Journal of Heredity 2003:94(3):212–217
DOI: 10.1093/jhered/esg051

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

K. M. Jacobson and E. Lester

From the Department of Biology, Grinnell College, Grinnell, IA 50112.

Address correspondence to Kathryn M. Jacobson at the address above, or e-mail: jacobsok@grinnell.edu.

Abstract

Welwitschia mirabilis is a monotypic member of the family Welwitchiaceae 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 (Bennetitales 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 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

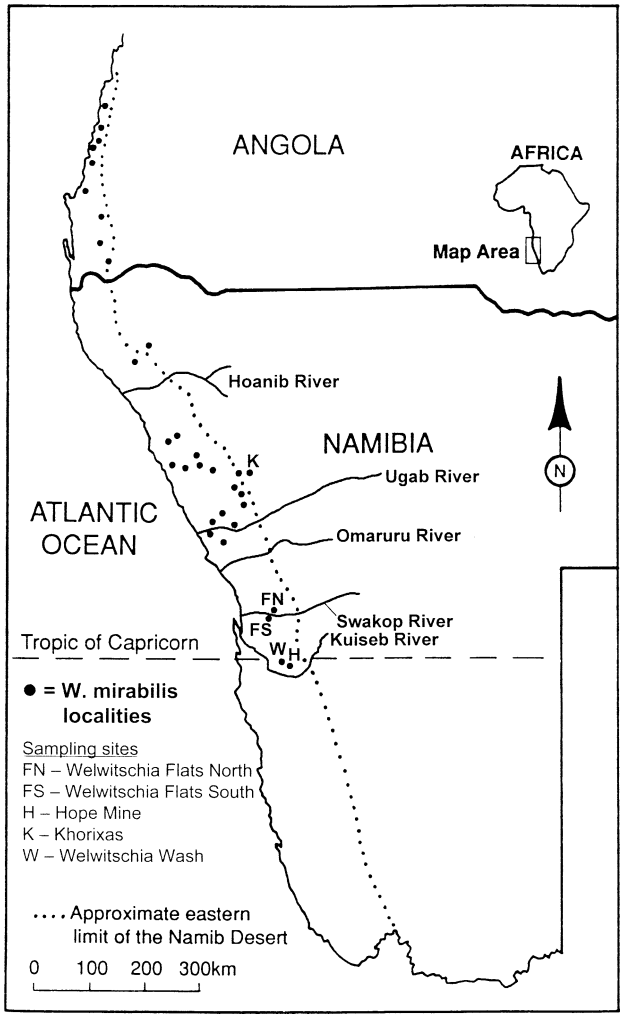


Figure 1. The distribution of *W. mirabilis* in Namibia and Angola according to Kers (1967). Samples were taken at labeled sites: W (Welwitschia Wash) and H (Hope Mine) along the Kuiseb River; FN (Welwitschia Flats North) and FS (Welwitschia Flats South); and K (Khorixas).

et al. 2000), and climatic events that favor seedling germination (rains in excess of 55 mm, or ephemeral stream flows) are infrequent throughout much of the plant's range. Populations are thus typically composed of obvious size cohorts representing several germination events occurring over the past millennium (Bornman 1972; Jürgens et al. 1997). When climatic conditions are appropriate, germination can occur in highly disturbed habitats such as streambeds in ephemeral watercourses and rocky scrapings from road works (Jacobson K, unpublished data). Recent studies have conclusively eliminated wind as a possible pollination vector, establishing flies as the predominant agent (Wetschnig and Depisch 1999). Seeds are winged, but being fairly large, probably serve only as agents of dispersal within populations (Henschel and Seely 2000).

