Genetic diversity in the monospecific Western Australian endemic, *Geleznovia verrucosa* Turcz. (Rutaceae)

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Allozyme diversity was surveyed at 16 loci across 16 populations of the monospecific, but morphologically diverse, endemic shrub *Geleznovia verrucosa* Turcz. (Rutaceae). Single-locus diversity measures portrayed *G. verrucosa* as a genetically depauperate genus (A , 1.4; P , 29.6%), with total genetic diversity (H_T , 0.304) partitioned between populations (D_{ST} , 0.175), rather than within (H_S , 0.129). Some 58% of the total genetic diversity was attributable to interpopulational differences. Although restricted distribution, bottlenecks and/or founder effects and small population size have probably contributed to the low levels of genetic diversity found within this genus, it is evident from this investigation that the genus is not monospecific. The partitioning of genetic diversity, and possible differences in reproductive strategy, suggest that the small- and large-flowered forms represent distinct taxa. Higher levels of divergence between the intermediate-form populations and increased heterozygosity at the *Lap* locus suggest that this form is of hybrid origin and has arisen through a series of hybrid events between the small and large forms.

Keywords: allozymes, disjunct populations, endemic, *Geleznovia verrucosa*, hybridization, population genetics.

Introduction

Geographically restricted species are often characterized by lower genetic diversity than more widely distributed species. This diversity is, however, often apportioned in much the same manner as that of more widespread species (Karron, 1987; Hamrick & Godt, 1989). Small population size, reduced gene flow, inbreeding, and founder effects all contribute to the lower genetic diversity of endemic taxa (Hamrick & Godt, 1989).

The Western Australian flora, particularly that of the south-west region, is distinguished by high species diversity and endemism (Hopper, 1979). Many evolutionary patterns occur across the landscape. Refugial species endure in the higher rainfall areas, whereas fragmented relictual species and suites of newly derived taxa exist in the more arid areas (Hopper, 1979). Many species are rare and locally endemic, the product of a long and complex evolution that has generated many distinct genetic systems (Hopper, 1979). Some of the

more well-known mechanisms include intraspecific chromosome variation (James, 1965; Coates & James, 1979), polyploidy (Bousfield & James, 1976), dysploidy (James, 1981; Stace, 1995) and complex hybridity (James, 1970).

The levels and distribution of genetic diversity within Western Australian endemics do not always concur with expectations. For example, although some rare and localized species such as *Banksia cuneata* (Coates & Sokolowski, 1992) and *Eucalyptus caesia* (Moran & Hopper, 1983) exhibit low levels of genetic diversity, other narrow endemics such as *Acacia anomala* (Coates, 1988) and *Stylidium coroniforme* (Coates, 1992) do not.

Genetic diversity has also been found to be apportioned both within (Coates & Hnatiuk, 1990; Byrne & James, 1991) and between (Moran & Hopper, 1983; Sampson *et al.*, 1988) populations. This genetic variability probably reflects the complex evolutionary history of the area, where recently evolved taxa coincide with more ancient relictual taxa.

Geleznovia verrucosa Turcz. (yellow bells) is a little-known woody shrub, endemic to the sand plains of Western Australia. It occurs as small disjunct popula-

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tions from Cape Range (21°50'S, 116°12'E) in the north of the state, to Dowerin (31°12'S, 117°02'E) in the eastern wheat belt (Hnatiuk, 1990; Keighery & Gibson, 1993). Although considered monospecific, *G. verrucosa* is morphologically diverse, with populations ranging from a taller (1–2 m) form with abundant, large flowers and leaves, to populations comprising shorter plants (0.5–1 m) with small, often solitary, flowers and leaves. Between these forms are a number of variable populations exhibiting intermediate characters. Although the different forms rarely co-occur, when they do, morphological integrity is generally maintained.

This research was initiated to investigate patterns of genetic diversity within and between the disjunct and morphologically variable populations of *G. verrucosa*, and to determine whether the different forms are genetically distinct and represent different taxa.

