http://dx.doi.org/10.7751/telopea19971010

The cytotaxonomy of four Tasmanian genera of Proteaceae

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Abstract

Wiltshire, R.J.E.[1] and Stace, H.M.[2] ([1]Department of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tasmania 7001, [2]Department of Botany, University of Western Australia, Nedlands, WA 6907) 1997. The cytotaxonomy of four Tasmanian genera of Proteaceae. Telopea *7(3): 181–185*. New chromosome number determinations for *Orites diversifolia* (2*n*=28) and *Cenarrhenes nitida* (2*n*=26) correct prior reports. We confirm *Agastachys odorata* with 2*n*=26 and *Bellendena montana* with 2*n*=10. These data indicate that *x*=15 is absent from subfamily Grevillioideae and family Proteaceae, and *x*=14 is absent from tribe Conospermeeae of subfamily Proteoideae, but *x*=5 is confirmed in subfamily Bellendenoideae.

Introduction

In Proteaceae the highest and the lowest chromosome base numbers were reported from two Tasmanian species, *n*=15 in *Orites diversifolia* and *n*=5 in *Bellendena montana* (Venkata Rao 1957a, 1957b, 1971). All other chromosomal reports in 65 genera of the family range between *x*=14 and *x*=7 (e.g. de Vos 1943; Darlington and Wylie 1955; Smith-White 1959; Ramsay 1963; Johnson and Briggs 1963, 1975). The two results have never been revisited, although that for *O. diversifolia* is discordant with other data in the genus *Orites* (otherwise *n*=14), and that for the Tasmanian endemic *Bellendena* is unusual in the subfamily Persoonioideae (usually *x*=7) in which it was formerly included (Weston 1995). Another Tasmanian endemic, *Cenarrhenes nitida*, was reported as *n*=14 (Ramsay 1963), a generally rare result in the tribe Conospermeeae and subfamily Proteoideae which includes a further Tasmanian endemic *Agastachys odorata* with 2*n*=26 (Venkata Rao 1957a). All four genera, of which three are monospecific, are included in cladistic studies of Proteaceae (Douglas pers. comm.). Accurate knowledge of cytological character states is relevant for interpreting phylogenetic hypotheses of relationship among primitive and advanced taxa of Proteaceae (Smith-White 1959; White 1978).

Methods

Fresh seeds of *O. diversifolia* were obtained from two localities in the vicinity of Hobart (Table 1), and germinated on moist filter paper. Root-tips were taken from *C. nitida* grown at the University of Tasmania. Rooted cuttings of *B. montana* were supplied by Royal Botanic Gardens, Hobart. Excised root-tips were pre-treated for 2.5 hours in saturated aqueous p-dichlorobenzene (BDH chemicals) or bromo-naphthalene, then fixed in 3:1 ethanol: acetic acid for 24 h, stored at 4°C in 70% ethanol, and stained overnight at 60°C with Snow's alcoholic carmine. Root-tip squashes were examined and cells in mitotic metaphase were photographed by Zeiss Axiophot bright-field microscopy using Kodak Imagelink ASA 6 film.