The range of *W. mirabilis* extends over a 150,000 km^2 area along the southwestern Namib coast from the Nicolau River in southern Angola to the Kuiseb River and Central Namib Sand Sea in central Namibia (Figure 1). Rainfall throughout much of the species' range is less than 50 mm and populations comprising 2 to more than 2,000 plants (Henschel and Seely 2000) are thus typically found in and along ephemeral watercourses. In the northern part of the species' Namibian range, however, plants can also be found in semiarid mopane savanna where rainfall averages 150–200 mm/year (Jacobson et al. 1993a). In the south, within the Namib Naukluft Park, the locations and extent of geographically discrete populations are fairly well known. In contrast, this information is only partially known for populations north of the Omaruru River. Kers's (1967) map (Figure 1) suggests that plants occur in discrete populations throughout the range, but our preliminary mapping efforts in the extensive roadless regions between the Ugab and Hoanib Rivers revealed that a number of these supposedly geographically discrete northern populations are in fact continuous.

As a first step in the design of an extensive phylogeographic study of *W. mirabilis*, this article reports on genetic variation between and within five locations in Namibia, using random amplified polymorphic DNA (RAPD) markers. These polymerase chain reaction (PCR)-derived markers are obtained using 10-base primers that amplify random genomic fragments throughout the genome (Williams et al. 1990), thus providing an efficient first assessment of genomic variation (Weising et al. 1995). It was essential to use PCR-derived molecular markers for this first analysis because the plant's protected status required use of minimal amounts of leaf tissue. Herein we (1) describe genetic variation and calculate genetic diversity within each population; (2) use cluster analysis (NTSYS) to create a phenogram illustrating the distribution of variation among the populations; and (3) use analysis of molecular variance (AMOVA) to determine whether patterns exist regarding the distribution of variation within populations, between populations, and between regions. We interpret these results in light of Gnetalean palynology and the geological record of western Namibia.

Materials and Methods

Site Descriptions

The Welwitschia Wash (23°36′54.5″S, 15°10′45″E) and Hope Mine (23°34′273″S, 15°15′533″E) sites are located at the southern limit of the species' range, just to the north of the Kuiseb River in the Central Namib Desert (Figure 1). These sites, referred to collectively as the Kuiseb sites, are approximately 6 km apart (Henschel and Seely 2000). Welwitschia Wash is a fairly small, discrete population of roughly 250 plants located in a single ravine. Hope Mine is a larger and more dispersed population that extends over at least 10 km^2 in numerous ravines in rugged canyon habitat. Hope Mine samples were taken from a ravine similar to Welwitschia Wash, containing roughly 100 plants in relatively close proximity. Average rainfall at both Kuiseb sites is between 25–55 mm/year and other vegetation is sparse,

Table 1. Description of genetic variation and genetic diversity in *W. mirabilis* populations sampled using RAPD markers

Sites	Welwitschia Wash (W)	Hope Mine (H)	Welwitschia Flats North (FN)	Welwitschia Flats South (FS)	Khorixas (K)
N	15	12	10	14	8
No. of loci/population	37	37	37	37	37
Percent variable loci/population	35%	43%	51%	30%	43%
Percent invariable loci/population	65%	57%	49%	70%	57%
G [percent unique genotypes/population (PD)]	100	100	92	93	100
D (Simpson's index of diversity)	1.00	1.00	0.98	0.99	1.00
E (genotypic evenness)	1.00	1.00	1.00	1.00	1.00

comprised primarily of perennial species (e.g., *Adenolobus pechuellii* (Kuntze) Torre & Hille., *Orthanthera albida* Schinz., and *Sutera maxi* Boiss).

Plants at the Welwitschia Flats South (22°45.781′S, 14°56.501′E) and North (22°40.434′S, 14°58.888′E) sites are located in shallow dry watercourses on wide gravel plains. Average rainfall is approximately 25–50 mm/year. Other perennial vegetation, comprising *Zygophyllum stapffii* Schinz, *Asclepias buchenaviana* Schinz, and *Arthraerua leubnitziae* (Kuntze) Schinz, is extremely sparse. Welwitschia Flats South is approximately 180 km north of the Kuiseb sites, with no intervening populations of the plant. The two Welwitschia Flats sites are approximately 18 km apart and separated by the ephemeral Swakop River. Although *W. mirabilis* plants are found in this intervening distance, they are sparse (more than 200–500 m apart).

