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Genetic diversity and phylogenetic relationships within Eucalyptus marginata (Myrtaceae)

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Abstract

The eucalypt species *Eucalyptus marginata* which is harvested for high quality timber comprises three subspecies recognized by morphological characters; *E. marginata* ssp. *marginata*, ssp. *thalassica*, and ssp. *elegantella*. Genetic diversity and phylogenetic relationships between the subspecies were examined using anonymous nuclear RFLP loci, with *Eucalyptus staerei* included as an outgroup in the phylogenetic analysis. The level of diversity within the nuclear genome was lower than that found in comparative studies with other eucalypts (A = 2.7, H_t = 0.345). Most of the variation occurred within the populations (96.9%, H_s = 0.334). The two populations sampled for each of ssp. *thalassica* and ssp. *elegantella* clustered together in the UPGMA analysis, however there was little differentiation between the three subspecies overall (D = 0.029). *Eucalyptus marginata* was clearly distinct from its closest relative *E. staerei* (D = 0.16). There is little genetic support for the separation of the subspecies.

Introduction

Eucalyptus marginata Donn ex Smith (Jarrah) is a forest tree in the south-west of Western Australia that produces high value timber that is durable, hard and fine-grained. It has a wide distribution from 100 km north of Perth to Albany on the south coast (Figure 1). The species is threatened by an introduced pathogen, *Phythophthora cinnamomi* (dieback), but there is resistance to the pathogen, and clonal lines have been developed from selected resistant trees (McComb *et al.* 1994). These resistant lines may be used in revegetation of disease affected sites, and for rehabilitation of sites following bauxite mining.

The level and structuring of genetic diversity within *Eucalyptus* marginata is unknown and RFLP markers were used to determine whether there are significant genetic differences between the three morphologically recognized subspecies; *Eucalyptus marginata* (subsp. *elegantella*, *thalassica* and *marginata* Brooker & Hopper), or between trees from the Northern and Southern forests, a division (approximately at Collie) informally recognized by foresters.

Materials and Methods

Fifteen populations were selected from throughout the range of *Eucalyptus marginata*, including two outlying populations, 14 and 15 (Figure 1). A population of a closely related species, *E. staeret* was also included from the Mount Frankland locality. Leaves from 10 individuals from each population were collected. The DNA was extracted as outlined in Byrne et al. (1998), digested with *BgHI* or *Eco*RV. Southern blotted, and hybridised with 30 nuclear probes from the eucalypt genetic linkage map (Byrne *et al.* 1995), according to Byrne and Moran (1994).

Gene diversity parameters were calculated using FSTAT (Goudet 2001), and GDA (Lewis and Zaykin, 2001). A UPGMA dendogram based on unbiased genetic distances (Nei 1978) was created, with *Eucalyptus staerei* included as an outgroup.



Fig. 1. Distribution and collection sites for *Eucalyptus marginata*. Distribution of the three subspecies shown by shading. Boundary between the northern and southern populations is indicated by the horizontal line at Bunbury.

Results

A moderate level of allelic diversity was found in *Eucalyptus marginata* (A = 2.7, P = 67.1%). The level of diversity was lower (H_e = 0.332, H_o = 0.298) than that found in comparative studies of other eucalypts (Table 1). The fixation index (Fis = 0.107) was more than double compared to other eucalypt species (Table 1) and indicates some degree of inbreeding within populations. The majority of genetic diversity was maintained within populations (Gst = 3.1%) and the differentiation between populations was low (Fst = 0.033). All populations of *E. marginata* clustered together and the species was clearly distinct from its relative, *E. staerei* (D = 0.16, Figure 2). There was little structuring within *E. marginata* and there was no separation of populations in the northern and southern regions. The two populations of the subspecies *elegantella* and *thalassica* clustered together but neither was distinct from the main subspecies *marginata* (Figure 2).

	Hs	Ht	Gst(%)	Fst	Fis	He	Ho
E. marginata	0.334	0.345	3.1	0.033	0.107	0.332	0.298
					(0.016)	(0.004)	(0.007)
E. kochii ¹	0.502	0.514	2.2	0.045	0.044	0.489	0.466
					(0.010)	(0.007)	(0.011)
E. loxophleba ²	0.402	0.418	3.8	0.089	0.046	0.377	0.370
					(0.012)	(0.009)	(0.009)
E. nitens ³	0.445	0.373	16.2	1.29	0.044	0.373	0.342
					(0.009)	(0.018)	(0.019)

Table 1. Genetic Diversity Parameters for *Eucalyptus marginata* and three other eucalypt species assayed using RFLP Analysis (Figures in parentheses indicate standard error).

1. Byrne (1999), 2. Hines and Byrne (2001), 3. Byrne et al. (1998)

Discussion

Eucalyptus marginata showed moderate levels of genetic diversity within populations but little differentiation between populations and no separation of populations from the northern and southern forest regions. There was no genetic differentiation between the three recognised subspecies that are also difficult to distinguish in the field. Significanneighbourhood structuring within populations leading to mating between close relatives is the likely cause of the high fixation index, and this is consistent with high levels of biparental breeding found in a mating system of *E. marginata* (Millar *et al.* 2000). The low level of genetic structuring within *E. marginata* suggests that there will be little genetic differentiation between natural populations and the resistant dieback lines that have been selected throughout the species' range. Use of these lines in rehabilitation of minesites and disease areas are unlikely to lead to significant change in the genetic structure of the surrounding forest.

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Fig. 2. UPGMA phenogram of populations of *Eucalyptus marginata*. denotes southern populations, denotes northern populations, denotes outlying populations.