

For Mary - thanks for all your help on this!  
With best regards  
Kathy

## A First Assessment of Genetic Variation in *Welwitschia mirabilis* Hook

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### Abstract

*Welwitschia mirabilis* is a monotypic member of the family Welwitschiaceae which, along with *Ephedra* and *Gnetum* species, comprises the gymnospermous order Gnetales. While the monophyly of this order is now widely accepted, the relationship of the Gnetales to other seed plants is still contentious. Despite the unique phylogenetic position of *W. mirabilis* and its extraordinary physiological and anatomical adaptations, little is known about the plant's phylogeny or its current distribution in isolated locations throughout the Namib Desert. As a preliminary step in the design of an more extensive phylogeographic study, we analyzed 37 random amplified polymorphic DNA (RAPD) loci from 59 plants distributed among five sites separated by distances of 6–440 km. Cluster analysis and analysis of molecular variance (AMOVA) revealed significant levels of variation within and between populations and little evidence of inbreeding. Genetic differences between populations reflect the geographic distances separating them. Three of the populations formed discernable genetic clusters, suggesting that little gene flow occurs between populations separated by  $\geq 18$  km. In contrast, gene flow is occurring between two populations separated by only 6 km, supporting previous observations that pollen dispersal is primarily local and that seeds are not readily windborne over the large distances separating most *W. mirabilis* populations. As a working hypothesis, we propose that *W. mirabilis* had a continuous distribution across its current range as much as 105 million years ago, and that as a consequence of subsequent drying trends and physical disturbance, populations became progressively isolated, accounting for their current distribution.

The important phylogenetic position, odd morphology, and unique ecological adaptations of *Welwitschia mirabilis* Hook. make it one of the most intriguing plant species on earth, and the subject of more than 250 articles published since Hooker's description in 1863 (Henschel and Seely 2000). Despite this attention, no studies have addressed micro-evolutionary or phylogeographical hypotheses regarding *W. mirabilis*. The species is endemic to arid and semiarid regions of western Namibia and Angola; individuals are among the oldest living plants, with ages estimated up to 3,000 years (Bornman et al. 1972; von Willert and Wagner-Douglas 1994). Extensive morphological and molecular phylogenetic studies confirm that the three genera comprising the Gnetales (*Welwitschia*, *Ephedra*, and *Gnetum*) are a monophyletic group [see the review by Price (1996)]. However, the relationship of Gnetales with other seed plants has been widely debated and is still not resolved. Phylogenetic studies in the mid-1980s and early-1990s using chloroplast genes and/or morphological characters suggested that Gnetales were a sister group to the angiosperms, and along with two fossil orders (Bennetiales and Pentoxylales) comprised the Anthophyte clade. Bootstrap values for this clade were, however, very low [see the review by Doyle (1996)], and recent work has now rejected the Anthophyte hypothesis

(Gugerli et al. 2001; Winter et al. 1999). From data derived from nuclear homeotic genes, mitochondrial genes, and larger molecular datasets, Gnetales are now thought to be a sister group to Pinaceae.

A classic phreatophyte, *W. mirabilis* plants in the Namib Desert meet their water needs by inhabiting ephemeral watercourses, accessing groundwater via an extensive deep root system (Bornman 1972; Eller et al. 1983; Giess 1969), and possessing xylem vessels that are highly efficient for transporting water (Muhammad and Sattler 1982). Transpirational water loss from this  $C_3$  plant (Eller et al. 1983; von Willert et al. 1982) is in excess of  $1 \text{ l/m}^2$  leaf surface/day (von Willert et al. 1982), approximately twice the quantity available in leaves at dawn (Eller et al. 1983), and high compared to other Namib Desert evergreen plants (von Willert et al. 1992). The only mechanism *W. mirabilis* uses to regulate water loss is stomatal closing during exceptionally hot, dry periods (von Willert and Wagner-Douglas 1994). While growth rates vary seasonally and in response to rain, growth of the large straplike leaves from the woody basal meristem is continuous throughout the life of the plant.

Despite high fertility (Bornman 1978; Bustard 1990), juvenile plants are rare. Seeds are frequently contaminated with a fungus that severely reduces viability (Cooper-Driver

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