Materials and methods

Sampling strategy

Sixteen populations from within the known distribution of *G. verrucosa* and representing the three morphological forms (small, intermediate and large) were selected for electrophoretic study (Fig. 1). Where possible, 20

plants at each population were mapped, tagged and the total number of plants counted/estimated. To compare genetic variation within the unusually large Hutt River population, two sites of 20 plants $\approx$ 500 m apart were selected. At Pleshkes, where the small and large forms co-occurred, 10 plants from each form were selected, as well as 20 plants along a transect across the integration zone between them. Inflorescences were collected from tagged plants during 1995, placed in sealed plastic bags and transported to the laboratory at 4°C, where buds of a similar age were frozen at –72°C. Allozyme electrophoresis was carried out on anther material from these buds.

Electrophoresis

Anthers were ground with 30 μ L of modified extraction buffer (Systma & Schaal, 1985) (50 mg mL⁻¹ PVP, 0.5 mM NAD, 0.3 mM NADP, 1.7 mM EDTA, 1.2 mM ascorbic acid, 0.1% BSA, 10% sucrose w/v and 0.7 mg mL⁻¹ dithiothreitol in 0.1 M Tris-HCl, pH 7), centrifuged for 1.5 min and 12 μ L of extract transferred to sample wells from which 1 μ L was loaded onto presoaked cellulose acetate plates (Titan III, Helena Laboratories, Beaumont, TX). The plates were transferred to running tanks maintained at 5°C and electrophoresed at 5 mA/gel. Running times varied from 30 to 50 min depending upon the enzyme system under analysis.

Eleven enzyme systems were assayed (Table 1). All systems were run in an 80 mM Tris-EDTA-maleic acid buffer (80 mM Tris, 1 mM Na₂EDTA, 1 mM MgCl₂, 6.4 mM histidine, 13.3 mM glycine, 3.4 mM glutamic acid, 3.7 mM aspartic acid, pH to 7.6 with maleic acid then pH to 8.1 with sodium hydroxide) except *Aat*, which was run in Tris-citrate buffer pH 8.1 (100 mM

Fig. 1 Distribution of *Geleznovia verrucosa* populations. Symbols: $\blacktriangle$ small, $\bullet$ intermediate, $\blacksquare$ large, $\blacklozenge$ mixed forms and transect. Population codes: A, Arinya; AW1, Ajana West 1; AW2, Ajana West 2; C1, Coorow 1; C2, Coorow 2; G, Geraldton; HH, Hawks Head; HR, Hutt River (sites 1 and 2); IR, Indarra Reserve; M, Maiseys; MH1, Meanarra Hill 1; MH2, Meanarra Hill 2; N, Nanns; P, Pleshkes (PL, large form; PH, transect; PS, small form); RB, Red Bluff; WB, West Binnu.

Table 1 Enzyme systems examined for allozyme electrophoresis

Enzyme	Abbreviation	EC code
Acid phosphatase	<i>Acp</i>	3.1.3.2
Aspartate aminotransferase	<i>Aat</i>	2.6.1.1
Esterase	<i>Est</i>	3.1.1.1
Glucose-6-phosphate isomerase	<i>Gpi</i>	5.3.1.9
Isocitrate dehydrogenase	<i>Idh</i>	1.1.1.42
Leucine aminopeptidase	<i>Lap</i>	3.4.11.1
Malate dehydrogenase	<i>Mdh</i>	1.1.1.37
Menadione reductase	<i>Mdr</i>	1.6.99.22
Phosphoglucumutase	<i>Pgm</i>	5.4.2.2
Phosphogluconate dehydrogenase	<i>6Pgd</i>	1.1.1.44
Shikimate dehydrogenase	<i>Skd</i>	1.1.1.25

Tris, 10 mM citric acid). Staining procedures followed Richardson *et al.* (1986), except that 4 mL of stain buffer were used with stain ingredients proportionally increased. Sixteen zones of activity were scored for nine enzymes with each zone assumed to represent a single enzyme locus: *Aat-1*, *Aat-2*, *Aat-3*, *Aat-4*, *Aat-5*, *Est-1*, *Gpi-1*, *Idh-1*, *Lap-1*, *Mdh-1*, *Mdh-2*, *Mdr-1*, *Mdr-2*, *Mdr-3*, *Pgm-1* and *Skd-1*. Alleles were designated by enzyme system, locus and allele. For example, *Aat-1^c* is allele *c* at locus 1 for aspartate aminotransferase.