The fifth site (20°26.799′S, 14°35.524′E), located on the Great Escarpment approximately 35 km west of Khorixas, is 440 km north of the Welwitschia Flats sites. The Khorixas site (mean annual rainfall of 150–200 mm) is in mopane savanna comprised of extensive perennial and annual grass cover (Jacobson et al. 1993a) and two species of trees, *Colophospermum mopane* (Kirk ex Benth) Kirk ex J. Leonard and *Terminalia prunioides* C. Lawson, in addition to *W. mirabilis* plants which are abundant. This site is on the eastern edge of what may or may not be a large contiguous northern population (Figure 1; see Introduction) that occurs above and below the Great Escarpment. There are numerous well-known *W. mirabilis* locations (for example, Brandberg, Messum, Burnt Mountain) between the Khorixas site and the Welwitschia Flats sites to the south (Figure 1), but these northern sites are separated from the four southern sites by a minimum distance of 250 km.

Molecular Methods

Single tissue samples (1 cm^3) were collected in July 1999 from young portions of leaves located near the meristem of 8–15 male and female plants chosen at random from each of five locations. The sizes of the sampled plants, measured as the diameter of the long axis across the meristematic core, ranged from 8 to 200 cm. Tissue was stored in CTAB buffer held at –20°C to 4°C whenever possible until DNA was extracted. A portion of each sample was chopped into small pieces, frozen, and homogenized in fresh CTAB extraction buffer. DNA extraction and quantification was performed as described previously (Jacobson et al. 1993b).

RAPD reaction mixtures (25 μl) consisted of 9 ng genomic DNA, 25 mM $MgCl_2$, 250 μM each dATP, dTTP, dGTP, and dCTP, 0.5 U *Taq* DNA polymerase (Promega), and 47 ng of each 10-mer (Operon Technologies, Alameda, CA). Amplifications were performed in an MJ Research PTC-150 minicycler with an initial 5 min denaturation cycle at 95°C, followed by 45 cycles of 37°C for 1 min (annealing), 36°C for 1 min (polymerization), and 93°C for 1 min (denaturation). Amplified fragments were separated by gel electrophoresis in 0.7% agarose with 1% Synergel (Diversified Biotech) and 1 × TAE. Gels were run for 4 h at 100 mV and stained with ethidium bromide. In addition to running water controls with each reaction setup, all reactions were performed twice to confirm that RAPD markers scored were reproducible (Hadrys et al. 1992). The dataset analyzed for this initial study used two primers (OPA-3 and OPA-9).

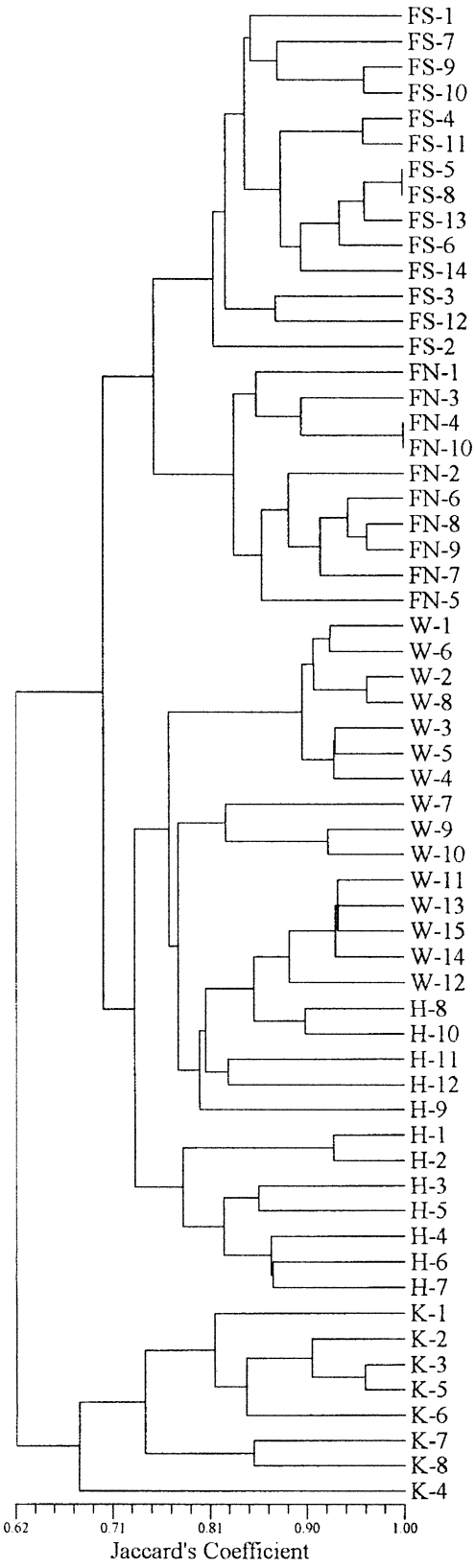
Bands generated from each sample were assigned a character state of 1 for presence or 0 for absence and organized in an Excel file. Data were imported into NTSYS (Rohlf 1988) and a similarity matrix was constructed using the Jaccard algorithm. A phenogram of the resulting matrix was generated using the unweighted pair group method (UPGMA), and the cophenetic correlation value was computed using the COPH function.

