CSIRO PUBLISHING
Australasian Plant Pathology, 2008, **37**, 154–161 www.publish.csiro.au/journals/app

Population structure of the fungal pathogen *Holocryphia eucalypti* in Australia and South Africa

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Abstract. *Holocryphia eucalypti* is a fungal pathogen that causes stem cankers on *Eucalyptus* species in South Africa and Australia. In South Africa it is considered opportunistic but in Australia it has been associated with occasional but serious disease problems. The aim of this study was to determine the genetic structure of a South African population of *H. eucalypti* and compare it with three Australian populations of the fungus. Seventy-two isolates from *Eucalyptus* spp. and clones in South Africa were compared with 30 isolates from *E. globulus* and 24 isolates from *Corymbia calophylla* in the south of Western Australia and 23 isolates from *E. dunnii* in eastern Australia. DNA of these isolates was amplified using eight pairs of microsatellite markers previously developed for *H. eucalypti*. Nei's gene diversity (H) showed that the eastern Australian population is the most genetically diverse and the Western Australian populations from *Corymbia* and *Eucalyptus* are somewhat less diverse. The South African population displayed the lowest genetic diversity. The high genetic diversity in the Australian populations supports the view that *H. eucalypti* is native to that region and was introduced into South Africa.

Additional keywords: bark canker, disease, diversity, forestry.

Introduction

Holocryphia eucalypti, previously known as *Cryphonectria eucalypti* (Venter *et al*. 2002; Gryzenhout *et al*. 2006), is a fungal pathogen causing a stem canker disease on *Corymbia* spp. and *Eucalyptus* spp. in mainland Australia (Walker *et al*. 1985; Davison and Coates 1991; Carnegie 2007), Tasmania (Yuan and Mohammed 1997; Wardlaw 1999) and *Eucalyptus* spp. in South Africa (Van der Westhuizen *et al*. 1993). In Australia, *H. eucalypti* causes bark cracks, cankers, dieback of coppice shoots and in severe cases, tree death has been reported (Walker *et al*. 1985; Old *et al*. 1986; Wardlaw 1999; Jackson 2004; Carnegie 2007). In South Africa, infection typically results in superficial cracks in the bark and, only occasionally, severe cankers have been reported under environmental conditions stressful to the trees (Gryzenhout *et al*. 2003). Kino exudation or damage to the cambium is rarely observed in South Africa. However, *H. eucalypti* has been found on *E. smithii* near Pietermaritzburg (KwaZulu/Natal province) where cankers extended into the cambium (Gryzenhout *et al*. 2003).

Eucalyptus species were introduced to South Africa from Australia in the early 1800s and there are now over 400 000 ha of these trees commercially propagated in plantations in the country (Anon. 2006). *E. grandis* is most commonly planted, while in subtropical areas, clones of hybrids between *E. grandis* and *E. urophylla* or *E. camaldulensis* are commonly planted. Breeding programs are used to improve wood quality and growth rates as well as resistance to pests and diseases (Denison and Kietzka 1993; Wingfield and Roux 2000).

Management and control of plantation diseases has been widely achieved via breeding for disease resistant species, hybrids and clones (Wingfield *et al*. 2001; Wingfield 2003). In order to effectively manage hybrids with different levels and sources of resistance, it is important to understand the genetic diversity of the pathogen population. This knowledge provides insight into the capability of the pathogen to overcome host resistance (McDonald and McDermott 1993; McDonald and Linde 2002). Processes such as mutation, gene flow, reproduction/recombination, population size as well as selection, result in increased diversity in a pathogen population (Taylor *et al*. 1999; McDonald and Linde 2002).

Molecular markers can give an indication of the processes occurring in populations of pathogens such as *H. eucalypti* and they can often provide insight into the origin of a pathogen, which contributes to quarantine legislation (Milgroom and Fry 1997). Co-dominant markers have been effectively applied in population genetic studies due to their high level of polymorphism and reproducibility (McDonald 1997). Microsatellite markers have been widely used to examine diversity, mode of reproduction, gene flow and speciation in many fungi (McDonald 1997; Burgess *et al*. 2004*a*, 2004*b*; Barnes *et al*. 2005).

