of *G. avellana*, for which it was not possible to find enough individuals. It would have been more convenient to have all ontogenetic stages of each species within the same community, but in these, as in other tree species, regeneration does not take place under the canopy of adult trees of the same species. Juvenile and adult stages of *E. coccineum* and juvenile trees of *L. hirsuta* were sampled at Cañadón de la Mosca, Nahuel Huapi National Park (41°32′S, 71°27′W, 819 m altitude; Fig. 1), where these species are present in a semi-open woodland that included *Maytenus boaria* and *Diostea juncea*. The adult *L. hirsuta* trees were sampled at Bahía Serena, San Carlos de Bariloche (41°06′S, 71°26′W, 782 m altitude; Fig. 1), where they form the woody stratum of the plant community, together with shrubs and trees of *Aristotelia chilensis* and *Maytenus boaria*. The sapling stages of *E. coccineum* and *L. hirsuta* were studied at the nursery of *Vivero Forestal Asociación Civil Sembrar*, San Carlos de Bariloche (41°06′S, 71°26′W, 810 m altitude; Fig. 1). Juvenile and adult stages of *G. avellana* were sampled at Lago Puelo National Park (42°05′S, 71°41′W, 250 m altitude; Fig. 1), where these trees develop under a semi-open canopy of *Austrocedrus chilensis*. All three stages of *L. ferruginea* were sampled at Puerto Blest, Nahuel Huapi National Park (41°02′S, 71°47′W, 780 m altitude; Fig. 1), where they inhabit clearings of a forest dominated by *Nothofagus dombeyi*.

The climate of the northern Argentinean Patagonia is dominated by a sharp west-east decreasing rainfall gradient across the Andes Mountains, ranging from up to 4500 mm on the western side to 700 mm or less on the eastern side (Conti, 1998; Fig. 1). The climate of the whole region is characterized by a marked seasonality in temperature and precipitation, with cold and wet winters, and mild and dry summers.

For at least 40 randomly selected individuals per species and ontogenetic stage, the distal end of the trunk shoot extended in the most recent growing season (2007–2008) was identified in August and September 2008 (winter). For each stage and species, the apical bud of 20 of these shoots was cut and dissected under stereomicroscope (Olympus SZH 10, up to 70×). The remaining twenty apical

Table 1
Basic characterization and sampling sites of sapling, juvenile and adult individuals of the four Proteaceae species studied. Mean and standard deviation are indicated for height and diameter at breast height. Tree nursery ■, Puerto Blest □, Cañadón de la Mosca ○, Lago Puelo ● and Bahía Serena ◇.