One drawback of RAPD markers is that their dominance precludes the use of *F* statistic-based analyses of population structure. Instead we used AMOVA (Excoffier et al. 1992), which has been used effectively to obtain a first assessment of genetic variation in other plant species using dominant RAPD markers (e.g., Buso et al. 1998; Gabrielsen and Brochmann 1998; Liao and Hsiao 1998). A distance matrix was also created using the Euclidean metric (Liao and Hsiao 1998), for use in AMOVA (Excoffier et al. 1992). AMOVA partitions the total genetic variation into specified hierarchical groupings. We conducted our AMOVA using three hierarchical levels: variation within populations, between populations, and between three regions [such as northern (Khorixas), south-central (Welwitschia Flats), and southern (Kuiseb)].

Results

Variation Within Populations

The two primers yielded 37 unambiguous RAPD loci from the 59 individuals, of which 30–51% were variable within each site (Table 1). The phenogram (Figure 2) based on



Jaccard similarities ($r = 0.80$, good fit) shows that there was considerable variation among the 59 plants. Likewise, AMOVA revealed that 64% of the total variation is accounted for within populations (Table 2), illustrating the high levels of genetic variation among individuals sampled. Populations were comprised primarily of plants with unique genotypes (Table 1), and only two pairs of plants had identical genotypes (FS 5 and 8, and FN 4 and 10) (Figure 2).

All estimates of genetic diversity within populations approach values of one (Table 1), further illustrating the genetically diverse structure of all five populations. Finally, PhiST, an analogue of the inbreeding coefficient F_{ST} (Excoffier et al. 1992), was low: 0.358 (Table 2).

Variation Between Populations

Plants from the Welwitschia Flats North and South sites and Khorixas form three site-specific genetic clusters in the phenogram (Figure 2). In contrast, plants from the two Kuiseb sites, Hope Mine and Welwitschia Wash, do not form discrete genetic clusters. Seven plants from Hope Mine form a cluster sharing 75% similarity, but five other plants cluster with Welwitschia Wash plants, sharing approximately the same level of similarity (74%) with these plants. The phenogram thus suggests that the Welwitschia Wash and Hope Mine sites might not be isolated from each other. Results from the AMOVA do not support this conclusion, however. The between-population differentiation among the five sampling sites is highly significant ($p < .001$) (Table 2). According to this analysis, despite considerable variation within each population, the five sampling sites are isolated from one another.

While partitioning the total variance among the three hierarchical levels using AMOVA confirmed that most of the variance is due to differences within populations (64%), the proportional variances attributed to within-region (24%) and between-region differences (12%) are also highly significant ($p < .001$) (Table 2). Reflecting the within-region analysis, the phenogram shows that the two Kuiseb populations form a cluster (72% similar), as do the Welwitschia Flats populations (73% similar). In addition, populations from these two southern regions form a distinct genetic cluster, separate from the northern Khorixas population (supporting the between-region AMOVA analysis).

