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Patterns of Genetic Variation in *in* and *ex situ* Populations of the Threatened Chilean Vine *Berberidopsis corallina*, Detected Using RAPD Markers

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Berberidopsis corallina Hook. f. (Berberidopsidaceae) is a threatened vine, endemic to the temperate rainforests of southern Chile. A RAPD analysis was carried out to assess the extent of genetic variation in remaining wild populations and in British cultivated plants, to assess the value of the latter for *ex situ* conservation. A total of 90 individuals (54 wild, 35 cultivated) were analysed, and a total of 54 polymorphic bands produced. A pairwise distance measure calculated from the RAPD data was used as input for principal coordinate analysis (PCO) and analysis of molecular variance (AMOVA). AMOVA indicated that an exceptionally high proportion of total genetic variation (54·8 %) was recorded among populations; pairwise Φ_{ST} comparisons showed that all the populations examined were significantly ($P < 0{\cdot}002$) different. PCO analysis highlighted clear differentiation between wild populations from the north and south of the natural range, further supported by a UPGMA dendrogram based on RAPD distances. Analysis of the extent of genetic variation within *ex situ* populations indicated that the variation within plants cultivated within Britain is comparable to that recorded in small natural populations; however, cluster and UPGMA analyses suggested that only populations from the northern part of the natural range of the species are represented in cultivation. These results are interpreted in the context of the recent biogeographical history of the area, and their implications for the development of *in situ* and *ex situ* conservation strategies for *B. corallina* are discussed.

Key words: Genetic conservation, conservation biology, temperate rainforest, Berberidopsidaceae, non-timber forest product.

INTRODUCTION

Berberidopsis (Berberidopsidaceae) is a bitypic genus comprising the Chilean endemic *B. corallina* Hook. f. and the Australian endemic *B. beckleri* (F. Muell.) Veldk. (Veldkamp, 1984). *Berberidopsis corallina* is an evergreen, vigorous scandent shrub or liana, capable of attaining heights of 15 m or more and smothering large shrubs and small trees. The species is endemic to the coastal forests of Chile (36° S to 41° S) extending over a range of 350 km between the provinces of Talca in the north and Llanquihue in the south. Throughout its range, *B. corallina* is most frequently found in remnant forest areas, often in damp places near watercourses. It occurs with a mixture of sclerophyllous vegetation characterized by the tree *Cryptocarya alba* (Molina) Looser and species from the Valdivian rain forests, the most important canopy tree being *Aextoxicon punctatum* Ruiz & Pav. *B. corallina* flowers from December to April, and fruiting starts in December (Veldkamp, 1984).

Much of the natural habitat of *B. corallina* has been destroyed by clearance for commercial forestry and agriculture, and as a consequence the species is now severely threatened (Smith-Ramírez, 1996). Meyer, collecting in 1966, suggested that the Puente Melizos in the Fundo Colcura, south of Concepción, was the last known wild locality of the species, but subsequently in 1976, Marticorena *et al.* found a surviving population in Quebrada Honda, south of Concepción (Veldkamp, 1984). The species is classified as 'Endangered' according to the IUCN categories of threat (Walter and Gillett, 1998), but given the current high rate of habitat loss, this assessment may be conservative. *Berberidopsis corallina* is valued by indigenous communities, particularly the Pehuenches, who use the stems to weave baskets that are sold commercially (Smith-Ramírez, 1996).

Berberidopsis corallina was first introduced to horticulture by Pearce in 1860 whilst working for the horticultural nursery firm Veitch & Son, based in Exeter and Chelsea in the UK. Pearce found the species in a forested ravine south of the coastal town of Lota in the province of Arauco, Chile, at the northern end of the species' range (Veldkamp, 1984). Plants from this collection were nursery-grown and sold to customers in Britain and Ireland, and *B. corallina* has become a frequently cultivated climber in British and Irish public and private gardens, where it is invariably planted on sheltered walls. Since its original introduction, it

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is likely that no other introductions have been made to British and Irish gardens until those undertaken in 1996 in connection with the current project.

Understanding patterns of genetic variation within plant species is of fundamental importance to the development of conservation strategies, both for defining appropriate units for *in situ* conservation and for developing effective sample collection strategies for *ex situ* conservation (Holsinger and Gottlieb, 1991; Hogbin and Peakall, 1999; Newton *et al.*, 1999). A large number of studies have been undertaken to assess the extent of genetic variation in threatened plant species (Hamrick *et al.*, 1991; Hamrick and Godt, 1996; Gitzendammer and Soltis, 2000). This reflects the importance accorded to the maintenance of genetic variation in conservation programmes, as its loss may reduce the evolutionary viability of populations by decreasing their ability to adapt to changing environmental conditions (Holsinger and Gottlieb, 1991; Ennos, 1996). However, relatively few studies have attempted to assess the extent of genetic variation in both *in* and *ex situ* populations of threatened plant taxa, even though such information is crucial to the development of integrated conservation strategies employing both approaches.