 10.1071/AP08004 0815-3191/08/020154

H. eucalypti was first found in Australia and is commonly found on native trees in that country (Walker *et al*. 1985; Davison and Coates 1991). For this reason it is assumed that the fungus in South Africa has an Australian origin (Nakabonge *et al*. 2005). However, there are no experimental data to support this view and nothing is known regarding the genetic diversity of the fungus in either country. Thus, the aim of the present study was to determine the population diversity of *H. eucalypti* in South Africa, using polymorphic microsatellite markers recently developed (Nakabonge *et al*. 2005) for this fungus.

Materials and methods

Fungal isolates

H. eucalypti was isolated from trees showing typical canker symptoms associated with this fungus (Fig. 1). All isolates (Table 1) were from individual trees growing either in plantations or natural forests in Australia and South Africa. Seventy-two isolates were obtained from *Eucalyptus* trees growing in plantations in South Africa, from an area of ~1000 km^2. Twenty-three isolates were obtained from plantation-grown *E. globulus* in Western Australia (WA) from an area of ~3000 km^2. Thirty isolates were obtained from native *C. calophylla* in WA from an area of similar size. Twenty-four isolates were obtained from eucalypt plantations in two eastern states of Australia (New South Wales, Queensland) and in the Australian Capital Territory.

Isolations

Isolates from South African trees were obtained from pieces of bark bearing fungal fruiting structures, which were placed in moist chambers to induce sporulation. Droplets of spores were picked up with a sterile needle and spread onto 2% malt extract agar (MEA) (20 g/L malt extract and 20 g/L agar, Biolab, Midland, Johannesburg) with 100 mg/L streptomycin sulfate (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany). After overnight incubation, single germinating spores were picked up using sterile needles and grown on fresh plates. Isolates of *H. eucalypti* from Australia (Table 1) were collected during routine surveys. Fruiting structures of the fungus are much less common in Australia than in South Africa, particularly in WA where only the asexual state of the fungus is found. Isolates from Australia were obtained by placing pieces of wood from the edges of cankers directly onto half-strength potato dextrose agar (one-half PDA; Becton, Dickinson and Co., Sparks, MD, USA). Fruiting structures of *H. eucalypti* form rapidly on this medium and pure cultures were obtained by streaking spores from a single structure onto fresh plates. Pure cultures of all isolates are maintained in the Culture Collection (CMW) of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa.

DNA extraction and microsatellite PCR

Isolates were grown in Petri plates on MEA at 26°C for 7 days. The mycelium was scraped from the plates, transferred to 1.5-mL Eppendorf tubes and DNA extracted as previously described by Nakabonge *et al*. (2005). Eight pairs of primers (Table 2), previously developed by Nakabonge *et al*. (2005) for *H. eucalypti*, were used to amplify the preferred microsatellite regions. The PCR reaction mixes and conditions were the same as those described by Nakabonge *et al*. (2005). The DNA concentrations of the PCR products were assessed visually by comparison with a known concentration of a 100-base pair marker

