GENETIC VARIATION IN WILD CHILEAN AND CULTIVATED BRITISH POPULATIONS OF *PODOCARPUS SALIGNUS* D. DON (*PODOCARPACEAE*)

T. R. Allnutt*, J. R. Courtis†, M. Gardner‡ & A. C. Newton†

The threatened Chilean conifer *Podocarpus salignus* D. Don is currently the focus of *ex situ* conservation efforts being undertaken by the Conifer Conservation Programme of the Royal Botanic Garden Edinburgh. To assess variation within *in* and *ex situ* populations of the species, leaf material collected from four wild populations was analysed by random amplified polymorphic DNA (RAPD). Amova of RAPD profiles indicated that 93% of the variation occurred within, rather than between, populations. Intraspecific genetic diversity, estimated using percentage polymorphic loci, Shannon's diversity index, and Nei's gene diversity, was relatively high (47%, 0.692 and 0.314, respectively). To assess genetic diversity in *ex situ* populations within the UK, RAPD analysis of parents and progeny at two Cornish arboreta was undertaken. The results provided evidence of novel hybridization with suspected paternal trees (*P. hallii* Kirk and *P. totara* G. Benn. ex D. Don) endemic to New Zealand. RAPD was found to be an effective tool for assessing the genetic structure of *P. salignus*, for providing a guide to future germplasm-sampling strategies, and for hybrid identification. Implications for genetic conservation of the species and the role of *ex situ* approaches are discussed.

Keywords. Amova, hybridization, phytogeography, RAPDs.

Introduction

Conifers of the southern hemisphere have often been described as relicts, being frequently of very limited distribution and restricted to marginal or adverse environments (Veblen *et al.*, 1995), making them particularly susceptible to threats posed by human activity. Although *in situ* methods are always preferable as a means of conservation, where the scope for such approaches is limited *ex situ* approaches may also be of value for the preservation of genotypes. Conifers from the temperate zones of the world frequently grow well in Britain, and the hospitable climate, combined with several centuries of developing horticultural expertise, make the UK potentially useful for the *ex situ* preservation of the genetic diversity of many threatened temperate conifers (Page & Gardner, 1994).

Podocarpus salignus D. Don (sect. *Capitulatis*; *Podocarpaceae*) is one such threatened southern-hemisphere conifer, endemic to the central and southern mountains

* Crop Genetics, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK.
† Institute of Ecology and Resource Management, University of Edinburgh, Kings Buildings, Mayfield Road, Edinburgh EH9 3JU, UK.
‡ Conifer Conservation Programme, Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK.

of Chile. *Podocarpus salignus* is listed as vulnerable on the Global Red List of threatened conifers (Farjon & Page, 1999); Belousova & Denisova (1992) also consider it to be threatened. The main threats to this species result from logging and destruction of suitable habitat, and the replacement of native forests by plantations of exotic species (Lara *et al.*, 1995), combined with its naturally limited distribution. Some of the largest known specimens are in Ireland, a notable example being one at Kilmacurragh Arboretum, County Wicklow (M.G., personal observation, 1997), which is indicative of its lack of *in situ* protection. There is no *in situ* conservation strategy for *P. salignus* in Chile and the species is not protected by legislation. Only one population is in a protected area: Reserva Nacional, Los Ruiles, Chile.

According to the *Flora de Chile* (Marticorena & Rodriguez, 1995), *P. salignus* is distributed along both the Andean and coastal mountain ranges of Chile from 35°50′–40°35′S. This is consistent with descriptions of other authors (Veitch, 1900; Bucholz & Gray, 1948; Silba, 1984), who suggested that the range extends from the Rio Maule in the north to Valdivia in the south. However, Hoffmann (1982) claims that the species reaches as far south as Chiloé, which is around 42°–43°S, and Veblen *et al.* (1995) also state 43°S as its southern limit. Its occurrence was also once mentioned as far north as the Peruvian Andes (Veitch, 1881). The altitudinal range of *P. salignus* is from close to sea level up to 900m (Bucholz & Gray, 1948).

