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Chemical and morphological study of a putative hybrid between *Luzuriaga radicans* and *L. polyphylla* (Monocotyledoneae: Luzuriagaceae)

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Abstract Patterns of headspace volatiles and flavonoids extracts from flowers of sympatric populations of *Luzuriaga radicans* Ruiz et Pav. (Monocotyledoneae: Luzuriagaceae), *L. polyphylla* (Hook.) J.F.Macbr., and a *Luzuriaga* taxon which presented some morphological characters of one of the species and other characters of the other species, suggest that this latter taxon is a hybrid between the two accepted species.

Keywords flower headspace volatiles; flower flavonoid patterns; hybridisation

INTRODUCTION

The genus *Luzuriaga* (Monocotyledoneae: Luzuriagaceae) is composed of four species: *L. marginata* Benth. & Hook.f., which grows in Chile, Argentina, and the Falkland Islands; *L. parviflora* Kunth, which is endemic to New Zealand; *L. polyphylla* (Hook.) J.F.Macbr., which is endemic to Chile; and *L. radicans* Ruiz et Pav., which grows in southern Chile and Argentina (Rodríguez & Marticorena 1987). Thus, this genus is a clear example of the floristic links between distant landmasses of the Southern Hemisphere. *Luzuriaga polyphylla* grows between c. 37 and 45°S and *L. radicans* between 35 and 45°S (Rodríguez & Marticorena 1987). Preliminary observations of these two species occurring sympatrically at the Valdivian temperate rainforests of Puyehue National Park revealed individuals that possessed some characters of one of the accepted species and other characters of the other species; those individuals also presented flowers with a scent similar to one of the species (the scent of the other species is unapparent) and anthers coloured as one of the species but not as the other. These observations suggested the occurrence of a putative hybrid between the two described species.

Flower headspace volatiles have been used to distinguish hybrids from parental species (Ishizaka et al. 2002). Flavonoid patterns have also been used to compare hybrids with their parents (Bhargava et al. 2005), and to distinguish between species and putative hybrids (Freeman et al. 1991; Wyatt & Hunt 1991; Heimler et al. 1993; Voirin et al. 1999; Menadue & Crowden 2000; Bayly et al. 2001), and between closely related taxa (Kellow et al. 2005). Given the differences detected in flower scent and colour of anthers, we analysed the volatiles in the headspace and the patterns of flavonoids in extracts of flowers of the sympatric *Luzuriaga* taxa mentioned.

MATERIAL AND METHODS

Study area

The study was carried out in the Puyehue National Park, Anticura sector, in southern Chile (40°40′S; 72°10′W; 364 m above sea level). The main forest type is the Valdivian temperate rainforest dominated

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by evergreen species such as *Eucryphia cordifolia* (Cunoniaceae), *Aetoxicon punctatum* (Aetoxicaceae), *Nothofagus dombeyii* (Nothofagaceae), and *Laurelia philippiana* (Atherospermataceae) (Muñoz 1980). The material collected was identified by Sebastián Teillier, Universidad Central de Chile. Voucher specimens were stored at the Herbarium of Universidad de Concepción (CONC).

Analysis of floral scents

Three flower samples of each taxon (between 40 and 70 g fresh weight per sample) were gathered from many individual plants in the same locality and placed inside a glass jar provided with an inlet and an outlet. At the inlet, a compressed air cylinder containing synthetic air made from extra pure oxygen and nitrogen, with no detectable organic impurities, was attached through a regulator which controlled the air flow. At the outlet, a column was attached which contained Porapak Q (100 mg). Volatile entrainment (c. 4 min per gram of sample, with an air flow of 0.5 L/min) commenced immediately after the flowers had been cut from the plants. The columns were taken back to the laboratory and the volatiles adsorbed on the Porapak Q eluted with 1 ml of dichloromethane. These extracts were analysed in a GC-MS (gas chromatograph: Hewlett-Packard model HP5891; mass spectrometric detector with integrated data system: Hewlett-Packard model HP5972) with a SPB-5 (25 m × 0.2 mm id) capillary column. Ionisation by electron impact (70 eV) was carried out at 280°C. The GC oven was programmed to remain at 50°C for 10 min, to increase up to 280°C at a rate of 5°C/min, and then to remain at 280°C for 45 min. The identification of compounds in the chromatographic profiles was achieved by comparison of their mass spectra with a library database, and was confirmed by comparison of retention indices with those of authentic standards or with values from the literature.