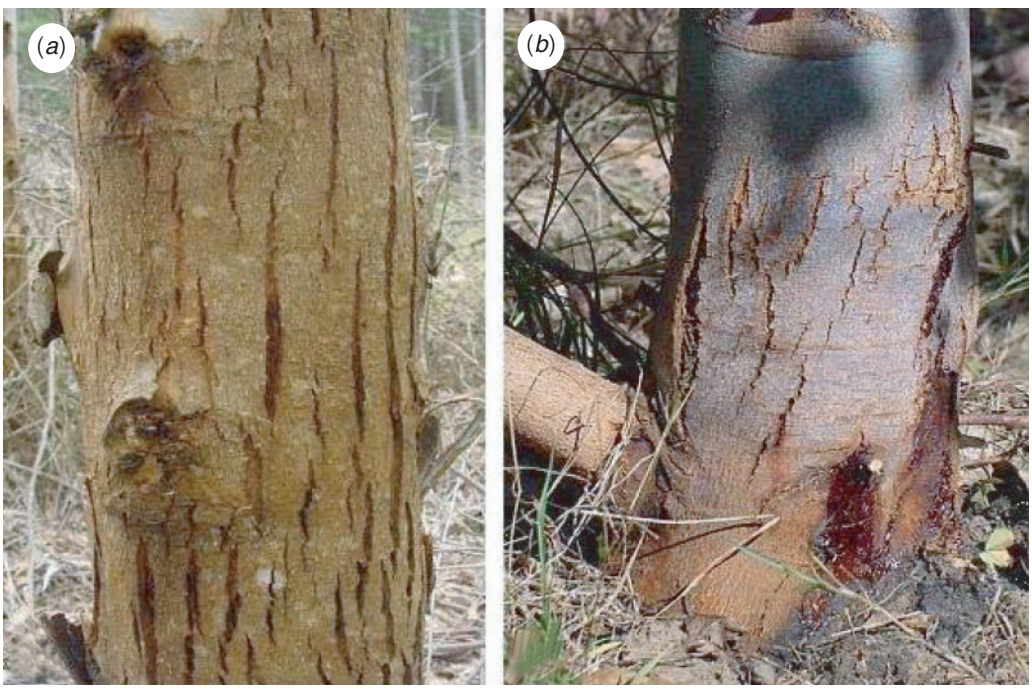

Fig. 1. Symptoms associated with *Holocryphia eucalypti* infection. (*a*) Cracks on *Eucalyptus* bark in South Africa. (*b*) Cracks and cankers on *E. dunnii* in eastern Australia.

Table 1. Isolates of *Holocryphia eucalypti* from South Africa and Australia used in this study

Isolate numbers (CMW)[A]	Origin	Code for origin	Host	Collector
18970, 18971, 18972, 18974, 18975, 18976, 19158, 19159	Nyalazi, KZN, South Africa	N	*Eucalyptus* (GC/GU clones)[B]	M. Gryzenhout
18985, 18977, 18986–18995, 18998, 18999–19002, 19021–19032, 19160–19162, 7034, 7035, 8541	KZN, South Africa	KZN	*Eucalyptus* (GC/GU clones)	M. Gryzenhout
18983, 18984, 18996, 18997, 19164, 19165, 19033, 18973, 18978, 18979, 18980–18982	Mpumalanga, South Africa	MPUM	*Eucalyptus* (GC/GU clones)	M. Gryzenhout
19003–19006, 19163, 19007–19020	Tzaneen, South Africa	TZ	*E. saligna*	M. Gryzenhout
15172, 15174	Albany, Western Australia (WA)	ALB	*E. globulus*	T. Jackson
15187–15191	Augusta, WA	AUG	*E. globulus*	T. Jackson
15167, 15168, 15173–15179	Brunswick Junction, WA	BJ	*E. globulus*	T. Jackson
15198, 15193–15197	Bunbury, WA	BUN	*E. globulus*	T. Jackson
15182–15186	Denmark, WA	DEN	*E. globulus*	T. Jackson
15180, 15181	Esperance, WA	ESP	*E. globulus*	T. Jackson
7038	Denmark, WA	DEN	*E. globulus*	M.J. Wingfield
15142–15148, 15153, 15154, 15156–15158, 15160–15164	Manjimup, WA	MAN	*Corymbia calophylla*	T. Paap
15166, 15165	Perth, WA	PER	*C. calophylla*	T. Paap
15152	Albany, WA	ALB	*C. calophylla*	T. Paap
15159, 15149–15151, 15155,	Denmark, WA	DEN	*C. calophylla*	T. Paap
6240, 6241, 6242	Canberra, Eastern Australia	ACT	*Eucalyptus* sp.	M.J. Wingfield
6268, 6673, 6683, 6687, 6693, 6695–6697	New South Wales, Eastern Australia	NSW	*Eucalyptus* sp.	M.J Wingfield
18689–18700	Brisbane, Eastern Australia	Qld	*E. dunnii*	G.Whyte

[A]CMW refers to the culture collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.
[B]GC, *E. grandis* × *E. camaldulensis* hybrid clone. GU, *E. grandis* × *E. urophylla* hybrid clone.