Discussion

This study of five locations revealed high levels of genetic diversity within populations and across the sampled portion

←

Figure 2. Phenogram generated using UPGMA, based on the similarity matrix using Jaccard's algorithm. Data used are 37 RAPD markers for *W. mirabilis* populations at Welwitschia Wash (W), Hope Mine (H), Welwitschia Flats North (FN) and South (FS), and Khorixas (K).

Table 2. AMOVA of 37 RAPD loci for 59 *W. mirabilis* plants from five sites within three regions: northern (Khorixas site), south-central (two Welwitschia Flats sites), and southern (two Kuiseb sites)

Source of variation	df	SSD	MSD	Variance component	Percent total variance	P^a	Phi statistics	Bartlet's statistics
Global								
Between regions	2	580.1	290.1	0.19	11.89	<.001	$\Phi_{CT} = 0.119$	2.17[b]
Populations/regions	2	377.6	188.8	0.39	23.93	<.001	$\Phi_{SC} = 0.272$	7.85[b]
Plants/populations	2178	2335.0	1.0	1.04	64.18	<.001	$\Phi_{ST} = 0.358$	
Within regions								
Between populations	4	957.7	239.4	0.53	33.96	<.001		
Within populations	2178	2335.0	1.0	1.04	66.04	<.001		
Between regions								
Between regions	2	580.1	290.0	0.42	26.00	<.001		
Within regions	2182	2712.6	1.2	1.20	74.00	<.001		

[a] Nonparametric randomization test (1,000 permutations).

[b] Significant: $P < .0001$.

of the species' range. The hierarchical analysis performed by AMOVA, as well as the cluster analysis, clearly showed that genetic differences between the populations studied reflect current geographic distances separating them. Despite the high levels of genetic variation, populations at three of the sites are discrete and readily discernable genetic entities. These data suggest minimal gene flow has occurred between populations separated by distances as small as 18 km, consistent with previous observations that pollen dispersal is primarily local (Wetschnig and Depisch 1999) and that the large, albeit winged, seeds are not readily windborne over the large distances currently separating the southern, central, and northern populations. In contrast, gene flow could be occurring between the two Kuiseb populations that are separated by only 6 km. Here, five plants from Hope Mine are more genetically similar to plants from Welwitschia Wash than to other plants at Hope Mine.

The Geological and Paleoclimatic Context for Subsequent Studies

Palynological studies suggest a peak in Gnetalean diversity in western Africa and eastern South America in the late Cretaceous, at what is now approximately the equator (20 degrees south of the equator then) (Crane and Lidgard 1989). It is thus plausible to hypothesize (in the absence of any data to the contrary) that proto-Welwitschia was present in this mesic region of Gondwanaland as early as the Cretaceous. Given that this phreatophyte possesses a number of enigmatic characteristics not typically associated with desert habitats (e.g., high rainfall requirements for germination, C_3 metabolism, and excessive transpirational water loss and associated high daily water demands), and grows continuously and lusciously when provided with warm and moist conditions in greenhouses (van Jaarsveld 1990), it is plausible that earlier habitats of *W. mirabilis* were more mesic than the current Namib habitat.

During the Cretaceous, plate tectonics radically transformed the landscape and climate of southwestern Africa [see the review by Jacobson et al. (1995)]. Volcanic activity in the northern part of the current range of *W. mirabilis* during the early Cretaceous resulted in massive lava flows. These flows covered large regions of what is now Brazil and northwestern Namibia (Martin 1961) prior to the separation of Africa and South America approximately 135 million years ago. The remainder of the Cretaceous was a period of erosion in western Namibia in association with the upheaval of the Great Escarpment (Ward 1987) and presumed high rainfall in the region. However, as the continents continued to separate, cold offshore currents pushing up from the south Atlantic began to dominate the regional weather patterns, as they do today, and the climate became more arid (Ward and Corbett 1990). Other than three brief periods of semiarid to temperate climate 8–12, 20–24, and 26–32 thousand years ago, all evidence suggests continuous arid conditions throughout the Tertiary and Quatenary (Lancaster 2002).