There have been no major ecological or genetic studies of *P. salignus* (Veblen *et al.*, 1995). However, there have been studies of the foliar epidermis (Barrera & Meza, 1991), frost resistance and foliar lipid compositions (Latsague *et al.*, 1992), and two papers on the biochemistry of *P. salignus* (Matlin *et al.*, 1984a,b).

Podocarpus salignus is a dioecious tree that reaches a height of 20m, and trunk diameters of nearly 2m, but that frequently remains a shrub. Its seeds are borne on swollen receptacles that are fleshy and red when ripe, and these are dispersed by birds (Enright *et al.*, 1995). It has a pendulous habit, 'giving the appearance of a willow' (Hoffmann, 1982), with willow-like foliage. It prefers damp areas, especially near watercourses, in the higher parts of mountain ranges; it is frequently found forming pure stands but is also associated with myrtaceous shrubs and trees (Veblen *et al.*, 1995). *Podocarpus salignus* is not abundant in any of the major forest zones of the temperate southern Andes (Veblen *et al.*, 1995). The wood of *P. salignus* is frequently used for carpentry and carving, being hard and scented with a clear yellow colour (Hoffmann, 1982).

Podocarpus salignus was discovered by Dombey, a French botanist who accompanied Ruiz and Pavón during their mission to Peru and Chile (1777–87), and was introduced into British gardens about the year 1849 (Veitch, 1900). Specimens in Cornwall are mentioned as having been collected by Richard Pearce in Chile in 1845 (Page & Gardner, 1994). *Podocarpus salignus* has not been widely planted in Britain, probably because in most places it exhibits poor growth and often remains a stunted bush (M.G., personal observation). In Cornwall, however, with its mild climate and heavy rainfall, specimens planted around the turn of the century are now almost 20m in height. The county also contains two gardens, Caerhays and

Tregrehan, both near St Austell, where groups of mature trees are reproducing. Two trees measured at the garden in 1927 were 8.5m and 6m high, although another report mentions a 9m specimen in 1916 (Thurston, 1930). Those at Caerhays are not mentioned. These breeding communities offer the opportunity of studying population dynamics in an *ex situ* environment.

The long-term conservation of a species is dependent upon the preservation of its evolutionary potential by maintaining genetic diversity (Hamrick *et al.*, 1991), enabling adaptation to future ecological changes, both biotic and physical (Ledig, 1996). Therefore, knowledge of the extent and distribution of genetic variation is critical to the development of effective conservation strategies (Hamrick *et al.*, 1991). The amount of genetic variation present within the species, and how this is partitioned among populations, can also enable management units (MUs; Holsinger & Gottlieb, 1991) or evolutionary significant units (ESUs; Moritz, 1994) to be defined, which help to provide a basis for conservation action (Newton *et al.*, 1999).

Random amplified polymorphic DNA (RAPD) analysis is now commonly used as a relatively simple tool for measuring genetic diversity and structure within many plant groups, including herbaceous (Nolan *et al.*, 1996) and woody angiosperms (Schierenbeck *et al.*, 1997), and conifers (Bucci & Menozzi, 1995; Heinze *et al.*, 1996). RAPD bands are normally inherited in a dominant Mendelian fashion (Williams *et al.*, 1990). This complicates measurement of genetic variation because of the difficulties of determining heterozygosity (Schierenbeck *et al.*, 1997). However, corrections to allele frequencies calculated from RAPDs (Lynch & Milligan, 1994) can substantially reduce error. In dioecious species such as *P. salignus*, errors due to selfing (leading to deviations from Hardy–Weinberg) are also reduced.