Table 2. Microsatellite DNA markers used to amplify South African and Australian populations of *Holocryphia eucalypti*

Primer pair	Fluorescent label	Sequence	PCR product size (base pairs)
5A-FF	NED	GGT CCA TCA GTC GTC TCA GC	240–336
5A-RR		GCA GCA ATG AGG TGC CTT GG	
7A-FF	VIC	CCT GAC AGA GAA GCG ACC CT	190–219
7A-RR		GCA TCA GCT CAG GGC ATA GAG	
9A-FF	VIC	CTG CTG ACA AGG ACG AGG AC	256–292
9A-RR		CGT TTC GTG GCT GGA TCT CG	
10A-FF	PET	CTC TTG CAG CCT CGG AGA CTG	388–403
10A-RR		GAG TGG CCA TAT TCA GCT TGG C	
5B-FF	NED	GTG TCG TCG CTC GCG AAT AG	342–377
5B-RR		CAG GAG AGG ACA TGC GAG AC	
2B-FF	PET	GCC CAA AGG ATG TGT GAA TGT G	216–232
2B-RR		CAA ACT GGC GGA TGA CAG GC	
1B-FF	6-FAM	GCA TCT CAA CAG TGC ACT CCA G	185–198
1B-RR		CAC ATA CAC TCT CAT AGC TCT CGG	

(Roche Molecular Biochemicals, Mannheim, Germany) on a 2% agarose gel stained with ethidium bromide, exposed to UV illumination.

PCR products were diluted for Genescan analysis based on the approximate sizes of the PCR products and the type of fluorescent label attached to the primers. Allele sizes were estimated by comparing the mobility of the microsatellite products with that of a LIZ 500 size standard (Applied Biosystems, Warrington, UK). Genescan analysis was executed using an ABI Prism 3100 DNA sequencer (Perkin-Elmer, Warrington, UK). The allele sizes for the DNA fragments were determined using a combination of the GeneScan 2.1 analysis software (Applied Biosystems) and GeneMapper (Applied Biosystems).

Genetic diversity and population differentiation

Isolates were scored based on allele size at each locus. This information was used to generate a multilocus profile or haplotype for each isolate. Identical haplotypes were treated as clones and removed and statistics were calculated for clone-corrected populations. Allele frequencies in each population were then calculated by dividing the number of times an allele occurred in the population by the population sample size. The

allele frequencies were used to calculate the gene diversity (Nei 1973), $H = 1 - \sum_k x_k^2$, where x_k is the frequency of the kth allele for each population using the program POPGENE (Version 1.31; Yeh *et al*. 1999). Differences in allele frequencies for clone-corrected populations were estimated by Chi-square tests (χ^2) (Workman and Niswander 1970). Allele frequencies of populations from the two hosts from WA were compared. Populations from South Africa, eastern Australian and WA were also determined to assess the level of gene diversity within these populations and the level of population differentiation between them.

Population differentiation (G_{ST}), as measured by theta (Weir 1996), was calculated between all pairs of clone-corrected populations in Multilocus (Version 1.3; Agapow and Burt 2001). The statistical significance was determined by comparing the observed G_{ST} value to that of 1000 randomised datasets in which individuals were randomised among the populations being compared. The number of migrants (M) that must be exchanged between populations for each generation, to give the observed G_{ST} value, was calculated using the equation $M = [(1/\theta) - 1)]/2$ (Cockerham and Weir 1993).

Genotypic diversity was calculated using the formula $G = 1/\Sigma [f_x (x/n)^2]$, where, n is the sample size and f_x is the number of genotypes (haplotypes) occurring $\times$ times in the population and G being the effective number of equally frequent haplotypes (Stoddart and Taylor 1988). The genotypic diversities between populations was compared by obtaining the maximum percentage of genotypic diversity using the formula $\hat{G} = G/N \times 100$, where N is the sample size (McDonald *et al*. 1994).

Genetic distance

The genetic distance between all *H. eucalypti* haplotypes from Australia and South Africa was calculated based on Nei's (1972) unbiased genetic distance. The distance matrix was generated

Table 3. Allele size (base pairs) and frequency at eight loci for clone-corrected populations of *Holocryphia eucalypti* from Western Australia on *Corymbia calophylla* (WAC) and *Eucalyptus globulus* (WAE), Eastern Australia (EA) and South Africa (RSA)

N, number of isolates; N(g), number of multilocus haplotypes; G, genotypic diversity (Stoddart and Taylor 1988); $\hat{G}$, percent maximum diversity

