

## Lowland valleys shelter the ancient conifer *Fitzroya cupressoides* in the Central Depression of southern Chile

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**Abstract** The location of glacial refugia of tree taxa in Patagonia is determined primarily using data from the fossil pollen record. These data suggest that cold-tolerant conifers such as *Fitzroya cupressoides* probably survived the Last Glacial Maximum in coastal areas of southern Chile, where vegetation types corresponded to those currently found at relatively high altitudes in the Chilean Coastal Range. Much of this region is thought to have been covered by ice. However, the question remains whether *F. cupressoides* could have persisted locally in ice-free areas within the Central Depression of Chile. In this area, the species has been almost eliminated by human activities that have occurred since the 16th century. Geographic patterns of isozyme variation within 21 populations of *F. cupressoides* indicated that lowland populations showed high within-population isozyme variation. In addition, lowland populations were clearly differentiated genetically from those on the coast or in the Andes. These results strongly suggest that populations of *F. cupressoides* persisted in the Central Depression throughout glacial times. This implies that ice caps in the south-western Andes were probably not continuous, but, instead, the existence of ice-free areas in lowland valleys allowed the local survival of cold-temperate woody taxa.

**Keywords** alerce; biogeography; *Fitzroya cupressoides*; glacial refugia; isozymes; Patagonia; Pleistocene; South America

### INTRODUCTION

#### Quaternary history of austral South America

Patagonia was affected by the climate change events that occurred throughout the Pleistocene. However, the distribution of tree species during the Last Glacial Maximum (LGM) is still a matter of debate. Information on vegetation changes throughout the Quaternary at mid latitudes in Chile (39–42°S) is derived primarily from the pollen record obtained from sites in the Chilean Lake District (Heusser & Flint 1977; Villagrán et al. 1996). However, the restricted number and location of fossil pollen deposits limits the amount of information available regarding the shifts of species' ranges that occurred. In southern South America,

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past and present biogeographical patterns are particularly complex, owing to the physical heterogeneity of the landscape and the oceanic influence of the Pacific. In addition, many taxa cannot be distinguished readily from one another on the basis of pollen morphology (Villagrán et al. 1996), complicating inferences about the biogeographic history of particular species.

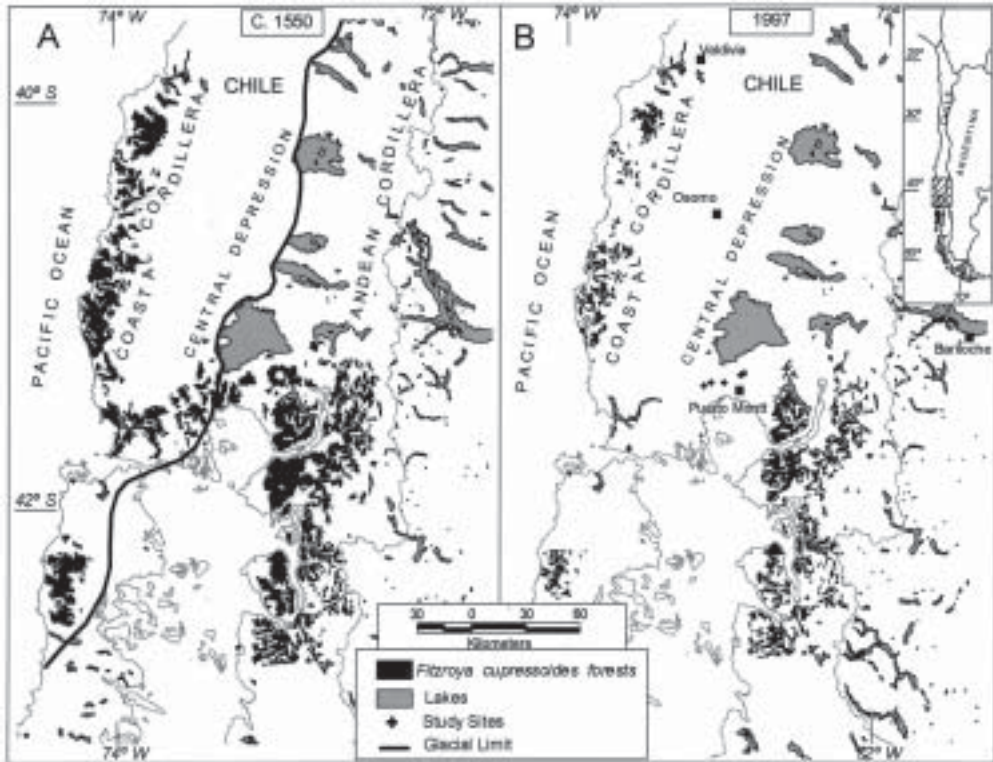
### **Glacial refugia of rainforest taxa**