The primary aim of this work was to assess the extent of genetic diversity within and between populations of *P. salignus* D. Don in Chile, and in doing so address the hypothesis that coastal populations are genetically isolated from Andean populations. The results will be used to guide the Conifer Conservation Programme (CCP) in future sampling strategies of wild populations of *P. salignus*. Further aims of the project were to ascertain if the provenance of the specimens in British cultivation could be determined, which would increase their utility as genetic stock for *ex situ* conservation programmes, and to analyse the two *ex situ* breeding populations in Cornwall to provide information on the parentage of seedlings. The occurrence of reproduction at Tregrehan amongst a population of four female *P. salignus* trees suggested that the progeny were possibly of hybrid origin, which has potentially important implications for the use of such populations in an *ex situ* conservation programme. *Podocarpus totara* and *P. hallii*, both from New Zealand and both growing nearby, were considered to be the only possible male parents; this was explicitly tested by RAPD analysis.

Materials and Methods

Samples

Five grams of fresh leaf material from *P. salignus* seedlings, saplings and trees was collected from each individual used in the analysis. This was either dried in silica

gel (Chase & Hills, 1991) or frozen at −20°C as soon as possible after collection. From each of the Chilean populations, between 7 and 12 samples were taken (Table 1), reflecting the small size of the extant populations. The locations of sample sites are shown in Fig. 1. All sexually mature adult trees were sampled from the *ex situ* populations in Cornwall together with 14 putative progeny (Table 2). At Tregrehan, single male trees of *P. hallii* and *P. totara* growing near the *P. salignus* were also sampled. DNA was isolated from all samples following the method described by Allnutt *et al.* (1998).

TABLE 1. Sampled Chilean populations of *Podocarpus salignus*

No.	Name	*n*	Latitude	Longitude	Altitude (m)
1	Angol	12	37°47′S	72°49′–72°52′W	556–800
2	Biobio	11	37°49′–37°55′S	71°35′–71°42′W	500–600
3	Valdivia	11	40°S	73°10′W	300
4	Chillan	7	36°51′S	71°38′W	850

n, number of samples.

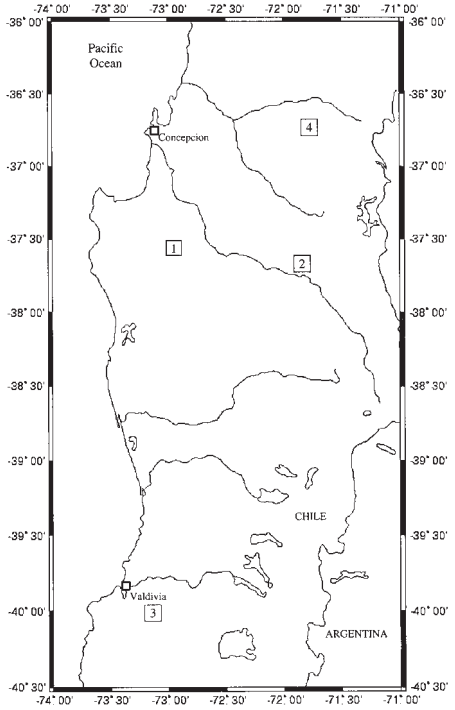

FIG. 1. Map of south central Chile showing the locations of *Podocarpus salignus* populations used for RAPD analysis, and the natural distribution of the species: 1–4, locations of populations.

TABLE 2. Tregrehan and Caerhays *Podocarpus salignus* samples

Tregrehan sample	Description	Caerhays sample	Description
1	F	1	F
2	F	2	F
3	F	3	F
4	*P. totara* M	4	M
5	*P. hallii* M	5	M
6	P (1)	6	M
7	P (1)	7	M
8	P (2)	8	P (1)
9	P (2)	9	P (1)
10	P (2)	10	P (1)
11	P (2)	11	P (1)
12	P (2)	12	P (1)
13	P (2)	13	P (2)
14	P (2)	14	P (2)
15	P (2)	15	P (2)
16	P (3)	16	P (2)
17	P (3)	17	P (2)
18	P (3)	18	P (3)
19	F (?)	19	P (3)
		20	P (3)
		21	F (?)