Our preliminary results support a working hypothesis that attributes the current fragmented distribution of *W. mirabilis* to aridification during the Tertiary and Quatenary, limiting the species' range from a widespread and contiguous paleodistribution throughout the region to locations with adequate moisture resources. Given that *W. mirabilis* is currently found both on top of and below the escarpment, and its pollination biology precludes long-distance dispersal, we propose further that the current range of the species may already have been established prior to the upheaval and down-cutting in this region more than 105 million years ago. While this study provides a first assessment of genetic variation in extant *W. mirabilis* populations, the high levels of RAPD marker polymorphism found preclude their use for effectively testing the proposed phylogeography that spans more than 100 million years. Studies are thus under way to test our hypothesis using loci that are evolving at appropriate rates, a more intensive sampling effort within populations,

and more widespread sampling of plants in the northern part of the range.

Acknowledgments

We gratefully acknowledge the Namibian Ministry of Environment and Tourism for granting permission to sample from *W. mirabilis* and to work within the Namib-Naukluft and Skeleton Coast Parks. Thanks also to the staff at the National Botanical Research Institute of Namibia for providing useful comments on our research proposal; the Desert Research Foundation of Namibia for critical logistical support for the fieldwork; Grinnell College and the Woodrow Wilson Foundation for financial support; and P. Jacobson, B. Schaal, M. Seely, and two anonymous reviewers for helpful comments on various drafts of the manuscript.

References

Bornman CH, 1972. *Welwitschia mirabilis*: paradox of the Namib Desert. Endeavor 31(113):95–99.

Bornman CH, 1978. *Welwitschia*: paradox of a parched paradise. Cape Town, South Africa: C. Struik.

Bornman CH, Elsworthy JA, Butler V, and Botha CEJ, 1972. *Welwitschia mirabilis*: observations on general habit, seed, seedling and leaf characteristics. Madoqua 1:53–66.

Buso GSC, Rangel PH, and Ferreira ME, 1998. Analysis of genetic variability of South American wild rice populations (*Oryza glumaepatula*) with isozymes and RAPD markers. Mol Ecol 7:107–117.

Bustard L, 1990. The ugliest plant of the world: the story of *Welwitschia mirabilis*. Kew Magazine 7:85–90.

Cooper-Driver GA, Wagner C, and Kolberg H, 2000. Patterns of *Aspergillus niger* var. *phoenicis* (Corda) Al-Musallam infection in Namibian populations of *Welwitschia mirabilis* Hook.f. J Arid Environ 46:181–198.

Crane PR and Lidgard S, 1989. Angiosperm diversification and paleolatitudinal gradients in Cretaceous floristic diversity. Science 246:675–678.

Doyle JA, 1996. Seed plant phylogeny and the relationships of Gnetales. Int J Plant Sci 157:S3–S39.

Eller BM, von Willert DJ, Brinkmann E, and Baasch R, 1983. Ecophysiological studies on *Welwitschia mirabilis* in the Namib Desert. S Afr J Bot 2:209–223.

Excoffier L, Smouse PE, and Quattro JM, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Gabrielsen TM and Brochmann C, 1998. Sex after all: high levels of diversity detected in the arctic clonal plant *Saxifraga cernua* using RAPD markers. Mol Ecol 7:1701–1708.

Giess W, 1969. *Welwitschia mirabilis* Hook. fil. Dinteria 3:3–55.

Gugerli F, Sperisen C, Buchler U, Brunner I, Brodbeck S, Palmer JD, and Qui YL, 2001. The evolutionary split of Pinaceae from other conifers: evidence from and intron loss and a multi-gene phylogeny. Mol Phylogenet Evol 21:167–175.

Hadrys H, Balick M, and Schierwater B, 1992. Applications of randomly amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol 1: 55–63.

Henschel JR and Seely MK, 2000. Long-term growth patterns of *Welwitschia mirabilis*, a long-lived plant of the Namib Desert (including a bibliography). Plant Ecol 150:7–26.