For cold-tolerant rainforest conifers such as the Cupressaceae, including the genera *Fitzroya* and *Pilgerodendron*, it has been suggested that the most likely location of glacial refugia was west of the Andes in the Chilean southern coastal range, from north-western Chiloé Island to the north (Villagrán et al. 1996). Pollen obtained from bogs at middle latitudes indicated that rapid forest expansion occurred simultaneously on both sides of the Andes after glacial retreat (Markgraf et al. 1996). However, the Quaternary fossil record cannot be used to determine whether rainforest taxa east of the Andes were derived by postglacial dispersal from western refugia, or if they survived locally throughout the glacial period on both slopes of the Andean Cordillera. This question was addressed by an analysis of current patterns of genetic variation in populations of *F. cupressoides* (Premoli et al. 2000), which suggested that the modern populations of *F. cupressoides* are derived from at least two separate glacial refugia in coastal Chile and on the eastern southern slopes of the Andes. In addition, western populations in Chile formed a genetically similar group. Whether this similarity indicates continuous geneflow among locally surviving populations or postglacial colonisation from a unique gene pool has not been determined. The possible location of refugia for *F. cupressoides* on the western slopes of the Andes therefore remains open to doubt.

Here we test the hypothesis that populations of *F. cupressoides* located in the Central Depression are derived by postglacial expansion from a single refugium in coastal Chile. If such expansion occurred in a stepwise fashion, then we hypothesise that it would have left a genetic pattern, traceable using either gene frequency clines or rare allele distribution (Wheeler & Guries 1982; Hewitt 1996). Additionally, if recolonisation during postglacial expansion occurred by the establishment of small populations, then this may have reduced genetic variability in newly colonised areas, which may still be detectable (Comps et al. 2001). In contrast, if more than one refugium existed, we expect them to be genetically differentiated, based upon current gene frequency distribution and comparable levels of genetic variation.

### ***Fitzroya* in the Central Depression**

In the Central Depression of Chile, *Fitzroya cupressoides* (Molina) I.M.Johnst. (alerce) persists today only in small remnants (Fraver et al. 1999) of a forest that was once very extensive (Fig. 1) (Donoso 1994). Since the 16th century, accessible *F. cupressoides* stands have been decimated by timber extraction, and by the widespread use of fire to convert forest to agricultural land. As early as 1850, much of the *F. cupressoides* forest that formerly occurred north of Puerto Montt had been cleared, and by 1890 only a few trees remained in this lowland area (Fonck 1896; Pérez 1958; Veblen et al. 1976; Donoso 1983). Thus, the connection between Andean and coastal range populations was removed (Armesto et al. 1995). In the present study, samples taken from remaining *F. cupressoides* populations in the Central Depression were analysed by protein electrophoresis. The samples were compared with other populations analysed previously (Premoli et al. 2000) from the Coastal Range and the Andes of southern Chile. The following questions were addressed: are *F. cupressoides*



**Fig. 1** Distribution of *Fitzroya* from **A**, historical records c. 1550 and **B**, current extent of forests in southern Chile.

populations in the Central Depression the result of long-term persistence or recent colonisation? How diverse is the gene pool of highly fragmented, remnant populations of *F. cupressoides* in the Central Depression?