The investigation had two aims: firstly, to examine the extent of genetic variation within and between the naturally occurring populations of *B. corallina*, and secondly, to assess the extent of genetic variation within accessions of the species cultivated in Britain and Ireland, with a view to developing an integrated conservation strategy for the species. We tested the following hypotheses: (1) fragmentation and degradation of the native forest habitat of *B. corallina* has resulted in a relatively low extent of genetic variation within wild populations; (2) a low number of founder individuals and successive loss of plants through the effects of cold winters (Krüssmann, 1976) has resulted in a relatively low extent of genetic variation within cultivated material; and (3) the plants cultivated in Britain and Ireland display closest genetic affinity with wild populations in the northern part of the species' range, from where material was originally collected.

To test these hypotheses, we employed RAPDs (random amplified polymorphic DNA) markers (Williams *et al.*, 1990) to assess the extent of genetic variation within *in situ* populations and *ex situ* accessions of *B. corallina*. RAPDs are generated using oligonucelotides (usually 10-mers) to amplify random DNA fragments from genomic DNA, and the bands generated are normally inherited in a dominant Mendelian fashion (Williams *et al.*, 1990). The wide availability of commercial primer sets, and the lack of dependence on sequence information, has led to the widespread use of RAPDs for assessing genetic diversity and structure within many plant groups, including herbaceous (Dawson *et al.*, 1993; Nolan *et al.*, 1996) and woody angiosperms (Gillies *et al.*, 1997, 1999; Schierenbeck *et al.*, 1997; Heaton *et al.*, 1999; Newton *et al.*, 1999) and conifers (Bucci and Menozzi, 1995; Heinze *et al.*, 1996; Allnutt *et al.*, 1999, 2001*a*, *b*). However some caution has been expressed concerning use of RAPD markers, particularly with respect to the reproducibility of results obtained (Newton *et al.*, 1999). Despite such problems, an increasing number of studies have successfully employed RAPDs for the assessment of genetic variation in threatened plant taxa, with the aim of informing conservation strategies, both *in* (Hogbin and Peakall, 1999; Maki and Horie, 1999; Newton *et al.*, 1999) and *ex situ* (Virk *et al.*, 1995; Allnutt *et al.*, 1998, 2001*a*).

MATERIALS AND METHODS

The precise distribution of extant wild populations of *B. corallina* is unknown. Historical information suggests that the species occurs predominantly in two areas, one in the north of Chile in Cordillera de Nahuelbuta (36° S to 39° S), and the other further south in Cordillera San Juan de la Costa (40° S to 41° S). The aim of this investigation was to sample material from the entire range of the species, and therefore a programme of exploration was undertaken, using the location of previous collections to guide the sampling programme. Veldkamp (1984) cited nine historical locations, seven in the northern part of its range and two in the south. To our knowledge, only four of these sites have been relocated during the last 10 years (Lequesne and Schlegel pers. comm.), including the type location, although a search of the latter site in 1996 undertaken as part of the present study failed to locate any plants. Two additional sites not mentioned by Veldkamp (1984) but known to exist locally were relocated, Caramávida (province Arauco) and Reserva los Queules (province Cauquenes). Samples of plants from these locations are designated here as populations 'C' and 'H' (Table 1). An attempt was then made to search for the *B. corallina* towards the southern extremity of its range, where it was successfully located (Gardner, 1996*a*, *b*, 1997). These plants are designated here as population 'D' (Table 1). A search was undertaken in the coastal forests west of Osorno in the vicinity of San Juan de la Costa to try to locate populations in addition to those that had been located previously (F and G). All likely locations, including the small community named 'pilfuco', which forms part of the vernacular name of *B. corallina* ('voqui pilfuco') in this region, were visited. In addition, one plant ('E') was received from a local nursery which allegedly came from the type locality (south of Lota)—the origin of the first introduction of the plant to Britain.

Leaf samples were obtained from 54 different plants sampled from six different populations at four main locations extending throughout the range of the species (Fig. 1, Table 1). In the case of climbers such as *Berberidopsis*, the extent of each genotype in a given population is not always clear because of the possibility of vegetative spread. For this reason care was taken to avoid duplicate collections from the same genotype, by sampling individual shoots separated by a minimum distance of 5 m. In the case of population D (see Table 1), samples were taken from nursery-grown plants that had germinated from seed collected from the wild; in all other cases samples were collected from plants growing in the field. In each case, as many samples as possible were collected; sample numbers were limited by the small size of the populations encountered.