F, female adult; M, male adult; P, progeny (with number of putative female parent indicated in parentheses).

RAPD reactions

The PCR reaction constituent concentrations and conditions were optimized for representative samples of *P. salignus*. All reactions were performed in a Perkin-Elmer GeneAmp 9700 Thermal Cycler, using a 10μl reaction volume. Optimum reactions contained 5–20ng template DNA, 5pmol primer, 0.5U *Taq* polymerase (Bioline UK), 100μM each dNTP (Sigma), and 1.5mM $MgCl_2$ with 1 × *Taq* buffer (16mM $(NH_4)_2SO_4$, 67mM Tris-HC1 (pH 8.8), 0.01% Tween 20). Forty-five PCR cycles, consisting of 1min denaturation at 94°C, 1min primer annealing at 40°C, and 2min extension at 72°C, were used. Samples were maintained at 4°C after the termination of the programme.

Twenty 10-mer (Operon Technologies) and six 15-mer (Genosys UK) primers were screened for production of clear RAPDs. Seven 10-mer primers and five 15-mer primers were selected for use in the study (Table 3). RAPDs were separated on 2% agarose gels and visualized by staining with ethidium bromide and illumination with ultraviolet light.

Only RAPD bands that could be scored unequivocally were counted in the analysis. This can be difficult between lanes separated by many others with differing intensities of PCR products. Generally only bands in size ranges between or close

TABLE 3. RAPD primers used in the analysis

Primer	Primer sequence
OPAL 6	AAGCGTCCTC
OPAL 12	CCCAGGCTAC
OPAL 17	CCGCAAGTGT
OPK 9	CCCTACCGAC
OPK 16	GAGCGTCGAA
OPK 17	CCCAGCTGTG
OPK 19	CACAGGCGGA
TAL 6	AAGCGTCCTCATCGA
TAL 9	CAGCGAGTAGTGTAG
TAL 17	CCGCAAGTGTAGCTA
TAK 03	CCAGCTTAGGGCAAA
TAK 18	CCTAGTCGAGCCTAA

to clearly visible monomorphic bands were scored. This greatly reduced the potential number of bands that could have been scored but, importantly, avoided mis-scoring of bands for which co-migration could not be certain.

Data analysis

Amplified DNA marker bands were scored in a binary manner as either present (1) or absent (0), and entered into a binary data matrix. The shared absence of a band was not scored as a shared character. Where allele frequencies were used, they were calculated from RAPD band frequencies following the methods and corrections employed by Lynch & Milligan (1994).

A simple pair-wise distance measure (Jaccard's) was calculated from the binary RAPD data using the formula (Sneath & Sokal, 1973):

$$D = 1 - (S_{ij}/T_{ij}),$$

where S is the total number of shared present band positions and T is the total number of band positions between *i*th and *j*th individuals. It is important to note that T is the number of positions that for i and j at least one band is present, therefore a shared absence is not scored as a possible band position. This reduces overestimation of distance where individuals have significantly fewer RAPD bands. These distances were used to perform principal co-ordinate analysis (PCO; Gower, 1966), which provides a graphical representation of the RAPD relationships between individuals. Population structure within and among populations was analysed using the above distances as input for AMOVA (analysis of molecular variance; Excoffier *et al.*, 1992). The AMOVA program generates 'Phi' statistics analogous to Wright's F_{st} (Wright, 1951) and therefore allows comparison of results with other studies.