## METHODS

Six populations of *F. cupressoides* in the Central Depression of southern Chile were sampled for genetic analyses. These populations were geographically isolated from each other and surrounded by land under various uses. Thirty randomly selected individuals at least 20 m apart were selected at each site to avoid sampling of the same genotype. This is relevant in the case of *F. cupressoides* as vegetative reproduction may occur by root suckering, tipping, or layering (Veblen et al. 1995). Fresh leaf tissue samples from short, newly produced branches were collected from each individual. These samples were kept cold until proteins were extracted in the laboratory using the buffer by Mitton et al. (1979). Horizontal isozyme electrophoresis on starch gels was performed following the methods developed for *F. cupressoides* by Premoli et al. (2000). For each enzyme system, loci and alleles were numbered sequentially from the most anodal to the most cathodal. Genotypes from all individuals and populations were used to measure the levels of within-population genetic variation by the mean number of alleles/locus ( $A$ ), the percent of polymorphic loci ( $P_L < 0.95$  and  $P_L < 0.99$ , those with frequency of the most common allele  $< 0.95$  or  $< 0.99$ , respectively),

the observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, and the number of private alleles ( $N_P$ ), i.e., those occurring at only one group of populations out of three locales (Coastal Cordillera, Central Depression, and Andes). Heterogeneity in allelic frequencies was tested by  $\chi^2$  tests (Workman & Niswander 1970). The degree of among-population divergence was measured by Wright's  $F_{ST}$  based on eight polymorphic loci (*Aco*, *Idh*, *Mdh2*, *Per2*, *Pgi2*, *6Pgd1*, *Pgm1*, *Skdh1*) using FSTAT v. 2.9.1. (Goudet 2000). Means and confidence intervals (CI 95%) for  $F_{ST}$  were computed by jackknifing and bootstrapping over loci, respectively, following Weir & Cockerham (1984). Mean  $F_{ST}$  calculated for six populations from the Central Depression was compared with the value for 21 populations (i.e., including Coastal Cordillera, Central Depression, and Andes populations) and 30 populations covering the entire species' range (i.e., plus extra-Andean populations in Argentina) (A. Premoli unpubl. data; Premoli et al. 2000).

Discriminant forward stepwise multivariate analysis on allelic frequencies at the most variable loci (*Aco1*, *Aco3*, *Idh-1*, *Mdh2-1*, *Mdh2-3*, *Me2-1*, *Per1-3*, *Per2-3*, *Pgi2-4*, *Pgi2-5*, *Pgm1-3*) was used to classify different *a priori* alternative grouping hypotheses of populations in the three locales (Coastal Cordillera, Central Depression, and Andes). Wilk's  $\Lambda$  test was used to illustrate whether the groups (locales in our case) present significant differences in the position of their centroids ( $\Lambda = 0$  means perfect discrimination whereas  $\Lambda = 1$  indicates no discrimination). Squared Mahalanobis distances between the group centroids (population averages) were calculated to study the discriminatory power between any two groups. Allelic frequencies were arc sine transformed for multivariate analyses. In addition, genetic distinctiveness of locales (Coastal Cordillera, Central Depression, and Andes) was analysed by modified Rogers distance ( $D_{ST}$ ) which was calculated for all pairwise comparisons of groups of populations (Wright 1978).

## RESULTS AND DISCUSSION

### Within-population isozyme variability

Reduced mean levels of within-population isozyme variability were not detected in the Central Depression populations. Polymorphism in the Central Depression as a group was similar to or slightly more than that recorded for other clusters of populations or the species as a whole. The lowland group of populations had 57% polymorphism, meaning that 12 out of a total of 21 loci were polymorphic in at least one population. This implies that one more locus (*Mnr2*) was also polymorphic, compared with the value of 52% previously reported for *F. cupressoides* (Premoli et al. 2000). Coastal and Andean groups of populations had only 11 and 4 polymorphic loci in at least 1 population, respectively. In addition, a total of 46 alleles were recorded in the Central Depression while 44 and 41 were present in the Coastal and Andean populations, respectively. At the population level, the populations studied were similar to each other and to other groups of populations (such as the Coast, Andes, or the entire species) in terms of the mean number of alleles per locus, average polymorphism, and heterozygosity (one-way ANOVA or Kruskal-Wallis on ranks in cases where data were not normally distributed, all  $P > 0.2$ ) (Table 1).