Locus	Allele length	Allele frequencies				Locus	Allele length	Allele frequencies			
		WAC	WAE	EA	RSA			WAC	WAE	EA	RSA
1B	185	–	0.083	0.214	–	7A	190	–	–	0.214	–
	191	0.833	0.833	0.357	–		196	–	–	–	0.077
	196	–	0.042	0.286	–		198	–	0.167	–	0.077
	198	0.167	0.042	0.143	1.000		203	–	–	–	0.846
2B	216	–	–	0.071	–		208	–	–	0.286	–
	218	–	–	–	1.000		211	0.222	0.208	–	–
	220	–	0.042	–	–		213	–	0.083	0.357	–
	222	0.833	0.875	–	–		215	0.111	0.042	–	–
	224	–	0.042	0.857	–		219	0.667	0.500	0.143	–
	232	0.167	0.042	0.071	–	8A	238	0.056	–	–	–
5A	240	0.056	0.042	–	–		250	–	–	0.286	–
	243	–	0.042	–	–		255	–	–	0.071	0.077
	250	–	–	0.071	–		257	0.111	0.042	0.071	0.231
	256	–	0.083	0.286	–		259	0.778	0.792	–	0.692
	259	–	–	0.286	–		262	–	0.042	–	–
	261	–	0.042	–	–		264	–	0.042	0.071	–
	265	0.167	0.042	–	0.077		276	–	0.042	–	–
	267	0.167	0.375	–	–		277	–	0.042	0.500	–
	269	0.333	0.208	0.143	–	9A	256	–	–	–	0.077
	271	0.111	0.083	–	–		260	0.056	–	–	–
	273	0.056	–	–	–		267	–	0.042	–	–
	279	0.056	0.042	–	–		277	0.772	0.667	0.286	–
	283	0.056	0.042	0.214	–		278	0.222	0.208	0.286	–
	320	–	–	–	0.462		284	–	0.042	0.214	–
	322	–	–	–	0.308		289	–	0.042	0.214	–
	336	–	–	–	0.154		292	–	–	–	0.923
5B	342	0.222	0.167	–	–	10A	388	0.056	0.125	–	–
	344	0.611	0.500	0.071	–		390	0.944	0.750	0.429	–
	358	0.056	0.083	–	–		392	–	0.042	–	–
	368	0.111	0.083	–	–		399	–	–	–	1.000
	373	–	0.042	–	–		403	–	0.083	0.571	–
	375	–	0.083	0.571	0.846	N(g)		18	24	14	13
	377	–	0.042	0.357	0.154	N		24	30	23	72
						No. alleles		28	45	30	17
						No. unique alleles		21		6	9
						G		15.15	16.69	10.04	2.55
						$\hat{G}$ (%)		63.15	55.65	43.67	3.55

using the program POPGENE (Version 1.31) and a tree constructed using UPGMA (unweighted pair-group method with arithmetic mean) in MEGA (Version 2.1; Kumar *et al.* 2001).

Results

Genetic diversity

A total of 28 alleles were amplified across the eight loci for the WA population from *C. calophylla* and 45 alleles for the population from *E. globulus*. Thirty alleles were amplified in the eastern Australian population and only 17 alleles were amplified from the South African population (Table 3). Locus 5A was the most polymorphic with a total of 16 alleles and locus 1B was the least polymorphic with a total of four alleles. The South African population was monomorphic at three loci. There were 21 unique alleles among the WA populations, the majority of which were rare (only occurring in one isolate); however, allele 222 at locus 2B was common as was allele 267 at locus 5A, allele 342 at locus 5B and allele 211 at locus 7A. There were six unique alleles in the eastern Australian population and allele 259 at locus 5A and alleles 190 and 208 at locus 7A had a frequency of greater than 25%. Of the nine unique alleles in the South African population, three had a frequency of greater than 90% and all except two were common (Table 3). In WA, more alleles were found in the *E. globulus* population. However, of the 28 alleles found in the *C. calophylla* population, 25 were also present in the *E. globulus* population and the three that differed were of very low frequency. Twenty-three of the 30 alleles present in the eastern Australian population were also present in WA, although the frequencies were very different (Table 3). Only 45% of the alleles found in the South African isolates were found in Australia and at very different frequencies. Monomorphic loci and unique alleles affect gene diversity, which was high for the Australian populations and low for the South African population (Tables 4 and 5).