Shannon's diversity estimates (Lewontin, 1972) were calculated to provide a relative estimate of the degree of variation within each population by using the formula:

$$S = -\Sigma p_i \log_2 p_i,$$

where p_i is the frequency of presence or absence of each RAPD band (treating each RAPD band as a single locus according to Lewontin, 1972). Note that we here denote Shannon's measure as 'S' not 'H', so as to avoid confusion with other measures of diversity such as heterozygosity, to which Shannon's is not directly comparable. Following convention for heterozygosity we therefore denoted Shannon's across the total sample, S_t, and for each (sub)population, S_s. Gene diversity (Nei, 1973) was also calculated using corrected allele frequencies (Lynch & Milligan, 1994).

Results

Chilean populations

The 12 primers used produced a total of 120 RAPD bands, of which 57 (47.5%) were polymorphic (Table 4). All trees sampled from Chilean populations were found to be unique genotypes. Genetic diversity measures calculated from polymorphic data are summarized in Table 5. Analysis of variance indicated that genetic diversity ($P > 0.05$), calculated using Nei's gene diversity or Shannon's diversity index, did not differ significantly among populations.

The first two PCOs for Chilean populations described 9.23% and 8.17% of the total variance (Fig. 2). Little structure was present in this analysis, as populations largely overlapped in the graph space. However, the second PCO reveals some separation of populations 2 and 4 from populations 1 and 3. This indicates some degree of distinctiveness between coastal and Andean populations.

Analysis of molecular variance gave a Phi_{st} of 6.95%, which was significantly greater than zero ($P < 0.001$). This indicates low but significant genetic differentiation among populations (Table 6). Pair-wise Phi_{st} values among populations were also all significant ($P < 0.05$; Table 7). An UPGMA dendrogram (Fig. 3) of pair-wise Phi_{st} values illustrates the degree of similarity between populations, and particularly the relative distinctiveness of population 4 – Chillan. In addition, populations from the east and Andes (2 and 4) are separated from coastal populations (1 and 3) irrespective of their geographical distance.

Ex situ *populations*

The same RAPD primers were used for assessment of the *ex situ* populations as for the Chilean populations. However, these yielded a reduced number of polymorphic markers (20) for diversity estimation and PCO/AMOVA analyses. Both diversity measures (H_T and S_T) were not significantly different between *ex situ* and Chilean populations ($P = 0.44$ and 0.39 respectively). These values excluded putative hybrid offspring in the *ex situ* sites.

TABLE 4. Per cent polymorphic RAPD loci (P) obtained from *Podocarpus salignus* in comparison with values from other tree species

Species	Sample	P (%)	Source
Podocarpus salignus	59	47.5	Present study
Fitzroya cupressoides	89	72.4	Allnutt *et al.* (1999)
Pilgerodendron uviferum	192	35.7	Allnutt *et al.* (unpublished)
Pinus chiapensis	138	24.5	Newton *et al.* (unpublished)
Picea mariana	75	50.0	Isabel *et al.* (1995)
Populus tremuloides	100	90.2	Yeh *et al.* (1995)
Gliricidia sepium	249	65.2	Chalmers *et al.* (1992)
Cedrela odorata	68	91.5	Gillies *et al.* (1997)

TABLE 5. Genetic diversity measures for *Podocarpus salignus.* 95% confidence intervals are given in parentheses

Population	H_T	H_S	S_T	S_S
Chile				
1	0.314 (0.036)	0.317 (0.055)	0.692 (0.092)	0.669 (0.092)
2		0.280 (0.051)		0.583 (0.090)
3		0.309 (0.049)		0.652 (0.080)
4		0.323 (0.050)		0.668 (0.090)
Cornwall*				
Tregrehan		0.287 (0.084)		0.612 (0.172)
Caerhays		0.353 (0.067)		0.753 (0.121)

* Values for British samples were calculated excluding progeny, which appear to have been of hybrid origin.