Leaf material from cultivated plants was obtained as a result of a request published in *The garden* (Anon, 1996).

TABLE 1. *Source of samples used in RAPD analysis*

Sample no.	Label	Source	Original source/location	Altitude (m)
1	A	Mrs M. Hayes, Argyll	–	
2	A	Abbotsbury Subtropical Garden, Dorset	–	
3	A	Mount Stewart Garden, Co. Down	–	
4	A	Mount Stewart Garden, Co. Down	–	
5	A	Pukeiti Rhododendron Garden, New Zealand	New Highleigh Nurseries, Sussex	
6	A	D. Davidson, Lancashire	Bodnant Gardens, Clywd	
7	A	Trengwainton, Cornwall	–	
8	A	Nymans Garden, Sussex	–	
9	A	Castlewellan, Co. Down	–	
10	A	Trewidden Estate Nurseries, Cornwall	–	
11	A	Mr R. Gilbert, Cornwall	–	
12	A	ARJ Partnership, Northamptonshire	Podington Garden Centre, Northamptonshire	
13	A	Pleasant View Nursery, Devon	–	
14	A	Mr R. Staples, Yorkshire	Bodnant Gardens, Clywd	
15	A	Mrs W. Spence, Hampshire	–	
16	A	Mrs C. Mackenzie, Highlands	–	
17	A	Mrs H. Pigott, Cornwall	Burncoose & South Down Nurseries, Cornwall	
18	A	Mrs S. Hesketh, Cheshire	–	
19	A	Mrs A Drummond, Surrey	–	
20	A	Mrs J. Campbell, Buckinghamshire	RHS Wisely Garden, Surrey	
21	A	Mr Clarke-Hall, Sussex	Ingwersen Nursery, Sussex	
22	A	G. Rhodes, Devon	–	
23	A	Mr & Mrs D & E Benton, Hampshire	–	
24	A	G. Hudson, Pembrokeshire	–	
25	A	Mrs S. Sandry, Avon	Tickenham Garden Centre, Somerset	
26	A	Mrs F. Casement, Co. Antrim	Langthorns Plantery, Essex	
27	A	S. Etheridge, Sussex	Brinkman's Nursery, Sussex	
28	A	Mrs H. Kirkland, Midlothian	–	
29	A	Mount Stewart Garden, Co. Down	–	
30	A	Castlewellan, Co. Down	–	
31	A	J. Harding, Sussex	–	
32	A	Mrs J. Tims, Lancashire	Architectural Plants, Sussex	
33	A	Mr R. Gilbert, Cornwall		
34	A	Mrs D. Brammall, Cumbria	RHS Wisely Garden, Surrey	
35	A	Logan Garden Trust, Wigtonshire	–	
36	B	Mt Tomah Botanical Garden, Australia	Arboretum, Universidad Austral de Chile	
37–44	C	Caramávida, Cordillera de Nahuelbuta	37° 40′ 51″ S, 73° 17′ 42″ W	120
45–47	D	San Juan de la Costa	40° 26′ 25″ S, 73° 30′ 1″ W	182
48	E	Arboretum, Universidad Austral de Chile	–	–
49–56	F	San Juan de la Costa	40° 30′ S, 73° 23′ W	200
57–70	G	San Juan de la Costa	40° 30′ S, 73° 23′ W	200
71	H	Reserva los Queules	35° 58′ 78″ S, 72° 42′ 36″ W	459
72–90	I	Villa las Araucarias	38° 27′ S, 76° 16′ W	640–700

Material was obtained from over 80 different localities together with information on the original source, if known, and the cultural conditions under which the plants were growing. From the information accompanying many of the samples it was often possible to trace the cultivated source, thereby helping us to avoid analysing samples that potentially represented the same genotype. In all, 35 samples were used from cultivated sources in Britain and Ireland. These were grouped together and treated as an individual 'population' (A) in the analyses, although it is recognized that these individuals do not constitute a population in the biological sense. One sample was received from Australia (sample 36, B) which originated from the arboretum of the Universidad Austral de Chile (no accurate data exist concerning the wild origin of this plant). In addition, plant E (sample 48) grown at the Universidad Austral de Chile was included; records suggest this originated from the same locality as Pearce's original collection. In all cases, leaves were dried in sealed plastic bags containing silica gel (S4883 silica, Sigma Chemical Company Ltd, Dorset, UK) and stored at 4 °C prior to DNA extraction, following Chase and Hills (1991).