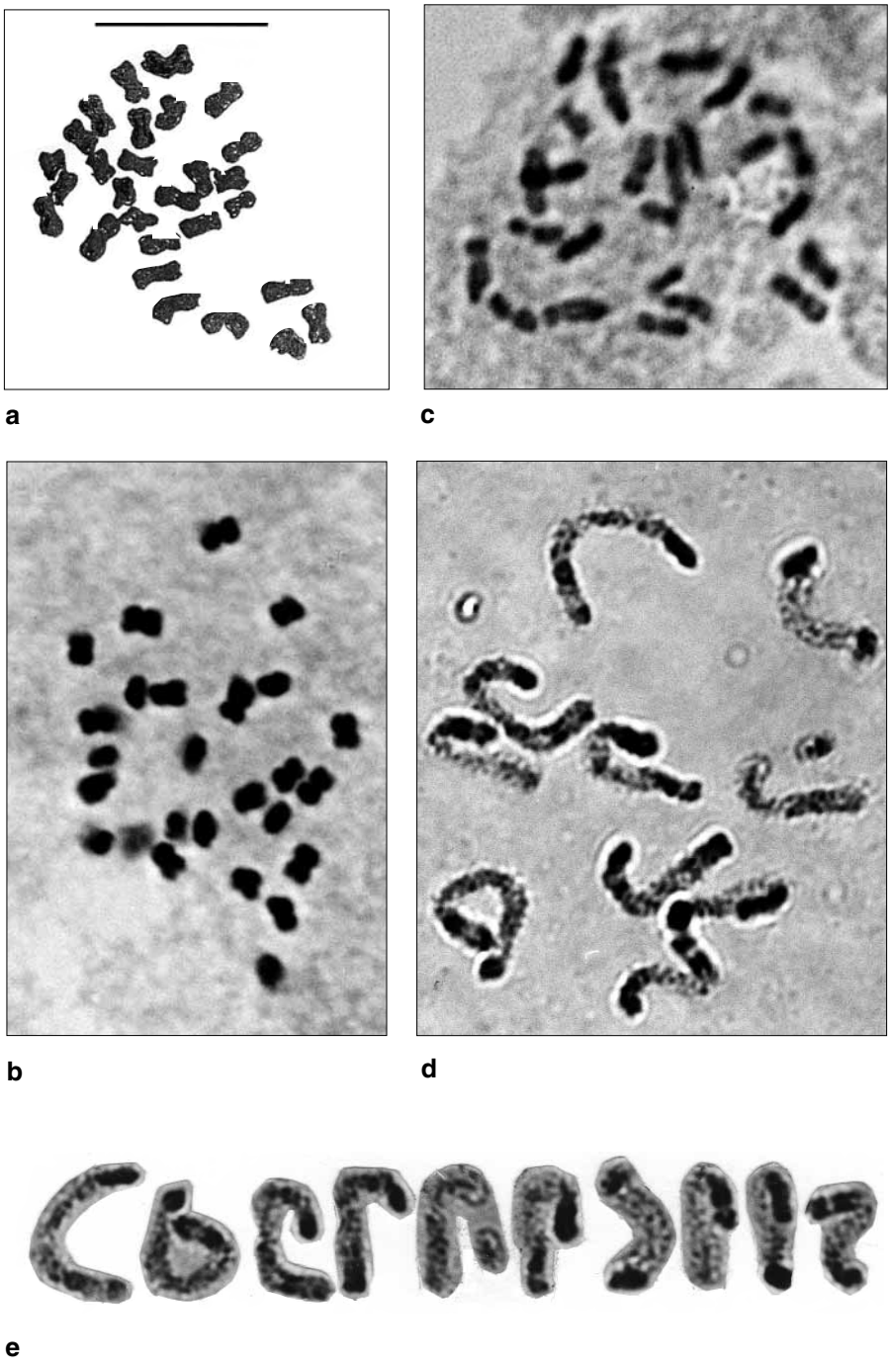

Fig. 1. Chromosomes in root-tip mitosis, all to similar scale (bar = 10 µm), with mean and total lengths of chromosomes in metaphase cells. **a,** *Orites diversifolia* (2n=28), mean 2.2 µm, total 61 µm, drawn from photograph. **b,** *Cenarrhenes nitida* (2n=26), mean 1.8 µm, total 48 µm. **c,** *Agastachys odorata* (2n=26), mean 3.1 µm, total 81 µm. **d, e,** *Bellendena montana* (2n=10), same cell, late prophase.

Results

Cells of *O. diversifolia* showed a maximum of 2*n*=28 well-stained chromosomes from both localities, and cells of *C. nitida* and *A. odorata* each had a maximum of 2*n*=26 chromosomes (Table 1). Metaphase chromosome sizes were relatively small (means 1.8–3.1 µm) and, allowing for differential contraction, were quite similar in all three species (Fig. 1 a, b, c) having a combined average total genome length of 63 µm (range 48–81 µm). Chromosome lengths such as these are typical for Grevillioideae and Proteoideae (Ramsay 1963; Johnson and Briggs 1963, 1975; Rourke 1972).

Cells from *B. montana* had 2*n*=10 chromosomes, those in late prophase were very long (10-15 µm), with a single markedly trabanted chromosome and possibly other heteromorphisms (Fig. 1 d, e). More contracted chromosomes of metaphase cells (not shown, mean chromosome length 6.7 µm) suggested three pairs of metacentrics and two shorter pairs of sub-telocentrics, but a complete karyotype of *B. montana* requires further study. However a preliminary assessment of the metaphase genome length of *B. montana* (total 67 µm) is close to the average for the previous three species with 2*n*=28 or 2*n*=26.