At Tregrehan, three distinct clusters are evident in the PCO analysis (Fig. 4): (i) all three female adults, the *P. totara* individual male and seedlings 6–9 (6 and 7 under female 1, 8 and 9 under female 2); (ii) ten seedlings (10–19); and (iii) the *P. hallii* individual male. Given the absence of *P. salignus* males in this population, PCO proximity suggests that seedlings 6–9 are *P. salignus* × *totara* hybrids and seedlings 10–19 are *P. salignus* × *hallii* hybrids. Also, two RAPD bands (amplified by primers TAL 9 and OPK 19) were found to occur only in *P. hallii* and a subset of the suspected hybrid progeny. Neither of these hybrids has previously been reported. Also, a sapling for which no putative female parent was assigned fell into this latter cluster and is therefore also likely to be a *P. salignus* × *hallii* hybrid.

Two clusters are evident in the Caerhays PCO (Fig. 5): (i) all adult female and male *P. salignus* with seedlings (8–12, those under female 1); and (ii) all other seedlings (13–21, under females 2 and 3). The distinctiveness of the latter cluster suggests that they are likely to be hybrids of unsampled *P. totara* or *P. hallii*. An unknown origin sapling in this population also falls into this putative hybrid cluster.

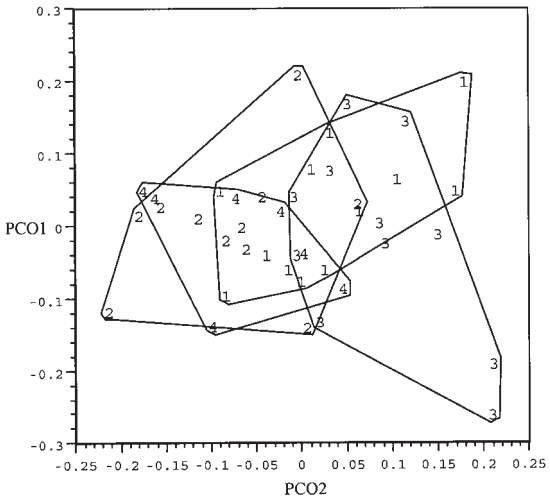

FIG. 2. Graph of the first two principal co-ordinates for Chilean *Podocarpus salignus* samples. Numbers refer to populations in Table 1. Each population is outlined.

TABLE 6. AMOVA of Chilean *Podocarpus salignus* populations, based on RAPD data

	d.f.	Sum of squares	Mean squares	Variance	Phi_{st}	*P*
Among	3	1.033	0.344	0.015	6.95%	<0.001
Within	37	7.256	0.196	0.196	93.05%	

TABLE 7. Pair-wise Phi_{st} values for Chilean populations of *Podocarpus salignus*. All distances were significant at $P<0.05$ (1000 replicate bootstrap)

1. Angol	–			
2. Biobio	0.062	–		
3. Valdivia	0.040	0.075	–	
4. Chillan	0.082	0.076	0.102	–

However, because no *P. totara* or *P. hallii* trees were found near the females, no paternity can be assigned at this site.

DISCUSSION

Genetic diversity

Total genetic diversity estimated by Nei's gene diversity, Shannon's diversity measure, and percentage polymorphic loci was generally high in wild Chilean populations of *P. salignus*, when compared with other RAPD studies (Table 4). For example, diversity values for *P. salignus* were generally intermediate between two other Chilean

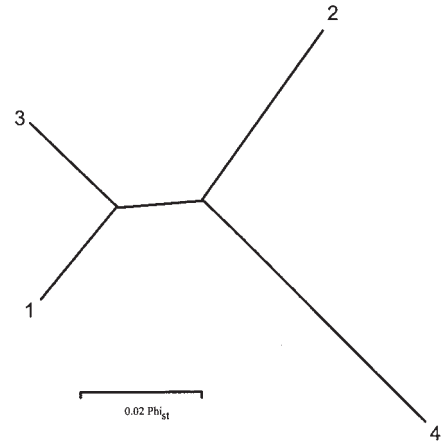

FIG. 3. UPGMA of pair-wise Phi$_{st}$ values for Chilean *Podocarpus salignus* populations 1–4.