Genetic analyses

The mean number of alleles per locus (A), proportion of polymorphic loci (P , 0.99 criterion), observed (H_o) and expected heterozygosity (H_e) were calculated using BIOSYS-1 (Swofford & Selander, 1981). Wright's (1978) F_{IS} , which is a measure of heterozygote deficiency or excess, was calculated using POPGENE version 1.2 (Yeh *et al.*, 1997). Total genetic diversity and its distribution within and between populations was estimated using Nei's (1973) gene diversity statistics and calculated using GENESTAT (Whitkus, 1985). Total gene diversity (H_T) was partitioned into mean gene diversity within (H_S) and between (D_{ST}) populations. The proportion of interpopulation differentiation (G_{ST}) was determined by $G_{ST} = D_{ST}/H_T$. Nei's (1972) genetic distance was used to generate the cluster analysis with standard error bars based on the unweighted pair-group average method using arithmetic means (UPGMA) with Ritland's (1989) Genetic Distance and Clustering (GD) program.

Results

Genetic variation within and among populations

Allelic frequencies varied among populations (Table 2). Although only three monomorphic loci were observed (*Idh-1*, *Mdh-2* and *Mdr-1*), in many populations, loci were either homozygous or the alternative alleles were at very low frequencies (e.g. *Aat-1^c* at Nanns, *Gpi-1^d* at Ajana West 2, *Gpi-1^b* at Coorow 1 and *Mdr-3^b* at Hutt River 2).

Single-locus diversity measures were also low (Table 3). The mean number of alleles per locus (A) was 1.4 (range 1.1–1.8), and the mean percentage of polymorphic loci (P) was 29.6% (range 6.3% to 62.5%). Observed heterozygosity (H_o , 0.055) was considerably lower than expected (H_e , 0.097). Populations exhibited marked differences in single-locus diversity measures. Heterozygous individuals were absent from the Ajana West 2, Hawks Head and Coorow 2 populations, and very low levels of heterozygosity characterized those at Ajana West 1 (0.006), Arinya (0.011), Coorow 1 (0.017),

Maiseys (0.016) and Hutt River 2 (0.022), where individuals with similar genotypes dominated. In contrast, populations at Meanarra Hill, Red Bluff and West Binu exhibited higher levels of diversity and approached panmixia. These results are also reflected in the F_{IS} values which varied in a similar manner across populations (range –0.025 to 0.387).

Genetic variation within and among forms

Forms also exhibited marked differences in single-locus diversity measures (Table 3). The percentage of polymorphic loci was lower in the small (26.1%) and intermediate (23.2%) forms than in the large form (36.3%). The large form also approached panmixia, whereas observed heterozygosity was considerably lower than that expected in the other two forms. This was again reflected by the fixation indices for each form.

Some loci showed fixed allelic or highly significant allele frequency differences among the morphological forms. Alleles *Aat-2^a* and *Aat-4^a* were restricted to the large form, except for the small form at Pleshkes, where both forms co-occurred (Table 2). The allele *Aat-3^a* was similarly restricted to the large form, except for small-form populations at Indarra Reserve and Pleshkes.

The distribution of alleles at Pleshkes where the small and large forms co-occurred strongly suggests a zone of hybridization. The small form at this site exhibited unique alleles *Aat-1^b* and *Aat-3^c* as well as other alleles restricted to the large form (*Aat-2^a*, *Aat-4^a*, *Aat-5^b* and *Aat-5^c*), indicating that introgression between the forms has been, and probably still is, occurring (Broadhurst, unpubl. obs.).

The mean total genetic diversity (H_T) was 0.304, with diversity distributed among populations (D_{ST} , 0.175) rather than within them (H_S , 0.129) (Table 4). The proportion of genetic diversity among populations (G_{ST}) was very high at 0.576, indicating that 58% of the total diversity was attributable to interpopulation differentiation.