Central Depression populations were highly heterogeneous in their allelic frequencies as detected by significant differences recorded in 12 out of 13 possible tests ( $\chi^2 = 679.5$ , d.f. = 115,  $P < 0.001$ ). Moreover, for the Coastal and Andean data sets, allelic frequencies were significantly different at 10 and 5 out of 12 cases, respectively ( $\chi^2 = 299.8$ , d.f. = 114;  $\chi^2 = 580.0$ , d.f. = 161,  $P < 0.001$ ). The high heterogeneity recorded throughout Central Depression populations was also reflected by the average divergence index  $F_{ST} = 0.112$  (CI<sub>95%</sub> = 0.079–0.132) which was within the range of that obtained for western populations in

Chile ( $F_{ST} = 0.124$ ;  $CI_{95\%} = 0.064\text{--}0.135$ ) and all tested populations of the species (average for 30 populations of *F. cupressoides*:  $F_{ST} = 0.125$ ;  $CI_{95\%} = 0.072\text{--}0.161$ ).

### Postglacial expansion from a single coastal refugium

These results suggest that dispersal from glacial refugia probably occurred in a sequential fashion, by the foundation of populations that probably consisted of a relatively low number of individuals. These population bottlenecks probably resulted in the loss of alleles during the migration process as well as in the lower genetic variation of more recently established populations compared with those of possible refugia. Population genetic theory predicts a limited effect of bottlenecks on gene diversity (i.e., expected heterozygosity) compared with loss of allelic richness. In particular, low frequency alleles tend to be lost by the combined effects of genetic drift and inbreeding acting in small populations (Nei et al. 1975).

Possible postglacial migration paths were examined by multiple regression analyses of allele frequencies and within-population genetic variability indices using longitude and latitude as independent variables. It was predicted that if glacial refugia existed in north-western Chiloé Island, as suggested by the pollen record (Villagrán et al. 1996), isozyme variation would decrease towards the north and the east (i.e., the Andes). Forward stepwise models revealed no significant geographic component for each of the 54 allele frequencies (including shared and rare alleles) or different measures of genetic variation tested. The lack of correlation between geographic and genetic patterns may be associated with the fact that *F. cupressoides* is a polyploid; duplicated copies of genes may buffer the effects of population bottlenecks.

**Table 1** Genetic variability at 21 loci in populations of *Fitzroya* from the Central Depression, Chile. Average values are compared with those for populations from the Coastal and Andean Cordillera. A, mean number of alleles/locus;  $P_L < 0.95$  and  $P_L < 0.99$ , the percent of polymorphic loci (95 and 99% criteria, respectively);  $H_O$ , observed heterozygosity;  $H_E$ , expected heterozygosity;  $N$ , the total number of sampled populations within each locale (Central Depression, Coastal, and Andean Cordillera);  $N_P$ , the number of private alleles that characterise different locales. Standard errors are in parentheses.

Population	A	$P_L < 0.95$	$P_L < 0.99$	$H_O$	$H_E$
Astilleros	1.6 (0.2)	28.6	42.9	0.093 (0.034)	0.102 (0.036)
Lahuen Ñadi	1.3 (0.1)	19.0	28.6	0.040 (0.022)	0.052 (0.022)
Manquemilla	1.4 (0.1)	23.8	38.1	0.048 (0.017)	0.072 (0.027)
Metrequén	1.4 (0.1)	38.1	42.9	0.079 (0.035)	0.083 (0.027)
Santa Teresa	1.3 (0.1)	14.3	28.6	0.072 (0.037)	0.068 (0.034)
Tepual	1.3 (0.1)	19.0	28.6	0.070 (0.037)	0.069 (0.033)
Central Depression $N = 6$ ; $N_P = 8$	1.4 (0.06)	23.8 (4.4)	34.9 (4.6)	0.067 (0.01)	0.074 (0.01)
Coastal Cordillera $N = 8$ ; $N_P = 4$	1.4 (0.05)	21.4 (3.5)	28.0 (2.9)	0.054 (0.008)	0.063 (0.007)
Andean Cordillera $N = 7$ ; $N_P = 2$	1.4 (0.07)	17.0 (1.7)	28.6 (5.4)	0.064 (0.006)	0.067 (0.007)
Species average $N = 30$	1.4 (0.03)	23.0 (1.9)	33.0 (2.0)	0.071 (0.005)	0.075 (0.005)