DNA was isolated from 0·5 g of dried plant tissue following the modified CTAB method of Doyle and Doyle (1990). DNA concentration was determined by comparison to standards on agarose gels and dilutions made in TE buffer to give 25 ng DNA μl^{-1} for RAPD reactions. RAPDs were performed in 10 µl reactions in a Perkin–Elmer GeneAmp 9700 thermal cycler using the following conditions: 50 ng template DNA, 10 pmol primer, 1 unit *Taq* polymerase, 200 µM each dNTP, 1·5 mM $MgCl_2$, reaction buffer (16 mM $NH_4)_2$ SO_4, 67 mM Tris–HCl (pH 8·8) and 0·1 % Tween-20. Forty-five PCR cycles were performed, each consisting of 94 °C for 1 min, 42 °C for 1 min, 72 °C for 2 min, followed by a final extension phase of 72 °C for 5 min.

To screen for RAPD primers, four samples (1, 45, 54 and 79) were used in reactions with 18 10-mer primers. Eleven

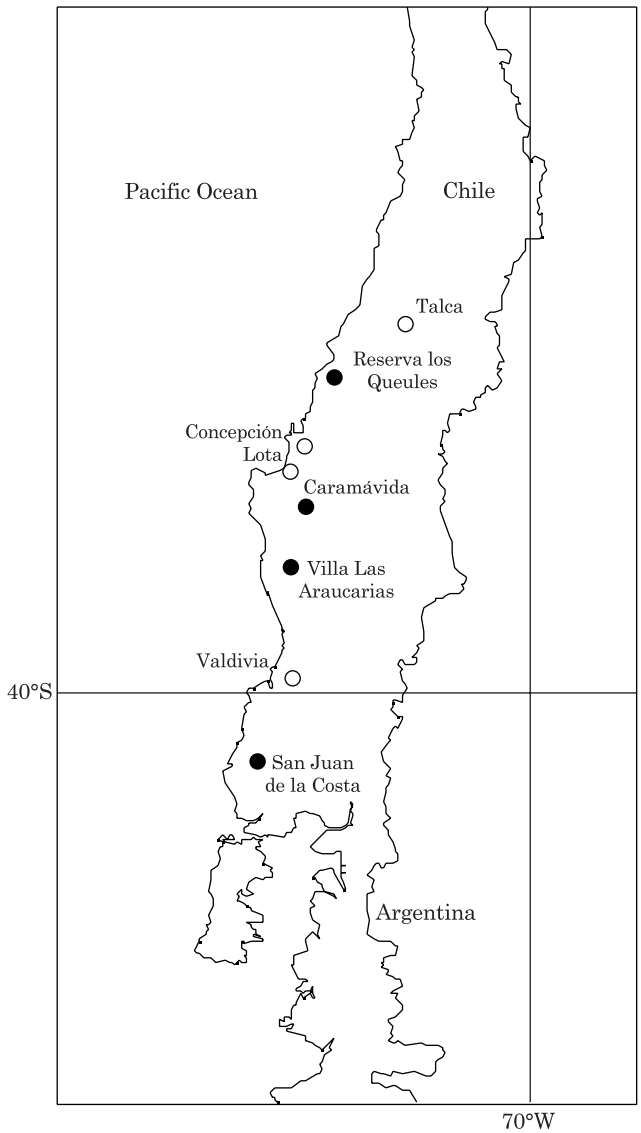

FIG. 1. Distribution map of samples included in the analysis. Filled circles indicate the location of the four main areas from which samples were collected in the field; empty circles indicate the position of nearby towns.

primers gave clear, reproducible banding patterns and were selected for further analysis (Operon OPB-03, 5, 7, 8, 9, 10, 11, 13, 15; OPAL-12 and 14). RAPD products were separated on 8 % acrylamide gels (29 : 1 acrylamide : bis-acrylamide) and visualized by staining with ethidium bromide and photographed over UV light. Only RAPD bands that could be scored unequivocally were counted in the analysis. Generally, only bands in size ranges between 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.

The data were recorded as presence (1) or absence (0) of a band for each marker in each individual sample. The overall percentage of polymorphism as a whole and for individual populations was calculated. Data were entered in a binary matrix, and population genetic structure was determined by calculating a simple distance matrix using the formula of Sneath and 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 the i^{th} and j^{th} individuals.

Genetic variation within and among the populations listed in Table 1 was determined by analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992). Jaccard (1908) distances were used as input for AMOVA (excluding shared absence) and 'ϕ' statistics, analogous to Wright's (1951) F_{ST}, were generated. AMOVA is the least biased method for apportioning variation among and within populations for RAPD data (Isabel *et al.*, 1995). The significance level for ϕ_{ST} was determined using 1000 bootstrap replicates. A UPGMA tree based on pair-wise ϕ_{ST} was generated to compare similarities between populations, excluding populations consisting of only one individual, and a UPGMA tree was also produced to illustrate the relationship between all samples based on Jaccard distances. Principal coordinate analysis (PCO) (Gower, 1966), based on Jaccard distances, was also performed to assess the degree of affinity between populations.