Gene diversity statistics generated according to morphological forms showed that the mean total genetic diversity (H_T) of the small form was lower (0.192) than that of the two other forms, with more of the diversity apportioned within populations (H_S , 0.122) rather than among them (D_{ST} , 0.070) (Table 4). Interpopulation differentiation accounted for 36% of this variation. Although genetic diversity measures for the large form were somewhat higher (H_T , 0.254), this form also partitioned genetic diversity within populations (H_S , 0.164) rather than among them (D_{ST} , 0.090), with similar interpopulation differentiation (G_{ST} , 35%). Total gene diversity in the intermediate form (H_T , 0.226) was similar to the large form but was apportioned

Table 2 Allele frequencies of polymorphic loci for the 19 *Geleznovia verrucosa* sites. See Fig. 1 for population codes

Locus	Allele	Small						Intermediate							Large					TR
		A	C1	C2	IR	M	PS	AW1	AW2	HH	HR1	HR2	N	WB	G	MH1	MH2	PL	RB	PH
<i>Aat-1</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	0.20	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	0.88
	<i>b</i>	–	–	–	–	–	0.80	–	–	–	–	–	–	–	–	–	–	–	–	0.15
	<i>c</i>	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–	–	–	–
<i>Aat-2</i>	<i>a</i>	–	–	–	–	–	0.20	–	–	–	–	–	–	–	1.00	1.00	0.83	1.00	0.35	0.75
	<i>b</i>	–	–	–	0.30	–	0.30	–	–	1.00	–	–	–	–	–	–	0.11	–	0.60	0.20
	<i>c</i>	1.00	1.00	1.00	0.70	1.00	0.50	1.00	1.00	–	1.00	1.00	1.00	1.00	–	–	0.06	–	0.05	0.05
<i>Aat-3</i>	<i>a</i>	–	–	–	0.10	–	0.40	–	–	–	–	–	–	–	1.00	1.00	1.00	0.70	0.45	0.70
	<i>b</i>	1.00	1.00	1.00	0.90	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	–	0.30	0.55	0.30
	<i>c</i>	–	–	–	–	–	0.10	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Aat-4</i>	<i>a</i>	–	–	–	–	–	0.50	–	–	–	–	–	–	–	1.00	0.72	0.78	0.80	0.60	0.85
	<i>b</i>	–	–	–	0.21	–	0.30	1.00	–	0.72	–	–	–	–	–	0.28	0.22	0.20	0.35	0.15
	<i>c</i>	1.00	1.00	1.00	0.78	1.00	0.20	–	1.00	0.28	1.00	1.00	1.00	1.00	–	–	–	–	0.05	–
<i>Aat-5</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	0.58	0.90	0.88	0.78
	<i>b</i>	–	–	–	–	–	0.11	–	–	–	–	–	–	–	–	0.10	0.31	0.10	0.12	0.14
	<i>c</i>	–	–	–	–	–	0.22	–	–	–	–	–	–	–	–	–	0.11	–	–	0.08
<i>Est-1</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	0.50	1.00	0.88	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	–	1.00	0.35
	<i>b</i>	–	–	–	–	–	0.50	–	0.12	–	–	–	–	–	–	–	1.00	1.00	–	0.65
<i>Gpi-1</i>	<i>a</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–	–	–
	<i>b</i>	–	0.05	–	–	–	0.22	–	–	–	0.12	–	0.43	0.42	0.30	0.37	0.50	–	0.15	0.20
	<i>c</i>	1.00	0.95	1.00	1.00	1.00	0.78	1.00	0.94	1.00	0.88	1.00	0.57	0.58	0.60	0.58	0.50	1.00	0.85	0.80
	<i>d</i>	–	–	–	–	–	–	–	0.06	–	–	–	–	–	0.10	–	–	–	–	–
<i>Lap-1</i>	<i>a</i>	–	–	–	0.10	–	–	0.68	0.83	0.72	0.06	0.15	0.70	0.16	1.00	–	–	–	0.05	–
	<i>b</i>	1.00	1.00	1.00	0.48	1.00	0.75	0.21	0.17	0.17	0.58	0.72	0.18	0.42	–	0.79	0.67	1.00	0.80	0.90
	<i>c</i>	–	–	–	0.42	–	0.25	0.11	–	0.11	0.36	0.13	0.12	0.42	–	0.21	0.33	–	0.15	0.10
<i>Mdh-1</i>	<i>a</i>	0.11	0.53	0.06	–	–	–	–	–	–	–	–	–	1.00	–	0.03	–	–	–	0.05
	<i>b</i>	0.89	0.47	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	–	1.00	0.97	1.00	1.00	1.00	0.95
<i>Mdr-2</i>	<i>a</i>	0.79	0.84	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	<i>b</i>	0.21	0.16	–	–	–	–	–	–	–	–	0.17	0.02	–	–	–	–	–	–	–
<i>Mdr-3</i>	<i>a</i>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	<i>b</i>	–	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–	–	–
<i>Pgm-1</i>	<i>a</i>	–	–	–	–	–	–	0.95	0.39	1.00	–	0.16	0.43	0.37	0.05	–	–	–	0.20	–
	<i>b</i>	1.00	1.00	0.71	1.00	1.00	1.00	0.05	0.61	–	1.00	0.84	0.57	0.63	0.95	0.89	0.94	1.00	0.80	1.00
	<i>c</i>	–	–	0.29	–	–	–	–	–	–	–	–	–	–	–	0.11	0.06	–	–	–
<i>Skd-1</i>	<i>a</i>	0.33	0.61	0.20	0.26	–	–	–	–	–	–	–	–	–	0.16	0.11	0.06	–	0.15	0.05
	<i>b</i>	0.67	0.39	0.80	0.74	0.87	0.94	1.00	1.00	1.00	0.72	0.44	0.47	0.77	0.84	0.56	0.56	0.75	0.82	0.85
	<i>c</i>	–	–	–	–	0.13	0.06	–	–	–	0.28	0.56	0.53	0.23	–	0.33	0.38	0.25	0.03	0.10