	Sapling			Juvenile			Adult		
	Height (m)	Diameter (cm)	Site	Height (m)	Diameter (cm)	Site	Height (m)	Diameter (cm)	Site
E. coccineum	0.2 ± 0.1	0.3 ± 0.1	■	2.1 ± 0.2	2.1 ± 0.5	○	4.5 ± 0.7	12.8 ± 3.9	○
L. hirsuta	0.3 ± 0.1	0.5 ± 0.2	■	2.2 ± 0.5	3.0 ± 0.8	○	5.4 ± 0.8	16.3 ± 5.0	◇
L. ferruginea	0.3 ± 0.1	0.3 ± 0.1	□	1.6 ± 0.8	2.6 ± 0.7	□	4.4 ± 0.6	10.5 ± 3.5	□
G. avellana	Not available			2.0 ± 0.6	3.0 ± 1.8	●	3.4 ± 1.2	9.5 ± 4.9	●
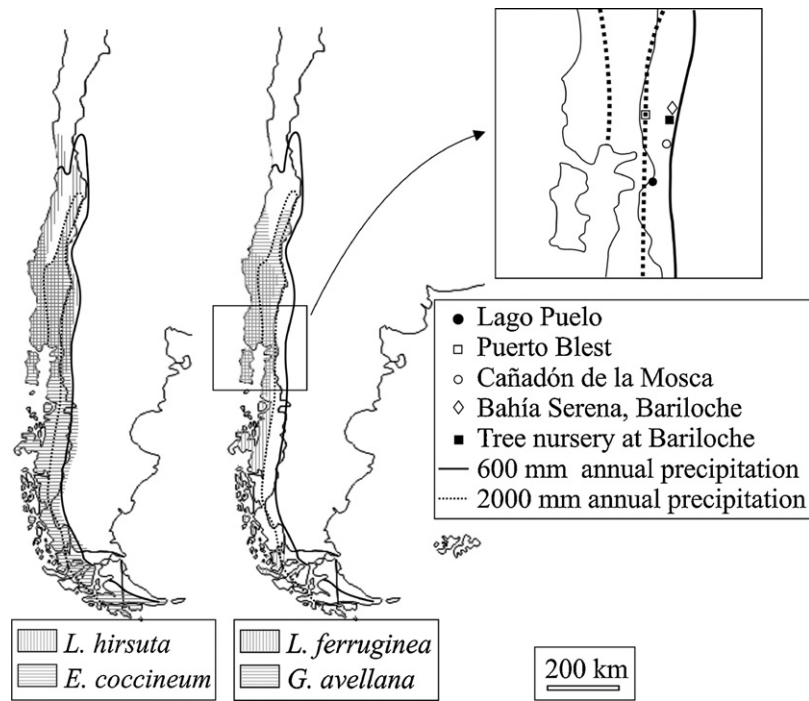
Fig. 1. Distribution maps of study species: left side *Embothrium coccineum* and *Lomatia hirsuta*; right side *Lomatia ferruginea* and *Gevuina avellana*. Annual precipitation isohyetes and study sites are indicated.
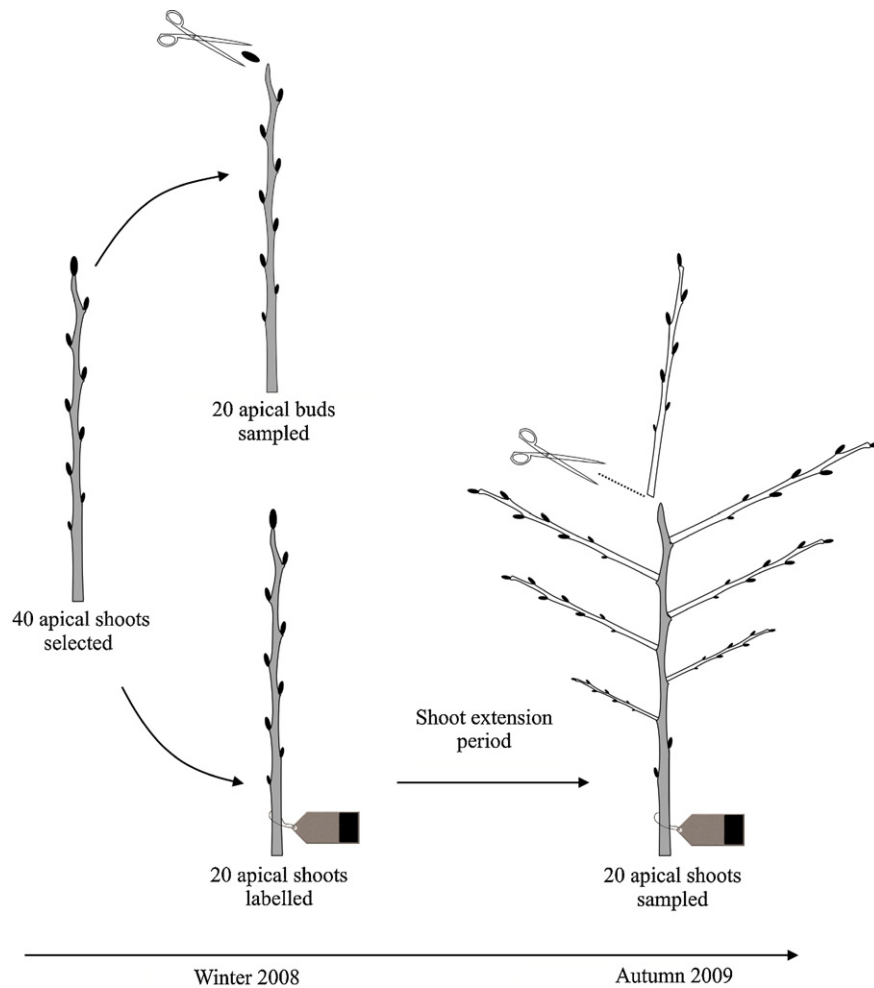
Fig. 2. Sampling scheme of the present study. Sample I: cutting of apical buds and labeling of apical shoots (winter 2008). Sample II: cutting of developed shoots from apical buds (autumn 2009).

buds per stage and species were labeled and collected in April 2009 (autumn), after the end of the 2008–2009 growing season (Fig. 2). For each dissected bud the numbers of cataphylls and green-leaf primordia were registered. In the case of labeled shoots (which derived from buds similar to those that were dissected), the number of developed metamers at the end of their extension was recorded. For each dissected bud, the number of preformed metamers was obtained as the sum of the numbers of cataphylls and green-leaf primordia.

Data analysis

The frequency distributions of the numbers of preformed metamers in apical buds or fully extended shoots were plotted separately for each growth stage and species. The dispersion of each of these distributions was assessed by means of the coefficient of variation (CV = standard deviation/mean $\times$ 100). Mann–Whitney's tests were used to evaluate differences between the numbers of metamers of buds and extended shoots, as some of the distributions deviated markedly from normality even after data transformation (Sokal and Rohlf, 1981). In order to determine whether the number of metamers per bud varied significantly among the ontogenetic stages of each species, Kruskal–Wallis' tests followed by *a posteriori* Dunn's tests (Q, for pairwise comparisons) were applied.

Results

Bud structure

Due to the relatively large sizes of buds and their components, and to the lack of exudates therein, bud dissections were clear and precise. In each case we were able to observe, after removing all primordia, the apical dome with no organs differentiated.