TABLE 2. *Percentage polymorphic RAPD bands recorded for both* in *and* ex situ *populations of* Berberidopsis corallina

Population	Sample size	% Polymorphism
A	35	17·24
B	1	–
C	8	17·24
D	3	3·44
E	1	–
F	8	12·06
G	14	22·41
H	1	–
I	19	22·41

For details of populations, see Table 1; for definition of populations included, see text.

RESULTS

A total of 58 clear, repeatable RAPD bands were scored. Of these, 25 were polymorphic (overall polymorphism = 43·10 %). No population-specific bands were observed. Several bands were exclusively shared between British cultivated material and northern Chilean populations. The polymorphism for individual populations ranged from 10·34 to 22·41 % (Table 2). Polymorphism in British cultivated plants was lower than in two Chilean wild populations (G and I), but higher than two wild populations (D and F) with relatively small sample sizes (Table 2).

Several identical RAPD patterns were obtained for the samples analysed. In the British material a total of 16 genotypes was recognized, while in northern wild populations 23 genotypes were present. Southern wild populations

TABLE 3. *AMOVA of RAPD genetic distances*

	d.f.	Sum of squares	Mean squares	Variance	% of total variance	*P*
Among populations	5	8·096	1·619	0·113	54·83	<0·02
Within populations	86	8·010	0·093	0·093	45·17	
Total	91	16·107				

TABLE 4. *Pair-wise ϕ_{st} values calculated by AMOVA*

	A	C	D	F	G	I
A	0·0000					
C	0·3852	0·0000				
D	0·6842	0·6513	0·0000			
F	0·6952	0·6548	0·2392	0·0000		
G	0·6456	0·5676	0·2506	0·3565	0·0000	
I	0·3947	0·2034	0·6188	0·6543	0·5569	0·0000

showed 19 distinct genotypes. Overall, 59 genotypes were found from 90 samples. This indicates that vegetative reproduction is common in the species. In cultivation, of course, this may frequently have been due to propagation via cuttings, which root easily.

AMOVA indicated that over half the genetic variation in the species is apportioned between, rather than within, populations ($\Phi_{st} = 54{\cdot}83\,\%$; Table 3). Pairwise comparisons of Φ_{st} values (Table 4) were all significant ($P < 0{\cdot}002$), indicating that all populations sampled differ significantly from each other genetically.

The first three principal coordinates described 10·02, 4·63 and 2·87 % of the total variance, respectively (Fig. 2). The total variation associated with these coordinates was relatively low, highlighting the existence of much unexplained variation. Thus, results must clearly be viewed with caution. The PCO analysis divided the individuals into two main groups which corresponded to geographical origin. The northern populations (C, H and I) and British cultivated plants constituted one main group, which was clearly differentiated from a second group which was exclusively composed of populations with a southern origin. In the case of the northern group, further separation was evident, with individuals from population A tending to be clustered separately from those of populations H and I, but not C (Fig. 2). Interestingly, the sample (E) from the putative original site of collection for British cultivated plants fell in the cluster containing British cultivated material.

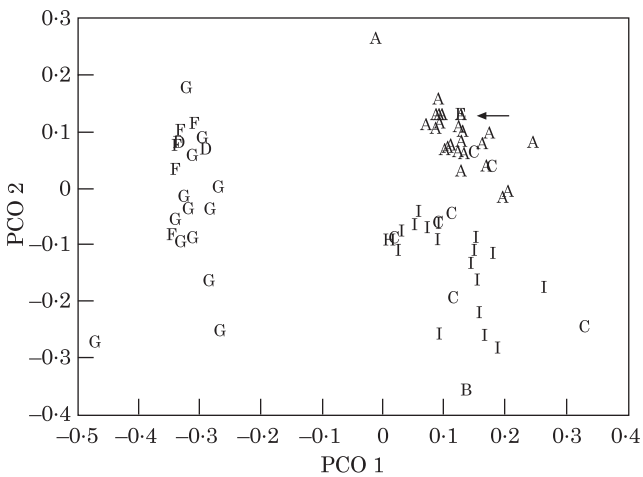

FIG. 2. Plot illustrating the results of the principal coordinate analysis (PCO) of *Berberidopsis corallina* samples, based on Jaccard distances. The first two principal co-ordinates of the PCO analysis described 10·02 and 4·63 % of the total variance respectively. Samples are denoted A to I according to Table 1. Arrow shows the position of sample E.