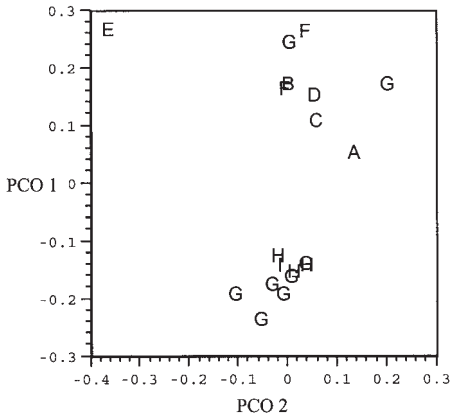

FIG. 4. Graph showing first two PCOs for Tregrehan *ex situ* population of *Podocarpus salignus*. A–C, female adults 1–3 respectively; D, *P. totara*; E, *P. hallii*; F, seedlings under female adult tree 1; G, seedlings under female adult tree 2; H, seedlings under female adult tree 3; I, sapling of no suspect female parent.

conifers in the *Cupressaceae*: *Fitzroya cupressoides* Molina I.M. Johnst. (Allnutt *et al.*, 1999) and *Pilgerodendron uviferum* (D. Don) Florin (Allnutt *et al.*, unpublished). This suggests that *P. salignus* has not undergone a genetic 'bottleneck' or suffered a severe loss of genetic variability as a result of reduction in range or population size. These results therefore contrast with those for *Pilgerodendron uviferum* in southern Chile, where low genetic variability was recorded within a number of small or geographically isolated populations (Allnutt *et al.*, unpublished).

In cultivation, despite the small numbers of trees sampled, variation was comparable with that observed in the wild populations. This is in marked contrast to *F. cupressoides*, where 89 individuals sampled from cultivation in the UK were found

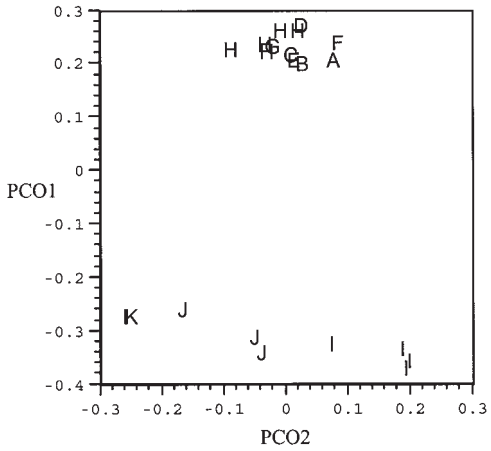

FIG. 5. Graph showing first two PCOs for Caerhays *ex situ* population of *Podocarpus salignus*. A–C, female adults 1–3 respectively; D–G, male adults 4–7 respectively; H, seedlings under female adult tree 1; I, seedlings under female adult tree 2; J, seedlings under female adult tree 3; K, sapling of no suspect female parent.

to contain only a single genotype (Allnutt *et al.*, 1998). *Podocarpus salignus* is therefore likely to have been introduced by seed or cuttings from several individuals, the genotypes of which have been maintained in cultivation, whereas the remaining trees of *F. cupressoides* must originate from cuttings from a single tree. This implies that the populations of *P. salignus* established within British gardens could be of value for the *ex situ* conservation of the species.

Population differentiation

Results for *P. salignus* were typical of wind-pollinated conifers in general, with a majority of genetic variation occurring within, rather than between, populations (Hamrick *et al.*, 1991). However, Phi_{st} for *P. salignus*, as estimated by AMOVA (6.95%), was much lower than the values recorded for the other Chilean conifers *F. cupressoides* and *P. uviferum* (14.4% and 18.6% respectively). This may be a consequence of bird dispersal of the seeds of *P. salignus*, which results in an increased likelihood of migration (Hamrick & Godt, 1989) compared with species with wind-distributed seeds, such as *F. cupressoides* and *P. uviferum*. This low Phi_{st} therefore indicates relatively high gene flow among populations compared with other Chilean conifers studied to date.