Analysis of flavonoids

After we collected headspace volatiles, flower samples were dried in the shade, taken back to the laboratory and milled. The flower powder (between 4 and 10 g per sample) was extracted with 250 ml of EtOH with agitation at room temperature for 24 h. The extracts obtained were concentrated to dryness under reduced pressure at 30°C. The residues were solubilised in 10 ml MeOH, of which 20 µl were used for each HPLC analysis. Separation was achieved with an analytical HPLC (Waters 600), using a reverse-phase Symmetry (5 µm particle size; 25 × 0.46 cm) column. Gradient elution was done with a mobile phase consisting of water (solution A) and MeOH (solution B) as follows: 0–5 min, isocratic elution 60%A/40%B; 5–30 min, linear gradient from 60%A/40%B to 40%A/60%B; and 30–45 min, isocratic elution 40%A/60%B. Detection was accomplished with a Waters 2996 diode-array-detector (DAD), and spectra were recorded at wavelengths between 200 and 800 nm. All compounds showed one band between 264 and 269 nm corresponding to band I of ring A of a flavonoid, and another band between 341 and 362 nm corresponding to band II of ring B of a flavonoid. The UV spectra and retention times of all flavonoids detected suggest they correspond to flavonol glycosides (Mabry et al. 1970; Martson & Hostettmann 2006). Quantitation was based on peak areas in chromatograms taken at 360 nm. The three samples collected were analysed independently.

Data analysis

Similarity in the composition of flower headspace volatiles and flower flavonoid extracts was compared with the Jaccard similarity coefficient, which uses presence-absence data, and with the Renkonen index, which uses quantitative composition data (Krebs 1989). In the latter, mean values of composition were used. Comparisons between the same taxon should give values of 1 and 100, respectively (complete similarity), and comparisons between different taxa should give values between 0 and 1, and between 0 and 100, respectively (partial similarity).

RESULTS AND DISCUSSION

Table 1 summarises and Fig. 1 illustrates some morphological features which differ between *L. polyphylla* and *L. radicans*. The putative hybrid shares characteristics with both accepted species, and closely resembles *L. polyphylla* except for the shape and dimensions of tepals.

Table 2 lists the compounds identified in the flower headspace volatiles. A total of 25 compounds were identified, six of which occurred only in *L. radicans*, nine only in *L. polyphylla*, and two only in the putative hybrid. These latter might have been produced as a result of new gene combinations in the putative hybrid. Of the nine compounds identified in the putative hybrid, three were shared with *L. radicans* and four with *L. polyphylla*. The two major compounds in the hybrid were also the major compounds in *L. polyphylla*.