The observations of buds formed after the end of the growing season, and of shoots extended from similar buds in the following growing season allowed the distinction of two bud types, depending on the species concerned. Apical buds of *E. coccineum* and *L. hirsuta* are composed of partially developed leaf primordia covered with ovate–lanceolate cataphylls, whose development is completed by the time bud are formed (Fig. 3A and B). Cataphylls and leaf primordia are clearly different both in consistency and color (yellowish-dun to red the former and whitish the latter).
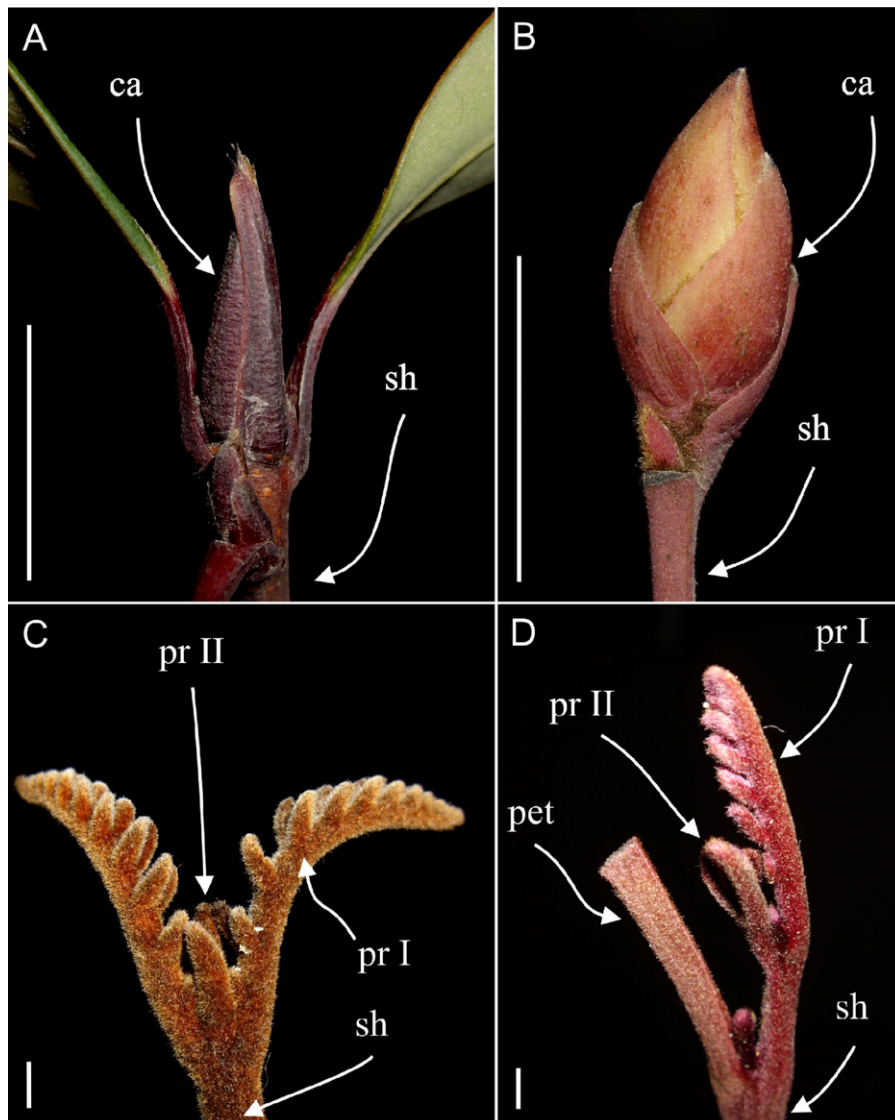
Fig. 3. General appearance of buds in Patagonian Proteaceae species. (A) *Embothrium coccineum*, (B) *Lomatia hirsuta*, (C) *Lomatia ferruginea* and (D) *Gevuina avellana*. *Abbreviations:* sh, bud-bearing shoots; ca, cataphyll; pr I, outermost leaf primordium; pr II, inner leaf primordium; pet, petiole of the leaf closest to the bud. Scale bars = 1 cm.

Table 2
Mean, median, coefficient of variation (CV), minimum (Min) and maximum (Max) for the number of cataphylls and the number of leaf primordia in terminal trunk buds for tree species of Proteaceae from northern Patagonia at three ontogenetic stages (sapling, juvenile and adult). N = number of samples.

	Sapling						Juvenile						Adult					
	N	Mean	Median	CV	Min	Max	N	Mean	Median	CV	Min	Max	N	Mean	Median	CV	Min	Max
E. coccineum	22						24						47					
Cataphylls		2.1	2	26	1	4		9.1	9	14	7	12		8	8	9	7	9
Leaf primordia		6.7	7	22	4	10		9.1	9	16	7	12		6.4	6	21	4	10
L. hirsuta	21						20						20					
Cataphylls		2.8	3	14	2	3		3.9	4	8	3	4		3.5	4	14	3	4
Leaf primordia		3.1	3	10	3	4		4	4	14	3	5		3.4	3	15	3	4
L. ferruginea	22						23						20					
Cataphylls		0	0	0	0	0		0	0	0	0	0		0	0	0	0	0
Leaf primordia		1	1	0	1	1		2	2	0	2	2		2	2	0	2	2
G. avellana							28						22					
Cataphylls		Not available						0	0	0	0	0		0	0	0	0	0
Leaf primordia								5.3	5	18	3	7		5.7	6	14	4	7

Apical buds of *L. ferruginea* and *G. avellana* lack cataphylls and consist entirely of primordia of green leaves. The margins of these primordia are rolled inwards, so that the adaxial epidermis cannot be seen from the outside; outer primordia are much larger than inner ones (Fig. 3C and D).