Amongst the cultivated material, sample 36 (B) from Australia was clearly differentiated from the other individuals sampled, indicating that it was quite distinct from all other plants. Given the unknown origin of this plant it must be assumed to be from a locality not sampled in this study. Although no bands were found to be specific to this sample, analysis of the results indicated that two bands that were present in virtually all samples were absent in this individual. It is therefore conceivable that slight DNA degradation may account for the isolated position of this sample.

PCO results were further supported by the production of a UPGMA tree (Fig. 3), which again clearly indicated the difference between samples from the northern and southern parts of the natural range of the species, the similarity between British cultivated samples and those from northern wild populations, and the distinctiveness of the Australian sample.

DISCUSSION

The most striking feature of these results is the high degree of genetic differentiation recorded between populations of *B. corallina*, with more than half of the total variation detected being attributable to differences between, rather than within, populations ($\Phi_{st} = 54{\cdot}8\,\%$). This value is exceptionally high compared to those derived for other

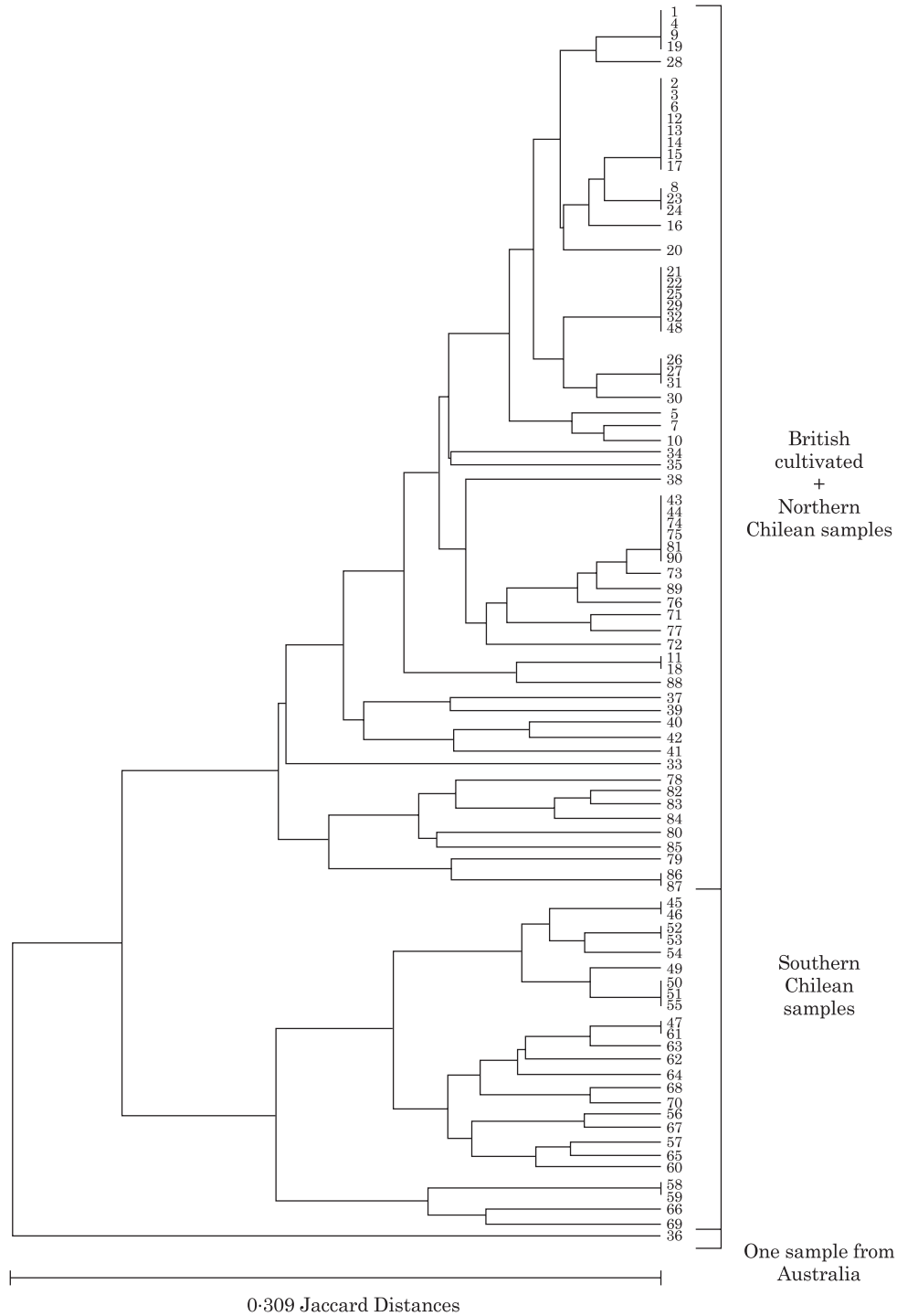

FIG. 3. UPMGA dendrogram showing the relationship between all the samples of *Berberidopsis corallina* analysed, based on Jaccard distances. For details of samples included, see Table 1.