Hooker JD, 1863. On *Welwitschia*, a new genus of Gnetaceae. Trans Linn Soc 24:1–48.

Jacobson KM, Jacobson PJ, and Miller OK, 1993a. The mycorrhizal status of *Welwitschia mirabilis*. Mycorrhiza 3:13–17.

Jacobson KM, Miller OK, and Turner BJ, 1993b. RAPD markers are superior to somatic incompatibility tests for discriminating genotypes in natural populations of the ectomycorrhizal fungus *Suillus granulatus*. Proc Natl Acad Sci USA 90:9159–9163.

Jacobson PJ, Jacobson KM, and Seely MK, 1995. Ephemeral rivers and their catchments: sustaining people and development in western Namibia. Windhoek, Namibia: Desert Research Foundation of Namibia.

Jürgens N, Burke A, Seely MK, and Jacobson KM, 1997. Desert. In: Vegetation of southern Africa (Cowling RM, Richardson DM, and Pierce SM, eds). Cambridge: Cambridge University Press; 189–214.

Kers LE, 1967. The distribution of *Welwitschia mirabilis*. Hook. F Svensk Bot Tidskrift 61:97–125.

Lancaster N, 2002. How dry was dry? Late Pleistocene palaeoclimates in the Namib Desert. Q Sci Rev 21:769–782.

Liao LC and Hsiao JY, 1998. Relationship between population genetic structure and riparian habitat as revealed by RAPD analysis of the rheophyte *Acorus gramineus* Soland. (Araceae) in Taiwan. Mol Ecol 7:1275–1281.

Martin H, 1961. The hypothesis of continental drift in the light of recent advances of geological knowledge in Brazil and in South West Africa. Geol Soc S Afr 64:1–47.

Muhammad AF and Sattler R, 1982. Vessel structure of *Gnetum* and the origin of angiosperms. Am J Bot 69:1004–1021.

Price RA, 1996. Systematics of the Gnetales: a review of morphological and molecular evidence. Int J Plant Sci 157:S40–S49.

Rohlf FJ, 1988. NTSYS-pc, numerical taxonomy and multivariate analysis system, version 1.40 Setauket, NY: Exeter.

van Jaarsveld E, 1990. The cultivation and care of *Welwitschia mirabilis*, the extraordinary caudiciform from the Namib Desert. Aloe 27:69–82.

von Willert DJ, Eller BM, Brinckmann E, and Baasch R, 1982. CO_2 gas exchange and transpiration of *Welwitschia mirabilis* Hook. f. in the central Namib Desert. Oecologia 55:21–29.

von Willert DJ, Eller BM, Werger MJA, Brinkmann E, and Ihlenfeldt HD, 1992. Life strategies of succulents in deserts. Cambridge Studies in Ecology. Cambridge: Cambridge University Press.

von Willert DJ and Wagner-Douglas U 1994. Water relations, CO_2 exchange, water use efficiency and growth of *Welwitschia mirabilis* Hook. f. in three contrasting habitats of the Namib Desert. Bot Acta 107:291–299.

Ward JD, 1987. The Cenozoic succession in the Kuiseb Valley, central Namib Desert. Geol Survey SW Africa/Namibia 9:1–124.

Ward JD and Corbett I, 1990. Towards an age for the Namib. In: Namib ecology: 25 years of Namib research, Transvaal Museum Monograph no. 7 (Seely MK, ed). Pretoria: Transvaal Museum; 17–26.

Weising K, Nybom H, Wolff K, and Meyer W, 1995. DNA fingerprinting in plants and fungi. Ann Arbor, MI: CRC Press.

Wetschnig W and Depisch B, 1999. Pollination biology of *Welwitschia mirabilis* Hook. f. (Welwitschiaceae, Gnetopsida). Phyton 39:167–183.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, and Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535.

Winter KU, Becker A, Munster T, Kim JT, Saedler H, and Theisen G, 1999. MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. Proc Natl Acad Sci USA 96:7342–7347.

Received July 23, 2002
Accepted January 30, 2003

Corresponding Editor: Jonathan F. Wendel