In *E. coccineum* and *L. hirsuta*, both the number of cataphylls and the number of leaf primordia per bud tended to increase from saplings towards juvenile trees, and to diminish in adult trees (Table 2). A similar but more pronounced gradient was found when analyzing the numbers of metamers of fully extended shoots throughout ontogenetic stages. In *E. coccineum* and *L. hirsuta*, the number of metamers per shoot tended to increase from saplings to juvenile trees (Q = 7.4, P < 0.05 for *E. coccineum*; Q = 7.4, P < 0.05 for *L. hirsuta*), and to diminish from juvenile to adult trees (Q = 6.3, P < 0.05 and Q = 4.1, P < 0.05, respectively). For *E. coccineum* and *L. hirsuta*, the number of metamers per shoot was similar in saplings and adult trees (Q = 1.7, P > 0.05 and Q = 1.5, P > 0.05; Fig. 4A–F). In *L. ferruginea* and *G. avellana* buds the numbers of leaf primordia were relatively constant in the three (*L. ferruginea*) or two (*G. avellana*) ontogenetic stages that were investigated (Fig. 4, Table 2).

Preformation and neoformation

For all three ontogenetic stages of *E. coccineum* and *L. hirsuta*, there was some degree of overlapping between the distributions of preformed metamers and fully extended shoot metamers. A significant proportion of the observed shoots included more metamers than the corresponding buds for both species (Fig. 4A–F). Nonetheless, some shoots of adult *E. coccineum* trees had less metamers than the lowest number of metamers found in buds of this species at the same ontogenetic stage (Fig. 4C). The numbers of metamers in the shoots presented higher dispersions (CV) than the numbers of metamers in the buds for all three ontogenetic stages of *E. coccineum* and *L. hirsuta*. In broad terms, the proportion of shoot metamers that could be considered neoformed was highest for juvenile trees, intermediate for saplings and lowest for adult trees for both species. In contrast, the distributions of metamers in buds and shoots were similar for each ontogenetic stage of *L. ferruginea* and *G. avellana*. In *L. ferruginea* the production of metamers was very low, with only one metamer per year in saplings and two metamers in juvenile and adult trees (Fig. 4G–I). *Gevuina avellana* trees produced five metamers at juvenile and adult stages (Fig. 4J–K).

Discussion

Bud morphology in Patagonian Proteaceae

The structure of the buds formed at the apex of fully extended shoots allows a clear classification of the four studied species of Proteaceae into two groups. The buds of *E. coccineum* and *L. hirsuta* include external cataphylls which completely cover the primordia of green leaves and apical meristems. In contrast, apical buds of *G. avellana* and *L. ferruginea* lack cataphylls and include only partially developed leaf primordia at their metamers. Therefore, from a traditional classification of buds (Bell, 1991), *E. coccineum* and *L. hirsuta* develop scaly buds while *G. avellana* and *L. ferruginea* form naked buds. Scaly buds are common in many temperate tree species with rhythmic growth, such as those in the families *Pinaceae*, *Rosaceae*, *Fagaceae*, *Nothofagaceae* and *Juglandaceae*, among others (Puntieri et al., 2002; Sabatier et al., 2003b; Souza et al., 2000). Naked buds seem to be more common in species belonging to families widely distributed in tropical areas, as *Myrsinaceae* (Nitta and Ohsawa, 1998) and *Lauraceae* (Throp et al., 1994), but are also present in species from temperate regions, as in many members of *Cupressaceae* (Grosfeld and Barthélémy, 2004) and some *Adoxaceae* (e.g., *Viburnum lantana*; Kollmann and Grubb, 2002). Typically, it is considered that cataphylls protect the fragile apical meristem and leaf primordia, mainly from low temperatures, wind, irradiation and desiccation (Kozlowski, 1971; Puntieri et al., 2007) although there is little empirical evidence in support of this assertion. A study on *Artocarpus* (Moraceae) showed that the removal of the outermost bud scales caused marked aberrations in the development of the organs covered by such scales (Potter 1891, cited in Hallé et al., 1978). On the other hand, the protective function of proximal cataphylls in many buds may be debatable due to their small size (see, e.g., Fig. 3B; and also axillary buds of *Nothofagus* spp.: Barthélémy et al., 1999). In the cases of *L. hirsuta* and *E. coccineum*, the majority of cataphylls are thick structures that are arranged in a compact way on the buds, so that attributing them a protective role seems to be justified.