TR, Transect.

Table 3 Single-locus diversity measures based on 16 loci at 19 *Geleznovia verrucosa* sites. See Fig. 1 for population codes

Form	Population and mean	<i>N</i>	<i>A</i>	<i>P</i>	<i>H</i> _o	<i>H</i> _e	<i>F</i> _{IS}
Small	A	16.2 (0.6)	1.2 (0.1)	18.8	0.011 (0.011)	0.063 (0.036)	0.154 (0.031)
	C1	19.4 (0.3)	1.3 (0.1)	25.0	0.017 (0.009)	0.086 (0.044)	0.152 (0.030)
	C2	19.4 (0.3)	1.2 (0.1)	18.8	0.000 (0.000)	0.054 (0.033)	0.188 (0.041)
	IR	19.5 (0.2)	1.4 (0.2)	31.3	0.053 (0.053)	0.122 (0.051)	0.222 (0.057)
	M	19.9 (0.1)	1.1 (0.1)	6.3	0.016 (0.016)	0.014 (0.014)	-0.010 (0.000)
	PS	9.5 (0.2)	1.8 (0.2)	56.3	0.047 (0.032)	0.261 (0.068)	0.387 (0.068)
	Mean	17.3 (0.3)	1.3 (0.1)	26.1	0.024 (0.020)	0.100 (0.041)	0.182 (0.038)
Inter	AW1	17.4 (0.7)	1.2 (0.1)	12.5	0.006 (0.006)	0.036 (0.031)	0.060 (0.016)
	AW2	17.9 (0.1)	1.3 (0.2)	25.0	0.000 (0.000)	0.069 (0.036)	0.250 (0.050)
	HH	18.4 (0.3)	1.2 (0.1)	12.5	0.000 (0.000)	0.054 (0.037)	0.125 (0.029)
	HR1	19.1 (0.3)	1.3 (0.1)	18.8	0.081 (0.049)	0.073 (0.042)	-0.021 (0.003)
	HR2	19.1 (0.3)	1.4 (0.2)	31.3	0.022 (0.016)	0.105 (0.044)	0.243 (0.039)
	N	20.7 (0.1)	1.4 (0.2)	37.5	0.071 (0.037)	0.133 (0.055)	0.172 (0.030)
	WB	18.8 (0.1)	1.3 (0.2)	25.0	0.135 (0.075)	0.124 (0.057)	-0.025 (0.029)
	Mean	18.8 (0.3)	1.3 (0.2)	23.2	0.045 (0.026)	0.085 (0.043)	0.115 (0.028)
Large	G	19.8 (0.1)	1.3 (0.2)	18.8	0.038 (0.038)	0.058 (0.033)	0.118 (0.030)
	MH1	18.7 (0.1)	1.6 (0.2)	43.8	0.105 (0.056)	0.146 (0.053)	0.150 (0.035)
	MH2	17.8 (0.1)	1.5 (0.2)	43.8	0.170 (0.082)	0.179 (0.059)	0.050 (0.060)
	PL	9.7 (0.2)	1.3 (0.1)	25.0	0.031 (0.031)	0.085 (0.040)	0.167 (0.044)
	RB	19.9 (0.1)	1.8 (0.2)	50.0	0.169 (0.053)	0.189 (0.053)	0.011 (0.020)
	Mean	17.2 (0.1)	1.5 (0.2)	36.3	0.103 (0.052)	0.131 (0.048)	0.099 (0.038)
Transect	PH	19.4 (0.3)	1.8 (0.2)	62.5	0.066 (0.028)	0.193 (0.045)	0.381 (0.060)
Mean		17.9	1.4	29.6	0.055	0.097	0.146