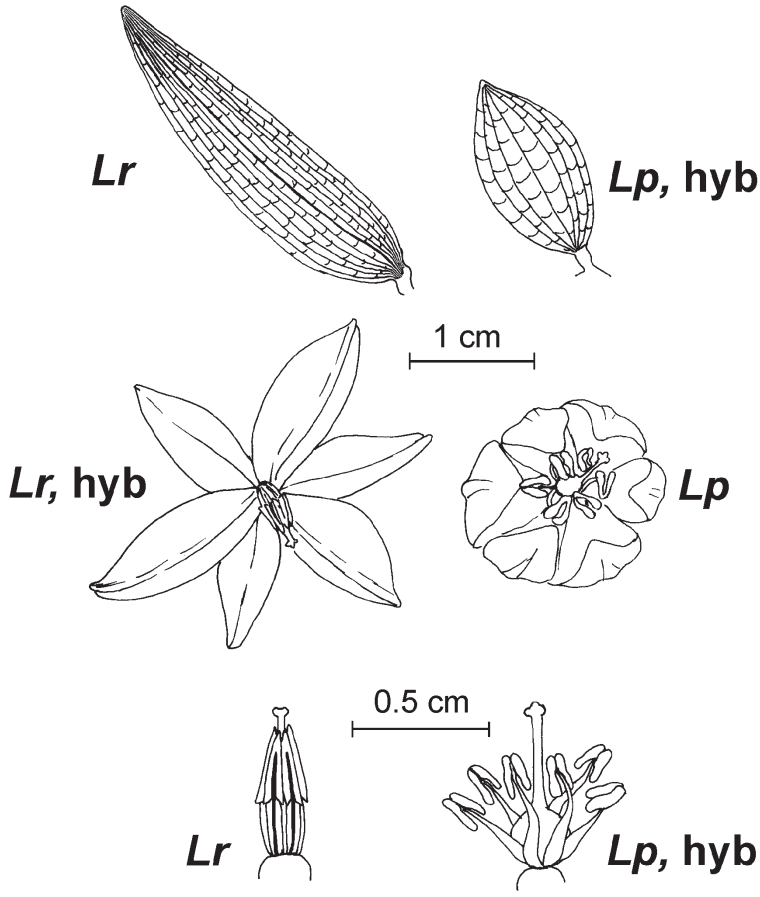

Fig. 1 Morphological characteristics of leaves, flowers, and reproductive organs of *Luzuriaga radicans* (*Lr*), *L. polyphylla* (*Lp*), and their putative hybrid (hyb).

Table 1 Some morphological characters of *Luzuriaga radicans*, *L. polyphylla*, and their putative hybrid, based on measurements of 50 individuals of each taxon collected at the Anticura sector of the Puyehue National Park, Chile.

Character	*L. radicans*	Putative hybrid	*L. polyphylla*
Leaf shape	falcate	straight	straight
Leaf length (mm)	20–40	8–25	8–25
Leaf width (mm)	4–10	3–14	3–14
Nerves in leaf (No.)	9–13	5–7	5–7
Grouping of flowers	cymes with 2–4 flowers (commonly 2)	solitary flowers	solitary flowers
Tepal shape	convex	convex	concave
Tepal length (mm)	16–18	19–21	12–13
Tepal width (mm)	5–7	6–8	9–11
Tepal width (internal/external)	>1	>1	<1
Ratio between length of tepals and stamens	3:1	2:1	2:1
Anthers	attached at base	versatile	versatile
Ratio between length of anthers and filament	>1	<1	<1
Connivent anthers	yes	no	no
Colour of anthers	orange	creamy	creamy
Flower aroma	strong	weak	unapparent

Table 2 Volatile compounds entrained from the headspace of flowers of *Luzuriaga radicans*, *L. polyphylla*, and their putative hybrid.

		GC area (%)[2]		
Compound	Retention index[1]	*L. radicans*	Putative hybrid	*L. polyphylla*
Methyl isobutyrate	706	6.3	–	–
Methyl butyrate	786	49.6	2.4	–
Hexanal	798	–	–	0.1
α-Pinene	937	–	–	0.6
6-Methyl-5-hepten-2-one	986	9.8	–	0.1
β-Myrcene	989	–	1.2	0.1
p-Methylanisole	1021	8.8	–	–
Limonene	1031	1.6	2.2	–
Benzyl alcohol	1037	–	–	1.6
(*E*)-Ocimene	1050	–	6.1	–
(*Z*)-Linalyl oxide	1080	–	–	0.1
Methyl benzoate	1097	–	–	0.1
Linalool	1108	–	57.1	83.1
Phenylethanol	1123	–	–	0.8
p-Dimethoxybenzene	1165	2.4	–	–
Benzyl acetate	1167	–	–	0.1
Methyl salicylate	1199	–	–	1.1
p-Methoxy benzylalcohol	1287	1.9	–	–
(*E*)-Methyl geranate	1323	2.3	–	–
Methyl *p*-methoxybenzoate	1377	8.9	1.5	–
Methyleugenol	1404	6.6	–	–
α-Farnesene	1509	–	1.2	0.7
(*Z*)-Nerolidol	1568	–	–	0.9
(*E*)-Nerolidol	1569	–	22.8	8.5
(*E*)-Farnesol	1722	–	2.3	–

[1]Retention indices on an SPB-5 column in reference to n-alkanes.
[2]Peak areas relative to total peak area. Means of three samples which did not differ by more than 10%.
–, not detected.