For the family Proteaceae, distributed mainly in temperate and subtropical regions, little information had been published until now regarding bud structure, despite the existence of detailed morphological studies on this family (Johnson and Briggs, 1975; Venkata Rao, 1969). In this study we found that, in the cases of *G. avellana* and *L. ferruginea*, leaf primordia are exposed to external conditions, without any kind of cover from specialized leaves, since their inception from the apical meristem. It may be wondered whether physiological, anatomical and/or morphological adaptations are involved in the tolerance of these primordia to cold and/or dry conditions. Their relatively large size (Fig. 3C and D) may in itself be thought of as a safeguard against frost and/or desiccation. There could also be adaptations to harsh conditions at cellular or subcellular levels (Le and McQueen-Mason, 2006). The heat balance of buds may also be regulated by the development of trichomes (abundant in metamer primordia of *G. avellana* and *L. ferruginea*) and epidermal pigmentation (Ehleringer and Björkman, 1978). In support of this idea, it has also been found that a high density of
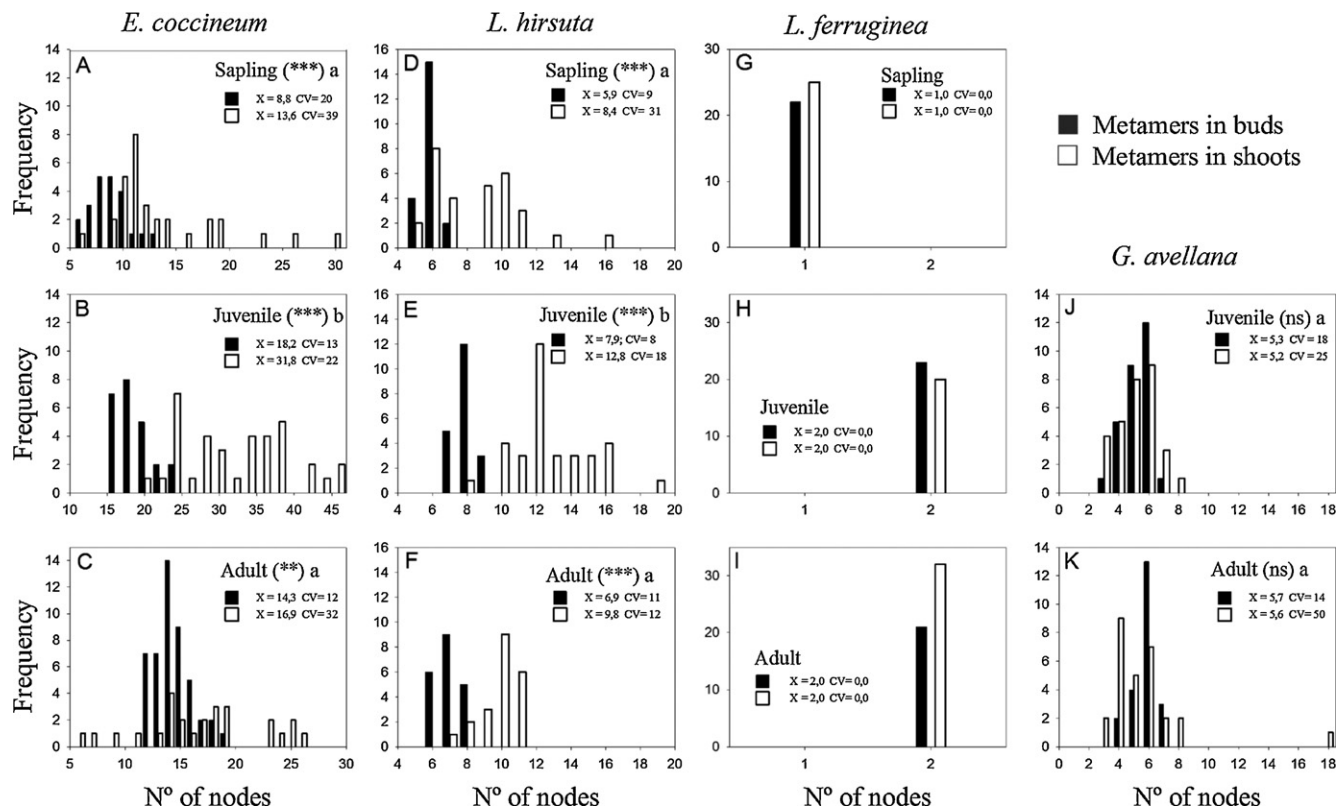
Fig. 4. Frequency distributions of the number of preformed metamers and the number the extended metamers for three ontogenetic stages of four species of Proteaceae. The mean and CV of the number of metamers are indicated for each distribution. The levels of significance of the differences (Mann–Whitney's test) between the distributions of preformed metamers and extended metamers ($**P < 0.01$, $***P < 0.001$, ns $P > 0.05$) are also indicated whenever such comparisons were possible. Statistically significant differences ($P \leq 0.05$) between ontogenetic stages of the same species regarding the number of metamers in shoots are indicated with different lowercase letters at the top-right corner of the graphs.

trichomes on the leaf surface is associated with decreased wettability of the leaf in some Patagonian species (Brewer and Nuñez, 2007), which could reduce the likelihood of bud freezing during the winter season. Other woody species typical of the Patagonian rainforests of Chile and Argentina, such as *Aextoxicon punctatum*, *Persea lingue*, *Laureliopsis philippiana* and *Azara* spp., share with *G. avellana* and *L. ferruginea* the development of naked buds with a dense cover of trichomes on their leaf primordia (A. Magnin, J. Grosfeld and J. Puntieri, unpublished). It may be hypothesized that the number of leaves that may be developed in one growing season would be inversely proportional to leaf size, so that the metabolic cost of resigning photosynthetic surface for the development of cataphylls would increase as mean leaf size increases. Therefore, the development of a dense layer of trichomes on naked buds would be, in temperate-cold regions, a cost-effective alternative to the development of cataphylls in plants with large leaves. More extensive studies on the relationship between bud structure and leaf size would be needed in order to evaluate the validity of this hypothesis at a wider geographical scale.