A total of 69 haplotypes were obtained when the three *H. eucalypti* populations from Australia and South Africa were combined. However, three were shared between the WA population from *C. calophylla* and the WA population from *E. globulus*, thus there was a total of 66 unique haplotypes. No haplotypes were shared between regions. The maximum genotypic diversity was 63.2% for the WA population from *C. calopylla*, 55.7% from *E. globulus*, 43.7% for the eastern Australian population and 3.6% for the South African population (Table 3).

Table 4. Gene diversity (H) and contingency χ^2 tests for differences in allele frequencies for the eight polymorphic microsatellite loci across clone-corrected populations of *Holocryphia eucalypti* collected from *Corymbia calophylla* and *Eucalyptus globulus* in Western Australia
There was no significant difference between allele frequencies at any loci

Locus	Gene diversity (H)		χ^2	d.f.
	Corymbia	*Eucalyptus*		
1B	0.28	0.30	3.2	3
2B	0.28	0.23	3.9	3
5A	0.81	0.79	3.2	10
5B	0.56	0.70	5.7	6
7A	0.49	0.67	4.3	4
8A	0.38	0.36	3.6	6
9A	0.43	0.51	8.4	5
10A	0.10	0.41	3.6	3
–	–	–	–	–
No. of haplotypes	18	24	–	–
Mean	0.41	0.50	–	–

Genetic differentiation and gene flow

The χ^2 tests for the eight microsatellite regions showed no significant difference in allele frequency at any loci between the WA population of *H. eucalypti*, which originated from two different, but closely related genera (Table 4). For the purposes of analysis, the lack of significant difference implies that these isolates can be combined to give a single population from WA. Conversely, when the populations from the different regions (Australia and South Africa) were compared, χ^2 tests were highly significant at all loci (Table 5). This is reflected in the G_{ST}, a statistic used to measure population differentiation. G_{ST} values were highly significant when comparing the populations from different regions (Australia and South Africa) and gene flow was very low (Table 6).

Genetic distance

The UPGMA tree constructed from the matrix obtained using Nei's (1972) genetic distance clearly separated the South African population from the Australian populations (Fig. 2). There was no

Table 5. Gene diversity (H) and contingency χ^2 tests for differences in allele frequencies for the eight polymorphic microsatellite loci across clone-corrected populations of *Holocryphia eucalypti* collected from Western Australia (WA), Eastern Australia (EA) and South Africa (RSA)

Locus	Gene diversity (H)			χ^2	d.f.
	WA	EA	RSA		
1B	0.31	0.72	0.00	53.5[A]	6
2B	0.27	0.25	0.00	121.0[A]	10
5A	0.83	0.76	0.66	99.9[A]	30
5B	0.67	0.54	0.26	55.9[A]	12
7A	0.65	0.72	0.27	97.4[A]	16
8A	0.36	0.65	0.46	55.4[A]	16
9A	0.50	0.75	0.14	82.4[A]	14
10A	0.31	0.49	0.00	89.4[A]	8
–	–	–	–	–	–
No. of haplotypes	39	14	13	–	–
Mean	0.49	0.61	0.23	–	–

Table 6. Pairwise comparisons of population differentiation, G_{ST}, (above the diagonal) and number of migrants, M, (below the diagonal) among clone-corrected populations of *Holocryphia eucalypti* collected from Western Australia (WA), Eastern Australia (EA) and South Africa (RSA)

	WA	EA	RSA
WA	–	0.328[A]	0.551[A]
EA	1.024	–	0.490[A]
RSA	0.407	0.520	–

[A]Indicates significant G_{ST} values, $P < 0.001$.

grouping of isolates according to the areas, plantations and plots sampled (Fig. 2). The majority of isolates from eastern Australia formed a distinct clade. However, one isolate from eastern Australia was placed in the predominantly WA clade and two WA isolates were grouped within the predominantly eastern Australian clade.