Table 1. New cytological reports for Tasmanian Proteaceae

Species	**2*n***	**Locality and voucher**
Orites diversifolia R.Br.	28	Snug Tiers, Tasmania (RJEW s.n., June 1996)
	c.28	Hartz, Tasmania (RJEW s.n., June 1996)
Cenarrhenes nitida Labill.	26	Bennetts Road, Hartz Mt., Tasmania (J. Read s.n.), cultivated at Department of Plant Science, University of Tasmania
Agastachys odorata R.Br.	26	Mt. Wellington, Tasmania (RJEW s.n., December 1996)
Bellendena montana R.Br.	10	Pine Lake, Central Plateau, Tasmania (RBG Hobart 96–113)

Discussion

The finding of 2*n*=28 (*x*=14) for *O. diversifolia* from localities around Hobart is entirely consistent with other data for *Orites*, indeed for the tribe Oriteae including *Neorites,* all *x*=14 (Venkata Rao 1957a, b; Johnson and Briggs 1975). This discounts the earlier report of *n*=15 for the species from near Hobart and in this respect *O. diversifolia* does not differ from other *Orites* species. In subfamily Grevillioideae the abundant *x*=14 is probably primitive (Smith-White 1959, Johnson and Briggs 1963), as supported by cytoevolutionary interpretations of recent phylogenetic studies (Douglas pers. comm.).

The provenance of Ramsay's *n*=14 *C. nitida* collection was stated as 'Tasmania. Wild.' and is not exactly replicable. The new result of 2*n*=26 (*x*=13) for the monotypic *Cenarrhenes* is relatively frequent in subfamily Proteoideae. Thus, outside subfamily Grevillioideae, *x*=14 is less common in Proteaceae than was previously thought (e.g. Stace 1995). In some recent phylogenetic models *Cenarrhenes* plus the New Caledonian *Beauprea* (*x*=11) and *Beaupreopsis* (*x*=11) are basal genera in Proteaceae that separate before the better resolved 'subfamily' clades and hence may be paraphyletic to subfamily Proteoideae (Douglas pers. comm.).

In taxonomic and phylogenetic treatments (Johnson and Briggs 1975; Douglas pers. comm.) the Tasmanian monotypic *Agastachys* (*x*=13) is always closely associated with the mainland genus *Symphionema* (*x*=10). Although this generic grouping may be basal in the subfamily Proteoideae, other genera suggest that *x*=13 and not *x*=10 may be plesiomorphic in this subfamily.

The 2*n*=10 from presumably a second locality for *B. montana* confirms the original report of *n*=5. The few but large chromosomes of *Bellendena* possibly constitute an advanced karyotype in Proteaceae (Johnson and Briggs 1975; White 1978; James 1981; Weston 1994), perhaps by a process of amalgamating smaller but more numerous chromosomes similar to those of the previous three taxa. However, *Bellendena* is now placed in the monotypic subfamily Bellendenoideae (Weston 1995) and is suggested to be the sister group to all other Proteaceae (Douglas pers. comm.). The apparently basal position of *Bellendena* in Proteaceae raises the question of the evolution of its unique karyotype. Is this a very primitive genome in Proteaceae, or is it an ancient but specialised genome and relictual from a much larger and cytologically more diverse group? Karyotypic comparison of Bellendenoideae (*x*=5) with another cytologically distinctive subfamily Persoonioideae (*x*=7) may clarify aspects of the evolution of their respectively few but large chromosomes in relation to those of other Proteaceae (*x*=14,13,12,11,10). The genera of Proteaceae from Tasmania reported here with *x*=14, 13 and 5, in three subfamilies Grevillioideae, Proteoideae and Bellendenoideae, indicate that early Gondwanan radiations of the family were associated with considerable chromosomal evolution.

Acknowledgments

We thank RBG Hobart and Greg Jordan for supplying plant materials, and Andrew Douglas, Bill Jackson and René Vaillancourt for discussions.

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Manuscript received 26 February 1997
Manuscript accepted 15 August 1997