plant species using similar approaches, including tree species native to the same area of Chile as *B. corallina*. For example, Allnutt *et al.* (2001*b*) found that 18·6 % of the variation recorded in the Chilean conifer *Pilgerodendron uviferum* Florin using RAPD markers was attributable to differences between populations, a value considered to be high for a wind-pollinated conifer. Lower values were recorded for two other Chilean conifers *Fitzroya cupressoides* I. M. Johnst. (14 %, Allnutt *et al.*, 1999) and *Podocarpus salignus* D. Don (7 %, Allnutt *et al.*, 2001*a*), again using RAPDs. Values available for RAPD studies of other tree species tend to be even lower, for example 1·8 % recorded for *Picea mariana*

Britton, Sterris & Poggenb. (Isabel *et al*., 1995) and 2·6 % for *Populus tremuloides* Michx. (Yeh *et al*., 1995). In a review of seven RAPD studies of different plant species, Hogbin and Peakall (1999) noted that variance among populations varied from 0–43 %, the higher values being recorded in species known to undergo selfing.

Recent analysis of the pattern of genetic variation in tree species using molecular markers has emphasized the importance of considering historical factors when attempting to explain current patterns of population differentiation (Newton *et al*., 1999). This is particularly relevant in the case of southern Chile which underwent extensive and repeated glaciation during the Pleistocene period (1·6–0·01 million years ago), resulting in the development of extensive ice sheets centred on the Andes (Villagrán *et al*., 1995). During glacial periods, forest areas are thought to have been restricted to refugia west of the Andes to latitudes between 36–42° S (Markgraf *et al*., 1995, 1996; Villagrán *et al*., 1995), particularly in the coastal mountain range of south-central Chile (Armesto *et al*., 1995). *Berberidopsis corallina* is one of many endemic plant species that are currently restricted to this area (Armesto *et al*., 1995), suggesting not only that its natural distribution was reduced by glaciation, but that the species displayed a limited capacity to spread from forest refugia following post-glacial climatic amelioration. Evidence from pollen analyses (Villagrán, 1991; Villagrán *et al*., 1995) and geomorphology (Veit and Garleff, 1995) suggest that forest refugia may have been limited in extent during the glacial period, being restricted to low elevations of the Coastal Range (Cordillera Pelada), the Cordillera de Nahuelbuta and the northern coastal range of Chiloé island (Armesto *et al*., 1995).

The restriction and isolation of forest habitats during glacial periods could have contributed significantly to the pattern of genetic differentiation detected in the current investigation. In this context, the striking difference between populations of *B. corallina* from the northern and southern parts of its range suggests that the species may have been restricted to at least two distinct refugia. Unfortunately, pollen analyses provide insufficient evidence to test this hypothesis further. However, comparable data are available for the Chilean conifers *Fitzroya cupressoides* (Allnutt *et al*., 1999) and *Pilgerodendron uviferum* (Allnutt *et al*., 2001*b*). In both cases, significant differences were detected using RAPD markers between populations towards the northern and southern parts of their natural ranges, coinciding with evidence from pollen analysis and suggesting the occurrence of multiple refugia for these species in coastal areas of Chile during the last ice age (Armesto *et al*., 1995; Villagrán *et al*., 1995; Allnutt *et al*., 1999, 2001*b*). However, as noted by Bossart and Prowell (1998), the relative impact of current *vs*. historical factors on patterns of genetic variation is difficult to evaluate.

The overall degree of polymorphism (43·1 %) was relatively low compared to values derived for other tree species using RAPDs. For example, levels of polymorphism of 100, 91·5, 90·2, 72·4 and 47·5 % were recorded for *Picea mariana* (Isabel *et al*., 1995), *Cedrela odorata* L. (Gillies *et al*., 1997), *Populus tremuloides* (Yeh *et al*., 1995), *Fitzroya cupressoides* (Allnutt *et al*., 1999) and *Podocarpus salignus* (Allnutt *et al*., 2001*a*) respectively. However, lower values have been recorded in the threatened plants *Zieria prostrata* J. A. Armstrong, ined. (37 %; Hogbin and Peakall, 1999) and *Haplostachys haplostachya* (Gray) St. John (42 %; Morden and Loeffler, 1999). The low values recorded here for *B. corallina* could have resulted from loss of genetic variation through inbreeding and/or genetic drift occurring as a result of restriction of the species to small, isolated populations. It is conceivable that degradation of remaining populations, as a result of harvesting or other habitat disturbance, has also had a negative effect on the extent of variation in remaining populations.