Inter, Intermediate; *N*, sample size per locus; *A*, average no. of alleles per locus; *P*, % polymorphic loci per population; *H*_o, observed heterozygosity; *H*_e, expected panmictic heterozygosity; *F*_{IS}, fixation index; SE in parentheses.

among populations (*D*_{ST}, 0.121) rather than within them (*H*_S, 0.105). These intermediate-form populations, with the highest interpopulational differentiation (*G*_{ST}, 54%), contributed significantly to the high overall interpopulation differentiation encountered within the complex, and to the differentiation between the small and large forms.

Table 4 Gene diversity statistics over all loci unbiased for sample size and population number for *Geleznovia verrucosa* forms and all populations

Form	<i>H</i> _T	<i>H</i> _S	<i>D</i> _{ST}	<i>G</i> _{ST}
Small	0.192	0.122	0.070	0.363
Intermediate	0.226	0.105	0.121	0.536
Large	0.254	0.164	0.090	0.354
Mean	0.304	0.129	0.175	0.576

*H*_T, total gene diversity; *H*_S, mean gene diversity within populations; *D*_{ST}, mean diversity between populations; *G*_{ST}, proportion of interpopulation differentiation.

Genetic divergence among populations

Average genetic distance (*D*) within the small and large forms was low, 0.071 and 0.070, respectively, but higher within the intermediate form (0.112). Distances between the intermediate and large forms were considerably higher (0.301) than those between the small and large forms (0.223) and the small and intermediate forms (0.122).

Relationships among populations based on estimates of genetic distance (*D*) are summarized in Fig. 2. Two distinct clusters were observed. The initial dichotomy separated the large-form populations at Pleshkes, Meanarra Hill, Red Bluff and Geraldton as a distinct group, within which the small form at Pleshkes was a discrete entity. The transect at Pleshkes (Pleshkes Hybrid) was also included in this group. The second group consisted of both the small and intermediate forms. All small-form populations, with the exception of Pleshkes Small, were clustered together with the intermediate form at Hutt River.

Fig. 2 Cluster analysis of *Geleznovia verrucosa* populations based on Nei's genetic distance (D) and UPGMA algorithm. Population clusters are statistically significant if the shaded bar is less than half the branch length (Ritland, 1989). Population and symbol codes in Fig. 1.

Discussion

Endemism and *G. verrucosa*

The levels of genetic diversity in *G. verrucosa* (P , 29.6%; A , 1.4) were consistent with those observed at the population level in other endemic taxa (P , 26.3%; A , 1.4; Hamrick & Godt, 1989). With few exceptions, *G. verrucosa* populations are small and disjunct, predisposing them to limited gene flow, genetic drift and fixation (Loveless & Hamrick, 1984; Hamrick & Godt, 1989). Such conditions are highly conducive to creating the population genetic structure and the low levels of genetic diversity observed. The extremely low levels of allozyme polymorphism exhibited by some populations, dominance of others by relatively few genotypes and significant population differentiation suggest that other factors also contribute to genetic diversity within *G. verrucosa*.

Bottlenecks, both natural and artificial, and/or associated founder effects may play a significant role in the patterns of genetic diversity observed in *G. verrucosa*. A recent investigation has highlighted this genus to be a disturbance opportunist, with smoke being an important germination factor (Dixon *et al.*, 1995; Roche, 1998). In addition, seed bank trials indicated that little seed remains in the seed soil bank after one year and that ants quickly predate much of the annual production (Roche, 1998). Under these circumstances, seedling recruitment following disturbance is likely to be from

limited seed resources primarily produced in the previous season, effectively generating natural bottlenecks.