Overall population pairwise genetic distance was analysed among locales, as it was hypothesised that the distance would tend to increase with the time elapsed since population establishment from possible source populations. Geographically distant populations had significantly lower average genetic distances and, thus, were genetically more similar to each other (i.e., between Andes and Coast,  $D_{ST} = 0.07$ ) than geographically closer populations (i.e., between Central and either Andes or Coast,  $D_{ST} = 0.13$  and  $0.12$ , respectively) (Kruskal-Wallis ANOVA on ranks, both pairwise tests  $P < 0.05$ ). Additionally, the latter were not significantly different from each other (Kruskal-Wallis ANOVA on ranks  $P > 0.05$ ). One possible explanation of these results is that genetic variants have been lost along the Coastal Range, so it may be the case that postglacial migration has occurred from a coastal refuge that is no longer extant. An alternative possibility is that the Central Depression was the source population from which genetic variants in common between the Coast and the Andes have been eliminated.

### **Local survival in multiple refugia?**

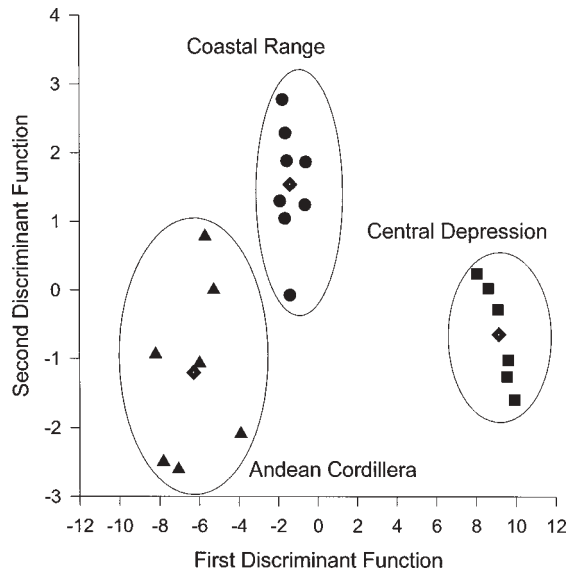
Low-frequency (rare) alleles were used to analyse biogeographical patterns of genetic differentiation among the Coastal Cordillera, the Central Depression, and the Andes by multivariate discriminant analysis. Populations sharing rare alleles are more likely to be the result of mutations that occurred in recent history or because more distantly related populations lost such alleles following divergence and isolation (Wheeler & Guries 1982). Discriminant analysis of 14 variables, 11 of which were entered in the forward stepwise model, yielded a highly significant distinction of the 3 locales;  $F_{(22,16)} = 7.4$ ,  $P < 0.0001$  (Fig. 2). The first function explained 96% of the total variance, and significantly discriminated among the position of the population groups centroids (Wilk's  $\Lambda = 0.008$ ,  $\chi^2 = 62.7$ , d.f. = 22,  $P < 0.0001$ ). Significant distances were obtained between all pairwise comparisons of centroids ( $P < 0.05$ ). The second discriminant function was not significant (Wilk's  $\Lambda = 0.4$ ,  $\chi^2 = 13.2$ , d.f. = 10,  $P = 0.21$ ).

Populations and locales (Coastal Cordillera, Central Depression, and Andes) were also characterised by the presence of genetic variants that in most cases were rare alleles (frequency  $< 0.1$ ). These were found at only one locale (private alleles) or at only one population (unique alleles). Eight private alleles were found in the Central Depression, 6 of which were unique alleles (data not shown). In contrast, 4, and only 1 private, alleles were recorded in the Coast and the Andes, respectively.

