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Evolution of leaf anatomy in arid environments – A case study in southern African *Tetraena* and *Roepera* (Zygophyllaceae) ☆

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ABSTRACT

The dry biomes of southern Africa (Desert, Nama Karoo and Succulent Karoo) are home to a rich and diverse xerophytic flora. This flora includes two morphologically diverse clades of Zygophyllaceae, *Tetraena* and *Roepera* (Zygophylloideae), which inhabit some of the most arid habitats in the region. Using a plastid phylogeny of Zygophylloideae we assess whether the evolution of putatively adaptive traits (leaf shape, vasculature, mode of water storage and photosynthetic type: C_3 versus C_4) coincides with the successful colonisation of environments with different drought regimes within southern Africa. Our results show general niche conservatism within arid habitats in *Tetraena*, but niche shifts from arid to more mesic biomes with longer and/or cooler growing season (Fynbos and Thicket) in *Roepera*. However, these distinct broad-scale biogeographical patterns are not reflected in leaf anatomy, which seems to vary at more local scales. We observed considerable variability and multiple convergences to similar leaf anatomies in both genera, including shifts between "all cell succulence" leaf types and leaf types with distinct chlorenchyma and hydrenchyma. Our survey of C_4 photosynthesis in the Zygophylloideae showed that the C_4 pathway is restricted to *Tetraena simplex*, which also having an annual life history and a widespread distribution, is rather atypical for this group.

1. Introduction

Plant adaptations to drought are as diverse as the environments in which they occur and often involve several anatomical, morphological and physiological specialisations that help to save water (Chaves et al., 2003; Fahn and Cutler, 1992; Levitt, 1972; Ludlow, 1989). Patterns of precipitation, including its seasonality and predictability, can vary greatly across geographic regions, which have a profound impact on the actual drought stress and subsequently on the composition of floras. Within southern Africa, a number of distinct biomes are recognised that share similar vegetation structures driven to a large extent by macroclimatic patterns (Mucina and Rutherford, 2006). The more arid regions of southern Africa fall under the 'Desert', 'Nama Karoo' and 'Succulent Karoo' biomes that receive less than half the mean annual precipitation of adjacent 'Fynbos', 'Thicket', 'Savanna' and 'Grassland' biomes. The Nama Karoo and Succulent Karoo share a 2 354 km long gradual boundary and have relatively low mean annual precipitation, but the rainy season differs (Nama Karoo: 208 mm/year, concentrated during the late summer/early autumn; and Succulent Karoo: 168 mm/year, mostly during the winter months; Rutherford et al., 2006). Summer rainfall in an arid environment increases the impact of aridity, creating a more hostile environment in the Nama Karoo than in the Succulent Karoo. The much drier Desert biome (<70 mm/year) borders the northern ends of Succulent Karoo and Nama Karoo with a winter-rainfall region in the western part and a summer-rainfall region in the eastern part, respectively. All three arid biomes of southern Africa are nevertheless home to diverse and rich floras. These include some well-represented families that are adapted either to avoid the worst climatic extremes (e.g. annuality in many Asteraceae, Fabaceae and Poaceae) or to resist them, by means of morphological, anatomical or physiological innovations that help to minimise water loss, maximise water uptake and improve water storage (e.g. clades from Aizoaceae, Apocynaceae, Crassulaceae, Euphorbiaceae, Geraniaceae and Zygophyllaceae).

Species of Zygophylloideae are found in a range of different southern African biomes and show a range of different drought

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adaptation strategies, hence representing an ideal subject with which to investigate adaptation to arid environments (Van Zyl, 2000). The members of the subfamily are also distributed across southern arid areas of Madagascar, Northern Africa, Asia and Australia. Bellstedt et al. (2012) concluded, on the basis of biogeographic analyses, that the clade originated in southern Africa, where two clades are particularly diverse: *Tetraena* Maxim. and *Roepera* A. Juss. Phylogenetic analyses have been published of Zygophylloideae in general (Beier et al., 2003; Sheahan and Chase, 1996, 2000) and of these two groups in particular (Bellstedt et al., 2008, 2012). To infer evolutionary processes acting within these clades, improved representation of species and resolution of relationships between them is needed.

Zygophylloideae drought adaptations are diverse. The leaves are often succulent to a greater or lesser degree, but can also be flat and coriaceous (Nyffeler and Eggli, 2010; Sheahan, 2007; Fig. 1). Both can represent adaptations to resist water loss: the coriaceous leaves of for example *Roepera hirticaulis* (Van Zyl) Beier & Thulin seem well-adapted to extremely dry conditions in the Succulent Karoo (e.g. El-Hadidi, 1972; Schulte et al., 1989; Sheahan, 2007; Van Zyl, 2000). Water loss and excess light is avoided by steep leaf angles (heliotropism) and reduced leaf canopy area (Van Zyl, 2000). *Tetraena decumbens* (Delile) Beier & Thulin and *Roepera cordifolia* (L. f.) Beier & Thulin (Fig. 1F) for example orientate their leaflets in conduplicate pairs (i.e. shading each other) during the hottest part of the day (Van Zyl, 2000). There are numerous examples of partial or complete leaf shedding and rapid re-sprouting (e.g. *Tetraena qatarensis* (Hadidi) Beier & Thulin, Ismael, 1983; or *Roepera morgsana* (L.) Beier & Thulin, Fig. 1D; Le Roux et al., 1989), and even annual life history (in *Tetraena simplex* (L.) Beier & Thulin, Fig. 1E). These are also observed for example in Ruschieae (Aizoaceae; Klak et al., 2013), and might increase long-term survival as drought avoidance strategies, particularly in seasonally arid habitats such as in the Nama Karoo (Sheahan, 2007; Van Zyl, 2000).

C_4 photosynthesis (PS) is known from the Zygophylloideae (Sage et al., 2011), representing an adaptive trait under high selective pressure in extremely arid desert environments (Christin and Osborne, 2014). However, few species of the subfamily have been screened for C_4 PS and *Tetraena simplex* is the only C_4 species currently known (*Zygophyllum simplex* L.; Crookston and Moss, 1972). In species of Sesuvioideae (Aizoaceae; Bohley et al., 2015), Portulacaceae (Ocampo et al., 2013) and Chenopodiaceae (e.g. Schütze et al., 2003; Freitag and Kadereit, 2014; Kadereit et al., 2014), C_4 PS is distinctively associated with drought adaptive leaf anatomical

Fig. 1. Members of *Roepera* and *Tetraena*. (A) *Tetraena segmentatum*, near Rosh Pinah, Namibia (photo: D. Bellstedt), (B) *T. madagascariensis*, near Faux Cap, Madagascar (photo: P. Wester), (C) *T. coccinea*, characteristic bifoliate leaves, greenhouse at University Mainz, Germany (photo: M. Lauterbach), (D) *Roepera morgsana*, near Eendekuil, South Africa (photo: M. Lauterbach), (E) *T. simplex*, near Aussenkehr, Namibia (photo: D. Bellstedt), (F) *R. cordifolia*, South Africa (photo: D. Bellstedt, (G) *R. swartbergensis*, Swartberg Pass, South Africa (photo: D. Bellstedt).