Several of the *G. verrucosa* populations may have also experienced artificial bottlenecks. Populations at Hawks Head, Geraldton and Nanns occur in disturbed habitats, those at Ajana West, Coorow, Indarra Reserve and West Binu populations are located on road verges, and at Pleshkes and Hutt River the populations are periodically disturbed when commercial cut-flower production falls. Many of these populations exhibited low levels of genetic diversity and departure from Hardy–Weinberg equilibrium (Table 3).

Levels of diversity within and among forms of *G. verrucosa*

The level of genetic divergence between the large form and the small and intermediate forms, the presence of alleles characterizing the various forms, and disparate apportioning of genetic diversity within the three forms, is highly suggestive of systematic separation. Differences in both the levels of genetic diversity and the fixation indices (F_{IS}) further suggest that contrasting reproductive strategies are employed, but insufficient levels of polymorphism among progeny have not allowed useful estimates of outcrossing to be made.

The low levels of genetic diversity and homozygote excesses observed in the small form are consistent with expectations of self-compatible species (Hamrick &

Godt, 1989). Other characters such as smaller less attractive flowers, abundant seed and distribution peripheral to that of the large form (Grant, 1975; Solbrig & Rollins, 1977; Cruden & Lyon, 1985; Ritland & Ritland, 1989), are further evidence of a selfing reproductive strategy. In contrast, the large form exhibits levels of genetic diversity concordant with predominantly outcrossed species (Hamrick & Godt, 1989), has larger and more abundant flowers and sets less seed, characteristic of an outcrossing, or at least mixed-mating reproductive strategy (Solbrig & Rollins, 1977; Lyons & Antonovics, 1991). Intermediacy in floral characters and gene diversity indices for the intermediate form suggest that this may be of hybrid origin between the small and large forms.

The apportioning of genetic diversity in the intermediate form strongly supports the notion of hybrid ancestry. The moderate total genetic diversity of the small and large forms resided within populations with relatively low levels of interpopulational differentiation. Such genetic diversity could readily be expected to reside within populations when taxa are compared. In contrast, the higher total genetic diversity of the intermediate form was apportioned between populations with 54% of the total genetic diversity attributable to interpopulational differences. This greater genetic diversity and population differentiation could reflect hybridization events generating a series of diverse hybrid products.

The relationships between populations based on genetic distances further suggest that the intermediate form is of hybrid origin and that several independent hybridization events have occurred (Fig. 2). For example, although the Ajana West populations are only 3 km apart, Ajana West 1 was closely allied with Hawks Head, 5 km west, whereas Ajana West 2 was closely related to Nanns, located 50 km south. The former populations are linked by the allele *Aat-4^b*, exhibited by most of the large-form populations, whereas the latter populations share the *Aat-4^c* allele, common in the small and intermediate forms. The presence of these contrasting alleles despite close proximity in the Ajana West populations may reflect separate hybridization events.

The major argument against the intermediate form being of hybrid origin is the disjunct nature of extant populations. The present distribution patterns indicate that little opportunity for hybridization exists, but such population disjunction may not always have been the case. *Geleznovia verrucosa* occurs within the transitional-rainfall zone, a species-rich region with a high number of infraspecific variants, cryptic species complexes and natural hybrids (Hopper, 1979, 1992). Plant evolution in this region is considered to have been largely driven by small population size, induced by recurrent late Tert-

ary-Quaternary events, in a heterogeneous soil mosaic (Hopper, 1992). Range expansion and contraction during these perturbations may have brought about a series of temporally and spatially distributed hybridization events, generating many novel genetic arrays and apportioning genetic diversity between rather than within the resultant hybrid populations. Unique selection within each location would further contribute to population differentiation.

This investigation has revealed the contribution of restricted distributions, population size, disjunction and bottlenecks to genetic diversity within *G. verrucosa*. More significantly, it has highlighted an enigmatic species complex comprised of two taxa, with strong evidence to suggest the evolution of a third through hybridization. Morphometric and reproductive analyses provide further evidence of systematic separation (Broadhurst, unpubl. obs.). Given the past climatic and geological history of the south-west of Western Australia, hybridization may be a much more significant feature of this flora than previously thought.

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