There seems little doubt that *B. corallina* is now severely endangered (Smith-Ramírez, 1996). As a result of the present study a total of 22 historical or current locations are now known for *B. corallina*; two of these are locations in which the species had not previously been recorded. Most locations consist of between two and approx. 20 individual clumps of stems, and in most cases the plants are surrounded by commercial plantations of *Eucalyptus* spp. and *Pinus radiata* D. Don. The intensity of threat to this species is illustrated by the fact that plants in the study site at San Juan de la Costa, which formed part of this study, were almost completely eliminated in the early part of 2000 by the deliberate burning of the habitat by the local landowner. Only the population in Reserva los Queules has any sort of state protection (Muñoz *et al*., 1996; Smith-Ramírez, 1996), but our observations indicate that only two individual plants remain there. Most of the other remaining populations are represented by only a few individuals. If loss of genetic variation is occurring, as the present results suggest, then the long-term evolutionary viability of the species must be in doubt, particularly when such losses are coupled with a high rate of habitat destruction.

Clearly conservation action is urgently required to safeguard remaining populations of *B. corallina*. The Chilean system of protected areas (SNASPE) covers 18 % of the area of the country, but of the 31 protected areas in central Chile, only four are located in the coastal regions (Lara and Fraver, 1997). There is an urgent need for the remaining coastal forests to be given government protection (Armesto *et al*., 1998). Given the genetic differentiation among populations recorded here, any *in situ* conservation strategy should aim to include populations from both the northern and southern part of the range of *B. corallina*, if the full breadth of genetic variation within the species is to be conserved. Moreover, the participation of local people is necessary for an effective *in situ* conservation programme, involving protection from browsing by cattle (Smith-Ramírez, 1996) and development of sustainable harvesting approaches (Gardner, 1996*b*). Observations suggest that if material is harvested appropriately the plant can resprout (Smith-Ramírez, 1996), offering the potential for sustainable production of materials for weaving.

For a species under such a severe degree of threat, *ex situ* approaches may also be appropriate as part of an overall conservation strategy (Hogbin and Peakall, 1999). One of the key objectives of this research was to evaluate the potential value of plants currently under cultivation in Britain, where individual plants have been known to survive

in gardens for more than 30 years (Gardner, 1996*b*). The results of the present study identified 16 different genotypes out of the 35 samples assessed. This degree of diversity was unexpected since it was hypothesized that the genetic variation would have narrowed since introduction, although the number of genotypes introduced by Pearce in 1860 is unknown. The original collection of *B. corallina* was thought to be in the form of seeds because Pearce collected his material in February (Veldkamp, 1984) which is the period in which *B. corallina* fruits in the wild. The hypothesis of seed origin is supported by the presence of a number of different genotypes in the cultivated material. These results contrast markedly with those obtained for individuals of the Chilean conifer *Fitzroya cupressoides* cultivated in Britain, all of which were found to be the same genotype, reflecting vegetative propagation since its introduction (Allnutt *et al.*, 1998).

Assessment of the genetic variation within the cultivated population suggests that the degree of polymorphism is comparable to that of a small wild population (i.e. eight individuals). This suggests that the cultivated population could be of some value for *ex situ* conservation. As part of the strategy of the International Conifer Conservation Programme (ICCP), which includes broadening the genetic diversity of threatened conifers and their associated species in cultivation (Rae and Gardner, 1993; Gardner and Thomas, 1996; Gardner, 1999), a programme has been initiated for the introduction into cultivation of genotypes from across the natural range of *B. corallina*. Owing to infrequent fruit-set in wild populations, only five out of the 30 genotypes collected were sampled as seed, the rest being propagated vegetatively. To date, genotypes from three wild populations (two from the north and one from the south of the range) have been propagated, and many of these are being distributed to locations within the ICCP's network of 'Safe Sites' in Britain and Ireland. The results of the current study will help to guide the ICCP in its future sampling strategies for *B. corallina*. As this research has recorded a high degree of genetic differentiation between wild populations there is clearly a need for the ICCP to embark upon further sampling from as many populations as possible. The genetic distinctiveness of the plant received from Australia (originally received from the Arboretum of the University of Valdivia, Chile) is enigmatic; it is conceivable that this represents a collection from a population that is now extinct. Alternatively, additional populations may await discovery, although on the basis of our field experience we suggest that the number of such populations is likely to be limited.

These results highlight the fact that the development of the *ex situ* population in cultivation in Britain could be of value to an overall conservation strategy for *B. corallina*. Such a strategy could potentially include restoration of populations which have been degraded, through reintroduction of plant material. However, any reintroduction must be undertaken at sites which are themselves protected; such protection is lacking at present. Care will also need to be taken to maintain the genetic integrity of populations both *in* and *ex situ*.

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