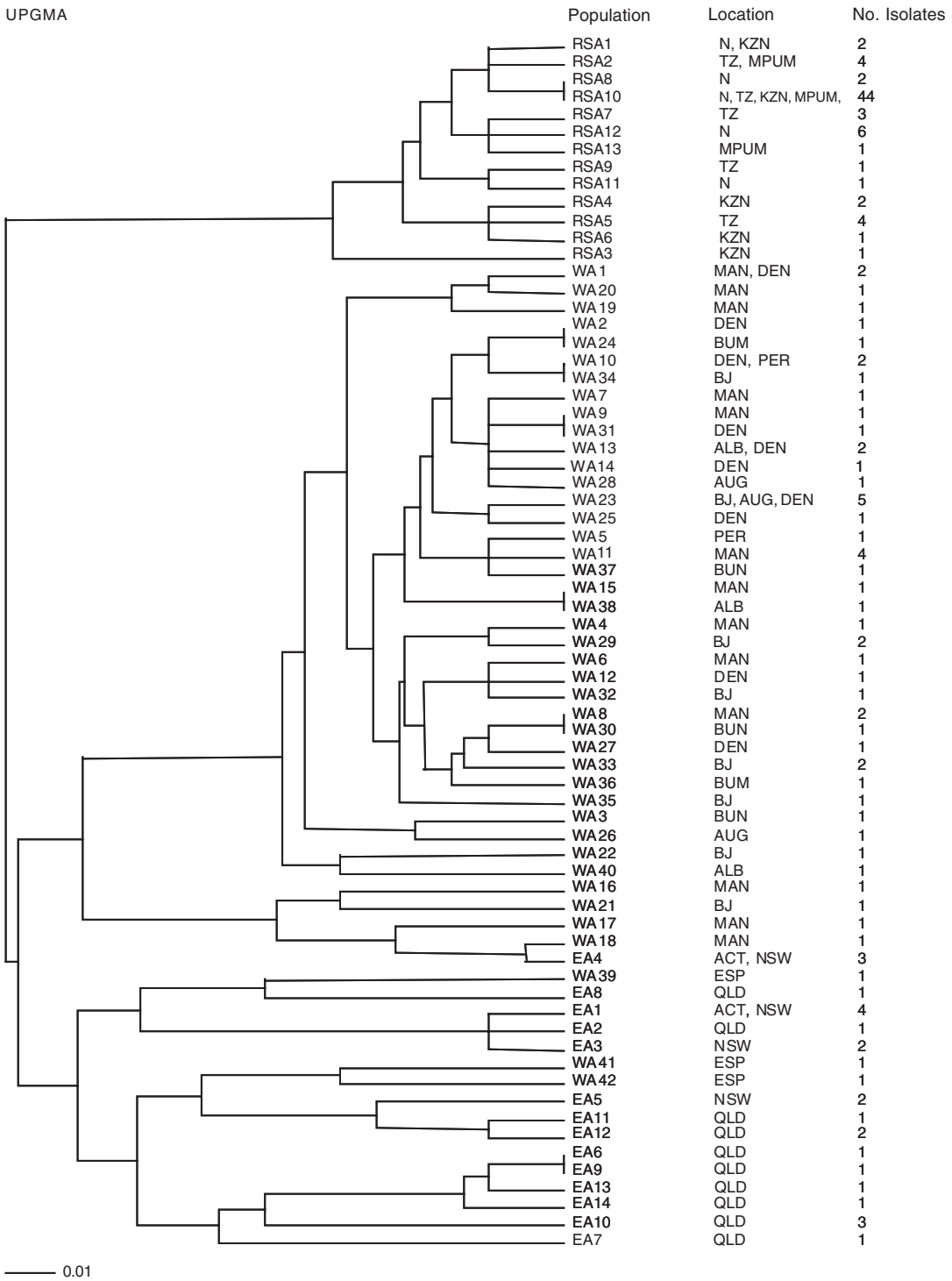

Fig. 2. Unweighted pair-group method with arithmetic mean dendrogram of *Holocryphia eucalypti* haplotypes from South Africa, Western Australia and eastern Australia constructed using clone-collected data obtained using eight polymorphic microsatellite markers.