Preformation and neoformation

This work gives evidence that trunk shoots of *E. coccineum* and *L. hirsuta* plants at sapling, juvenile and adult stages may consist entirely of metamers that were preformed in the growth season preceding that of their extension. Other shoots of these species include, in addition to those metamers that were preformed, a variable number of neoformed metamers. In both species, the numbers of neoformed organs seem to be lower than those of preformed organs, and the proportion of neoformed organs higher

at the juvenile ontogenetic stage than at sapling and adult stages. The fact that some shoots of adult *E. coccineum* trees had numbers of metamers lower than the numbers of metamers of buds in equivalent positions, may be explained by the abortion of leaf primordia or leaves during or after shoot extension. The apparently spontaneous death of leaf primordia has also been observed in *Nothofagus* species and could be related to harsh environmental conditions during shoot extension in Patagonia (Souza et al., 2000).

The development of preformed and neoformed organs has been observed in a number of tree species with rhythmic growth (Davidson and Remphrey, 1994; Guédon et al., 2006; Kozlowski, 1971; Puntieri et al., 2000; Souza et al., 2000). Several possible links between neoformation and ecological adaptations have been proposed. For instance, neoformation has been associated with an opportunistic response of plants typical of early successional stages (Davidson and Remphrey, 1994). Other authors have suggested that neoformation is part of a strategy aimed at exploiting favorable conditions of unpredictable occurrence (Guédon et al., 2006). In temperate regions, the production of neoformed organs would make axis extension possible for as long as environmental conditions are favorable, and after the end of preformation extension (Davidson and Remphrey, 1994; Guédon et al., 2006). The ability to develop shoots with neoformed metamers often depends on the position of the shoot in the plant, as well as on the ontogenetic stage that is expressed by an individual plant at a given time (Barthélémy et al., 1997; Davidson and Remphrey, 1994; Guédon et al., 2006). Some studies indicate that, for a given species, the development of neoformed organs reaches its highest level at the trunk of vigorously growing juvenile trees (Guédon et al., 2006; Souza et al., 2000). The variations observed in the present study in the production

of neoformed leaves among different developmental stages of *E. coccineum* and *L. hirsuta* are consistent with these observations.

In *L. ferruginea* and *G. avellana*, the number of metamers of the shoots at the end of the extension period did not exceed the number of preformed metamers in the winter buds. For this reason, we conclude that annual shoots in these species would be of entirely preformed nature. In both species, the numbers of preformed metamers were low and exhibited little variation throughout plant ontogeny. The development of a low number of preformed organs in these species is consistent with the large size of their leaves and is a common trait in many tropical evergreen trees (Gill and Tomlinson, 1971). However, we observed that very vigorous shoots of both *L. ferruginea* and *G. avellana*, such as those of root suckers and trunk-base sprouts (i.e. total reiterations sensu Hallé et al., 1978) may include more metamers than the maximum numbers of preformed metamers reported here. This suggests that under certain conditions, these species could increase the degree of preformation and/or develop neoformed organs.

Morphology and distribution

Qualitative morphological differences between *E. coccineum* and *L. hirsuta*, on the one hand, and *G. avellana* and *L. ferruginea* on the other, may be related to the establishment of these species at different sites. This view is based on the distributions of these species. *Gevuina avellana* and *L. ferruginea* are distributed only in areas with little water deficit in summer and, probably, with more moderate temperatures (see Conti, 1998). Both species are more common on the western side of the Andes, where the predominantly westerly winds are more likely to bring humidity and moderate extreme temperatures throughout the year. *Embothrium coccineum* and *L. hirsuta* may also inhabit these areas, but are able to reach drier areas where temperatures are more extreme. For these species, the development of specialized leaves for bud protection during the dormancy period and their capacity to profit from unpredictably favorable conditions through neoformation would be related to their wide ecological amplitudes compared to those of *L. ferruginea* and *G. avellana*. This is consistent with the differences in distribution area (wide vs. narrow) and bud structure (scaled vs. naked) observed between two northern hemisphere *Viburnum* species (Kollmann and Grubb, 2002).

Throughout the evolution of the Proteaceae family in different continents, significant variation in vegetative morphology has taken place. Part of this variation, including leaf shape, size and phyllotaxis, has been pointed out in several previous publications (Heywood, 1993; Johnson and Briggs, 1975; Venkata Rao, 1969). Variations in axis structure (including preformation and neoformation) and of bud structure have been incorporated in few taxonomic studies on this family (Weston and Crisp, 1994). It is likely that this information has not been registered for many species of Proteaceae. It is worth to notice that these attributes differ in two sympatric species of the same genus: *L. ferruginea* and *L. hirsuta*. Moreover, these species appear to differ in some aspects of their leaf anatomy (Torres, 1996) as well as in the basic architectural pattern of crown construction (data available upon request to the authors). Intuitively, it may be asked whether these two species have been properly classified. The genus *Lomatia* includes about 12 species, most of them native to the Australian continent, where some *Lomatia* species resemble *L. hirsuta* in leaf morphology and phyllotaxis, whereas others resemble *L. ferruginea* in the same traits (Weston and Crisp, 1994). It would be very interesting to carry out studies incorporating the morphological features considered in this study and a mapping of these traits across molecular-derived phylogenies, for a worldwide spectrum of species within the Proteaceae family. This would help in understanding their evolutionary and ecological relationships.