### **Long-term persistence of *F. cupressoides* in lowland valleys**

The fact that levels of genetic variation found in the Central Depression are comparable to that measured in the entire species, together with their clear distinction from other Coastal and/or Andean populations, and the presence of an elevated number of private alleles, strongly suggest that lowland valleys in southern Chile sheltered *F. cupressoides* throughout glacial times. Therefore, *F. cupressoides* probably survived locally in different glacial refugia along its range on the western slopes of the Andes, much as was hypothesised for populations on the eastern slopes of the Andes (Premoli et al. 2000). Information from the pollen record (Heusser & Flint 1977; Villagrán et al. 1996) as well as spatial patterns of genetic variation (Premoli 1998; Allnutt et al. 1999; Premoli et al. 2000) suggested that western refugia were most likely located in the Coastal Cordillera. Pollen deposits from the height of the glaciation dated by radiocarbon between 20 160 and 23 020 years BP (Denton 1993) indicate that vegetation in northern Chiloé Island and in the lowlands of the Lake District was dominated by non-woody elements. Rather, it was characterised by a mosaic of north-Patagonian forest with small amounts of cold-tolerant conifers such as *Fitzroya* and

**Fig. 2** First and second functions used to differentiate by multivariate discriminant analysis among different *a priori* grouping schedules of populations of *Fitzroya* from the Coastal Cordillera (circles), the Central Depression (squares), and the Andes (triangles) using arc sine transformed allelic frequencies at 11 rare alleles. Centroids (population averages) are depicted by rhomboids.



*Pilgerodendron* (not pollen-distinguishable) and subantarctic moorland (Villagrán et al. 1996). However, these vegetation types correspond to those currently found on the mountaintops of the Chilean Coastal Range (Armesto et al. 1996). Consequently, full-glacial conditions probably resulted in altitudinal lowering of the cold-resistant elements (Magellanic moorlands, *Nothofagus*, and conifers), which survived the last glaciation within microclimatically favourable habitats (Markgraf et al. 1995, 1996) at low elevation sites within the Lake District, between 40 and 43°S (Villagrán et al. 1996; Villagrán 2001). Under this scenario, *F. cupressoides* persisted locally in lowland areas such as the Central Depression during the height of the glaciation. Prolonged isolation may have resulted in the elevated genetic divergence of Central Depression populations from other locales such as those in the Coast or the Andes.

Evidence of long-term climatic change in the Central Depression is available from a floating 1229-year tree ring chronology dating back to approximately 50 000 <sup>14</sup>C years BP that was derived from *F. cupressoides* wood remnants. This evidence suggests that comparable cycles in tree growth occurred between the interstadials of the last glaciation (which were relatively warm) and current interglacial conditions. Therefore, similar climatically driven processes appear to have affected the radial growth of *F. cupressoides* since the Late Pleistocene (Roig et al. 2001). Consequently, the evolutionary history of the species, together with the genetic characteristics of *F. cupressoides* may reflect long-term persistence in particular habitats of austral South America.

On the other hand, the pollen record from the Lake District shows that conifers became dominant during the Early Holocene (c. 12 000 BP), at the same time that waterlogged soils and swampy habitats were widespread in the Central Depression. Thus, following deglaciation, *F. cupressoides* could have rapidly colonised ice-free areas from local refugia in the Central Depression. This could have been interpreted from pollen evidence as range expansion from coastal refugia (Villagrán 1988; Armesto et al. 1995). However, Central Depression populations today probably represent only a sample of the original gene pool that inhabited lowland areas, which in turn could have been the main source for postglacial expansion of *F. cupressoides* in southern Chile.

Small and isolated stands of *F. cupressoides* in the Central Depression are considered “to represent an extreme case of habitat fragmentation” (Fraver et al. 1999) given that an extensive conifer forest was apparently once continuous throughout the Central Depression between the Coastal Range and the Andes (Donoso 1983). These forests must have competed in magnificence with California’s coast redwood forests (Simmons & Vale 1975), and for more than two decades ecologists have been requesting legal protection to avoid further impoverishment of the *F. cupressoides* gene pool (Veblen et al. 1976). Lowland populations in southern Chile appear to be maintaining levels of within-population genetic polymorphism and among-population gene flow rates typical for the species as a whole, as indirectly estimated by  $F_{ST}$ . These results further emphasise the importance of preserving remnant stands of *F. cupressoides* in the Central Depression. The genetic distinctiveness of these populations highlights the fact that conservation efforts directed only at the species level may result in the loss of genetic diversity.

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