Discussion

Microsatellite markers specifically developed for *H. eucalypti* were used effectively in this study to compare populations of the fungus from eastern Australia, WA and South Africa. Two populations collected from different hosts in WA showed no significant differences, indicating a lack of host specificity. Australian populations showed high gene and genotypic diversity compared with very low gene and genotypic diversity within the South African population. As *H. eucalypti* is thought to be native to *Eucalyptus* spp. in Australia and the only record of occurrence outside Australia is in South Africa (Van der Westhuizen *et al*. 1993), the low diversity observed in South Africa is indicative of an introduced pathogen. However, over 50% of the alleles in the South African population were unique, suggesting that they were introduced from a region of Australia not sampled in the present study.

The forestry landscape in WA has been greatly altered in recent years following the signing of Regional Forestry Agreements, which have seen a shift in focus from native forests to plantations (www.rfa.gov.au/rfa/national/nfps/, verified 18 January 2008). In the last 15 years, 300 000 ha of Tasmanian blue gum (*E. globulus*) have been planted in WA (National Forest Inventory 2007). These plantations are closely associated with remnant native forests or state forests. For this reason, it is perhaps not surprising that no barrier to gene flow was found for *H. eucalypti* isolated from planted *E. globulus* and the native *C. callophylla*.

The South African population of *H. eucalypti* exhibited extremely low genotypic diversity, low gene diversity and a low number of alleles. Forty-four of the 72 isolates had the same multilocus haplotype. This haplotype was widely distributed throughout the regions sampled. This is particularly interesting as the sexual state of the fungus is commonly encountered in South Africa (Van der Westhuizen *et al*. 1993; Venter *et al*. 2002; Gryzenhout *et al*. 2003). Low genotypic diversity, combined with a low number of alleles, despite the presence of the sexual state, are commonly observed when there have been limited introductions of a fungus into a new area (Barton and Charlesworth 1984; McDonald 1997). While reproduction is sexual, *H. eucalypti* is probably homothallic as is commonly found in other relatives of this fungus (Milgroom *et al*. 1993). Under these circumstances, it would also be expected that alleles are linked (Milgroom *et al*. 1993; McDonald 1997).

In contrast to the South African population of *H. eucalypti*, those from Australia show high gene and genotypic diversity. They also have a much higher number of alleles, considering the relatively small population sample sizes compared with that of South Africa. The hypothesis that *H. eucalypti* originates from eucalypts in Australia is thus supported by our findings. However, our results are inconclusive regarding the distribution of *H. eucalypti* in Australia. Two scenarios are possible, first that *H. eucalypti* is native to eastern Australia and spread to WA. This is supported by the absence of the teleomorph in WA and the geographical barrier separating the regions. WA is separated from eastern Australia by 3000 km of desert. This desert has been an effective barrier to gene flow in flora and fauna since the early Tertiary period (Beadle 1981; Boland *et al*. 1984). The observation that 23 alleles (but no haplotypes) are shared between the two regions could be indicative of recently human assisted gene flow through the movement of infected nursery stock. The second scenario is that *H. eucalypti* is native to both the east and west of Australia. The high diversity in WA is more indicative of a natural population than an introduced one, unless there have been numerous repeated introductions. However, more isolates are needed from eastern Australia, particularly from Tasmania, to elucidate this.

Due to the high proportion of unique alleles in the South African population, and divergent allele frequencies between Australia and South Africa, the populations from the two continents are separated by large genetic distances and form separate clades. Thus, there are no Australian haplotypes with a similar multilocus profile to those found in South Africa in the populations used in this study. We suspect the source of *H. eucalypti* may be from a region in Australia not surveyed in this study. This strongly supports the view that *H. eucalypti* is native to the Australian continent, although it is still unclear whether it is native to WA.

Acknowledgements

We acknowledge financial support provided by members of the Tree Protection Cooperative Program, the THRIP initiative of the Department of Trade and Industry, South Africa, Third World Organisation for Women in Science and Makerere University, Uganda. We thank Trudy Paap, Tania Jackson and Gilbert Whyte for providing Australian isolates of *H. eucalypti*, collected during the course of their own studies, and Dianne White for the subculturing and maintenance of these isolates. The University of Pretoria is also acknowledged for financial support of an ongoing collaborative research project between the Forestry and Agricultural Biotechnology Institute, University of Pretoria and Murdoch University, Perth, Western Australia.

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Manuscript received 16 August 2007, accepted 18 December 2007