References

Barthélémy, D., Caraglio, Y., 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. Ann. Bot. 99, 375–407.

Barthélémy, D., Caraglio, Y., Costes, E., 1997. Architecture, gradients morphogénétiques et âge physiologique chez les végétaux. In: Bouchon, J.D.P., Barthélémy, D. (Eds.), Modélisation et simulation de l'architecture des plantes. INRA Editions, Paris, pp. 89–136.

Barthélémy, D., Puntieri, J., Brion, C., Raffaele, E., Marino, J., Martinez, P., 1999. Características morfológicas y arquitecturales de las especies de *Nothofagus* Blume (Fagaceae) del norte de la Patagonia Argentina. Bol. Soc. Arg. Bot. 34, 29–38.

Barthélémy, D., Caraglio, Y., Sabatier, S., 2009. Crown architecture of valuable broadleaved species. In: Spiecker, H.H.S., Markkonen-Spiecker, K., Thies, M. (Eds.), Valuable Broadleaved Forests in Europe. Brill, Boston, pp. 87–101.

Bell, A., 1991. Plant Form: An Illustrated Guide to Flowering Plant Morphology. Oxford University Press, Oxford.

Brewer, C.A., Nuñez, C.I., 2007. Patterns of leaf wettability along an extreme moisture gradient in western Patagonia, Argentina. Int. J. Plant Sci. 168, 555–562.

Cabrera, A.L., Willink, A., 1980. Biogeografía de América latina, 2nd ed. Acme S.A.C.I, Buenos Aires.

Conti, H., 1998. Características climáticas de la Patagonia. In: Correa, M. (Ed.), Flora Patagónica. INTA, Buenos Aires, pp. 31–47.

Costes, E., Sinoquet, H., Kelner, J., Godin, C., 2003. Exploring within tree architectural development of two apple tree cultivars over 6 years. Ann. Bot. 91, 91–104.

Davidson, C.G., Remphrey, W.R., 1994. Shoot neoformation in clones of *Fraxinus pennsylvanica* in relation to genotype, site and pruning treatments. Trees 8, 205–212.

Diggle, P.K., 2002. A developmental morphologist's perspective on plasticity. Evol. Ecol. 16, 267–283.

Donoso, C., 2006. Las especies arbóreas de los bosques templados de Chile y Argentina. Marisa Cuneo Ediciones, Valdivia.

Donoso, C., Escobar, B., 2006. Lomatia hirsuta. In: Donoso (Ed.), Las especies arbóreas de los bosques templados de Chile y Argentina. Marisa Cuneo Ediciones, Valdivia, pp. 248–253.

Donoso, C., Utreras, F., 2006. Lomatia ferruginea. In: Donoso (Ed.), Las especies arbóreas de los bosques templados de Chile y Argentina. Marisa Cuneo Ediciones, Valdivia, pp. 344–347.

Douglas, A., 1995. Affinities. In: McCarthy, P. (Ed.), Flora of Australia. CSIRO, Melbourne, pp. 6–14.

Ehleringer, J., Björkman, O., 1978. Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa*. Oecologia 36, 151–162.

Escobar, B., Donoso, C., Souto, C., Alberdi, M., Zúñiga, A., 2006. Embothrium coccineum. In: Donoso (Ed.), Las especies arbóreas de los bosques templados de Chile y Argentina. Marisa Cuneo Ediciones, Valdivia, pp. 233–245.

Gill, A., Tomlinson, P.B., 1971. Studies on the growth of red mangrove (*Rhizophora mangle* L.) 3. Phenology of the shoot. Biotropica 3, 109–124.

Grosfeld, J., Barthélémy, D., 2004. Primary growth and morphological markers of interannual growth limits in Cupressaceae from Patagonia. Bot. J. Linn. Soc. 146, 285–293.

Guédon, Y., Puntieri, J., Sabatier, S., Barthélémy, D., 2006. Relative extents of preformation and neoformation in tree shoots: analysis by a deconvolution method. Ann. Bot. 98, 835–844.

Hallé, F., Martin, R., 1968. Etude de la croissance rythmique chez l'Hevea (*Hevea brasiliensis* Müll-Arg. Euphorbiacees-Crotonoidees). Adansonia 8, 475–503.

Hallé, F., Oldeman, R.A.A., Tomlinson, P.B., 1978. Tropical Trees and Forests: An Architectural Analysis. Springer, Berlin.

Heuret, P., Meredieu, C., Coudurier, T., Courdier, F., Barthélémy, D., 2006. Ontogenetic trends in the morphological features of main stem annual shoots of *Pinus pinaster* (Pinaceae). Am. J. Bot. 93, 1577–1587.