Despite the relatively low degree of differentiation between populations, pair-wise comparison of Phi_{st} values indicated that populations from Andean and coastal regions display a significant degree of genetic differentiation. This could result from currently restricted gene flow between these two regions, or gene flow that was limited at some stage in the past (Newton *et al.*, 1999). These regions are separated by the

lower altitude, drier Central Valley, which could present a barrier to gene flow. Alternatively, this genetic differentiation may be the consequence of historical isolation, as occurred during the Pleistocene ice ages (Allnutt *et al.*, 1999; Newton *et al.*, 1999), which interglacial gene flow has not obscured.

Genetic structure of ex situ *populations*

Among the seedlings growing around the base of adult female trees at Tregrehan and Caerhays, a large proportion appeared to be hybrids of the New Zealand native species *P. hallii* and *P. totara*. These hybrids have not previously been reported. Given the geographic and temporal separation of *P. salignus* and *P. totara*/*hallii*, the formation of hybrids is surprising. Differences in karyotype number between *P. salignus* (2n = 40) and *P. hallii* and *P. totara* (2n = 34) (Hair, 1963) would lead to the expectation that the hybrid seedlings will be sterile, although this has not yet been tested. Precise parentage of trees at the UK sites could not be determined owing to an absence of deterministic RAPD bands, except for the paternal contribution of *P. hallii* to seedlings in cluster (ii) of Fig. 4. However, the similarity among individuals evident in the PCO plots does imply familial associations (e.g. seedlings 'F', Fig. 4, and seedlings 'J', Fig. 5).

The only previously described hybrids within the genus all occur amongst endemic New Zealand species: *P. hallii* × *nivalis*, *P. hallii* × *totara* (Allan, 1961); *P. acutifolius* Kirk × *totara* and *P. acutifolius* × *nivalis* (Webby *et al.*, 1987). The only other known hybrid within the *Podocarpaceae* is *Lepidothamnus laxifolius* (Hook.f.) Quinn × *intermedius* (Kirk) Quinn, also in New Zealand (Quinn & Rattenbury, 1972). The results of this study suggest that hybridization may be possible between many more *Podocarpus* species than previously considered. Although the current results strongly suggest that hybridization has taken place, use of species-specific markers (for example using microsatellites; Newton *et al.*, 1999) and wider sampling would enable the hybrid origin to be defined with greater precision, by indicating the precise parentage of each individual.

Implications for conservation

Although the genetic differences between remaining wild populations of *P. salignus* are relatively slight, the current analysis suggests that these differences are significant, and should therefore be considered in the development of future conservation strategies. Genetic data such as those presented here can be used as a basis of defining management units for conservation (Newton *et al.*, 1999), which are used to define areas requiring individual conservation attention, and for informing the movement of germplasm between areas during ecological restoration efforts. The existence of genetic differentiation between populations also has clear implications for germplasm collection for *ex situ* conservation approaches; ideally the full range of natural variation should be sampled.

The interfertility of Chilean and New Zealand *Podocarpus* species shows the possible risks of losing the genetic integrity of species in *ex situ* collections, and of the use of non-native *Podocarpus* species for forestry or horticulture in countries that have indigenous members of the genus. This discovery indicates that the progeny of *ex situ* conifers should not be used for reintroduction programmes unless they have been produced under controlled conditions, supporting the CCP's aim of developing controlled greenhouse pollination of young conifer specimens for seed production. The possibility of hybridization emphasizes the importance of using material of known wild origin in conservation schemes, as hybrid progeny from Tregrehan has already been distributed for horticultural purposes as *P. salignus*. The results of this investigation also highlight the value of the RAPD technique for assessing conservation issues such as these in threatened tree species.

Acknowledgements

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