The analysis of flavonoids in flower extracts is summarised in Table 3. A total of 21 flavonoids were detected, three of which occurred only in *L. radicans*, three only in *L. polyphylla*, and two only in the putative hybrid. Twelve flavonoids were shared by *L. polyphylla* and the hybrid, and five flavonoids were shared by *L. radicans* and the hybrid. The major flavonoid in the hybrid was also the major compound in *L. polyphylla*.

Values of Jaccard coefficients and Renkonen indices are given in Table 4. Whether presence-absence data or quantitative compositions are used for establishing comparisons between the three taxa studied, values of coefficients for interspecific comparisons are the lowest, pointing to the intermediate nature of the hybrid, while values for the coefficients for *L. polyphylla*-putative hybrid comparisons are consistently the highest. Taken together, the data point to the occurrence of a hybrid between *L. radicans* and *L. polyphylla* which resembles more closely *L. polyphylla*.

Both *L. polyphylla* and *L. radicans* are self-incompatible and totally allogamous (Riveros 1991; Arroyo & Humaña 1999). A 3 yr study in the Valdivian temperate forests of Chiloé Island (42–42.5°S, 73.35°W, 30 m above sea level), not far from our study site, showed that both species are pollinated exclusively by *Bombyx dahlbomii* and *Diphaglossa gayi* (Smith-Ramírez et al. 2005), two native hymenopterans of Chile whose distribution closely resembles that of the *Luzuriaga* species studied (Toro 1986). The co-occurrence of both *Luzuriaga* species at our study site, the coincidence of insect species pollinating them at a nearby site, and hence their likely occurrence in our study site are compatible with the local formation of a hybrid.

Table 3 Flavonoid patterns from flower extracts of *Luzuriaga radicans*, *L. polyphylla*, and their putative hybrid.

		HPLC area (%)[1]		
Retention time (min)	UV maxima (nm)	*L. radicans*	Putative hybrid	*L. polyphylla*
1.31	269, 345	9.4	7.5	8.0
1.65	265, 351	2.9	5.0	–
1.79	264, 348	3.7	–	–
2.05	266, 346	–	3.1	3.6
2.22	266, 347	–	3.7	10.0
2.65	265, 347	38.7	2.4	2.5
3.04	266, 347	–	7.2	8.0
3.49	266, 348	35.4	3.0	4.4
3.89	266, 348	–	5.5	6.1
4.36	266, 347	–	–	1.3
5.04	265, 345	–	25.4	–
5.20	265, 345	–	–	15.8
5.99	265, 345	–	28.6	29.9
6.27	265, 350	2.0	–	–
6.92	266, 348	4.9	–	–
7.42	265, 346	1.0	1.4	1.6
7.99	266, 352	–	–	0.2
11.07	265, 341	–	2.0	–
11.51	265, 342	–	3.0	4.5
15.09	265, 345	–	0.5	0.7
19.17	266, 362	–	0.2	0.3

[1]Peak areas relative to total peak area. Means of three samples which did not differ by more than 10%.

Table 4 Jaccard and Renkonen indices for comparisons involving compositions of headspace volatiles and flavonoids of flowers of *Luzuriaga radicans*, *L. polyphylla*, and their putative hybrid.

	Headspace volatiles		Flavonoids	
Comparison	Jaccard coefficient	Renkonen index	Jaccard coefficient	Renkonen index
L. radicans – *L. polyphylla*	0.045	0.10	0.211	16.4
L. radicans – putative hybrid	0.188	5.7	0.278	17.3
L. polyphylla – putative hybrid	0.210	68.5	0.632	67.1

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