Heywood, V.H., 1993. Flowering Plants of the World. BT Batsford Ltd., London.

Johnson, L.A., Briggs, B.G., 1975. On the Proteaceae: the evolution and classification of a southern family. Bot. J. Linn. Soc. 70, 83–182.

Kollmann, J., Grubb, P.J., 2002. *Viburnum lantana* L. and *Viburnum opulus* L. (*V. lobatum* Lam., *Opulus vulgaris* Borkh.). J. Ecol. 90, 1044–1070.

Kozlowski, T.T., 1971. Growth and Development of Trees: Seed Germination, Ontogeny and Shoot Growth, vol. I. Academic Press, London.

Le, T.-N., McQueen-Mason, S., 2006. Desiccation-tolerant plants in dry environments. Rev. Environ. Sci. Biotechnol. 5, 269–279.

Mabberley, D.J., 2009. Mabberley's Plant-book: A Dictionary of Plants, their Classification and Use, 3rd ed. Cambridge Univesity Press, Cambridge.

Nitta, I., Ohsawa, M., 1998. Bud structure and shoot architecture of canopy and understorey evergreen broad-leaved trees at their northern limit in East Asia. Ann. Bot. 81, 115–119.

Parodi, L.R., 1959. Enciclopedia argentina de agricultura y jardinería. Acme, Buenos Aires.

Puntieri, J., Souza, M.S., Barthélémy, D., Brion, C., Nuñez, M., Mazzini, C., 2000. Preformation, neoformation, and shoot structure in *Nothofagus dombeyi* (Nothofagaceae). Can. J. Bot. 78, 1044–1054.

Puntieri, J., Stecconi, M., Barthélémy, D., 2002. Preformation and neoformation in shoots of *Nothofagus antarctica* (G, Forster) Oerst. (Nothofagaceae) shrubs from northern Patagonia. Ann. Bot. 89, 665–673.

Puntieri, J., Grosfeld, J., Stecconi, M., Brion, C., Barthélémy, D., 2007. Bud and growth-unit structure in seedlings and saplings of *Nothofagus alpina* (Nothofagaceae). Am. J. Bot. 94, 1382–1390.

Rawat, D.S., Gaur, R.D., 2004. Floral preformation in alpine plants of the Himalaya. Curr. Sci. 86, 1481–1482.

Rowe, N., Speck, T., 2005. Plant growth forms: an ecological and evolutionary perspective. New. Phytol. 166, 61–72.

Sabatier, S., Baradat, P., Barthélémy, D., 2003a. Intra-and interspecific variations of polycyclism in young trees of *Cedrus atlantica* (Endl.) Manetti ex. Carrière and *Cedrus libani* A. Rich (Pinaceae). Ann. For. Sci. 60, 19–29.

Sabatier, S., Barthélémy, D., Ducousso, I., 2003b. Periods of organogenesis in mono- and bicyclic annual shoots of *Juglans regia* L. (Juglandaceae). Ann. Bot. 92, 231–238.

Seleznyova, A., Thorp, T., White, M., Tustin, S., Costes, E., 2003. Application of architectural analysis and AMAPmod methodology to study dwarfing phenomenon: the branch structure of 'royal gala' apple grafted on dwarfing and non dwarfing rootstock/interstock combinations. Ann. Bot. 91, 665–672.

Sleumer, H., 1984. Proteaceae. In: Correa, M. (Ed.), Flora Patagónica 4a. INTA, Buenos Aires, pp. 20–27.

Sokal, R., Rohlf, F., 1981. Biometry, 2nd ed. Freeman & Co., New York.

Souto, C.P., Premoli, A.C., Reich, P.B., 2009. Complex bioclimatic and soil gradients shape leaf trait variation in *Embothrium coccineum* (Proteaceae) among austral forests in Patagonia. Rev. Chil. Hist. Nat. 82, 209–222.

Souza, M., Puntieri, J., Barthélémy, D., Brion, C., 2000. Bud content and its relation to shoot size and structure in *Nothofagus pumilio* (Poepp. et Endl.) Krasser (Nothofagaceae). Ann. Bot. 85, 547–555.

Throp, T.G., Aspinall, D., Sedgley, M., 1994. Preformation of node number in vegetative and reproductive proleptic shoot modules of *Persea* (Lauraceae). Ann. Bot. 73, 13–22.

Torres, F., 1996. Anatomía foliar en Proteaceae sudamericanas con énfasis en las esclereidas. Plantula 1, 1–43.

Venkata Rao, E., 1969. Studies in the Proteaceae XIII. Tribe Grevilleeae. Proc. Indian Acad. Sci. 35, 471–486.

Weston, P.H., Barker, N.P., 2006. A new suprageneric classification of the Proteaceae, with an annotated checklist of genera. Telopea 11, 314–344.

Weston, P.H., Crisp, M., 1994. Cladistic biogeography of waratahs (Proteaceae, Embothrieae) and their allies across the pacific. Aust. Syst. Bot. 7, 225–249.

Weston, P.H., Crisp, M., 1996. Trans-Pacific biogeographic patterns in the Proteaceae. In: Keast, A.M.S. (Ed.), The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes. SPB Academic, Amsterdam, pp. 215–232.