

Pleistocene refugia and recolonization routes in the southern Andes: insights from *Hypochoeris palustris* (Asteraceae, Lactuceae)

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Abstract

Hypochoeris palustris (Phil.) De Wild. is a species growing in the southern Andean chain. To elucidate potential Pleistocene refugia and recolonization routes in the southern Andes, we analysed amplified fragment length polymorphisms (AFLPs) in 206 individuals in 21 populations of *H. palustris* from the coastal Cordillera, the central, northern, and eastern ranges of the southern Andes, and Patagonia. Populations from the coastal Cordillera harboured more private AFLP fragments, and exhibited a higher frequency of polymorphic fragments as well as higher Shannon diversity than all other areas investigated. The comparison among pooled AFLP profiles of each region revealed that the central Andean ranges shared most fragments with populations from the margins of the distributional area in the Andes, in the N, E, and S (Patagonia). Phenetic analysis indicated close relationships among populations of the central ranges. Populations of the coastal Cordillera were shown to be highly differentiated from the Andean populations. It is very likely therefore that (1) *H. palustris* recolonized the central ranges of the southern Andes from nearby refugia, possibly unglaciated areas N, E, and/or S of its present distributional area; (2) the postglacial spread of *H. palustris* in the central ranges of the southern Andes occurred rapidly; and (3) the coastal Cordillera served as a refugium for *H. palustris*, but these populations did not contribute to the recolonization of the central Andean ranges.

Keywords: amplified fragment length polymorphisms (AFLPs), Andes, Compositae, phylogeography, Quaternary biogeography, South America

Received 4 May 2004; revision received 13 September 2004; accepted 22 September 2004

Introduction

The flora of the southern Andes of South America exists along a chain of high mountains that have been affected by tectonics, volcanism and Pleistocene glaciation (Simpson-Vuilleumier 1971; Simpson 1975, 1979, 1983; Villagrán *et al.* 1995, 1998; Thorson 1999; McCulloch *et al.* 2000). At the beginning of the Miocene, the southern Andes began its uplift which continues to the present day (Ramos 1989). Paralleled with evolution of the major mountain system are recent Pleistocene glaciations (Hollin & Schilling 1981; Clapperton 1993; McCulloch & Bentley 1998; Hulton *et al.*

2002; Sugden *et al.* 2002). At the tip of South America, large glacial ice fields covered broad areas from c. 36° S–56° S; today, a more reduced glacial region of c. 46° S–55° S still persists in southern Chile (Hollin & Schilling 1981; Hulton *et al.* 2002). Also, local glaciers were located in the southern Andean chain during the Pleistocene N of closed ice fields (Hollin & Schilling 1981; Clapperton 1993; Hulton *et al.* 2002). Superimposed on these events have been repeated volcanic disturbances with local impact. Numerous explosions of ash and lava have occurred since Pleistocene times, the historically recent ones being well documented (González-Ferrán 1994).

The biogeographical effect and stimulus for speciation and intraspecific differentiation of the Pleistocene have been well documented in Europe and North America (e.g. Hewitt 1996, 2000; Comes & Kadereit 1998; Petit *et al.* 2003), but much less in South America. In Europe, massive ice

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sheets covered the Alps, Pyrenees and Carpathians. The flora was pushed into refugia mainly south of these glaciated regions (Hewitt 1996, 2000; Petit *et al.* 2003; Stehlik 2003; Tribsch & Schönswetter 2003). In North America, the ice sheet also pushed the flora southward, but because the mountains run north–south and not east–west (as in Europe), fewer barriers to migration existed (Abbott *et al.* 2000). In South America, the impacts of glaciation have been less well documented, although it is clear that much local glaciation occurred that caused extinction–migration of the flora northward and to lower elevations (Simpson-Vuilleumier 1971; Simpson 1975, 1979, 1983; Villagrán *et al.* 1995, 1998; Thorson 1999; McCulloch *et al.* 2000). Specific effects of volcanism have been underinvestigated, although impacts on vegetation because of historical eruptions have received some attention (Tremetsberger *et al.* 2003a).

Understanding the impacts of Pleistocene glaciation on population differentiation in the southern Andes requires choosing a specific plant group that is distributed in this region. *Hypochaeris* (Asteraceae, Lactuceae) contains about 60 species, of which more than 40 are restricted to South America, and approximately two-thirds of these occur in Chile and Argentina. Molecular phylogenetic studies have revealed that the group came from the Old World, probably from the Mediterranean region during the Pliocene or Pleistocene; possibly through long-distance dispersal (Samuel *et al.* 2003; Tremetsberger *et al.* unpublished). After colonization, a variety of habitats in southern South America offered opportunities for speciation, resulting in a multitude of taxa adapted to different habitats from sea level to over 5000 m.

Hypochaeris palustris nowadays occurs in areas which were glaciated during the Pleistocene (Figs 1 and 3), as well as in unglaciated, hence presumably refugial areas. Both empirical reconstruction (Hollin & Schilling 1981) and modelling (Hulton *et al.* 2002) of the Last Glacial Maximum (LGM) in southern South America show that the coastal Cordillera, which parallels the main Andean Cordillera in Chile, was ice-free from latitude 42° northwards (Fig. 1). Populations of *H. palustris* in this area could have served as source populations for postglacial colonization of the Andes in southern South America. Likewise, populations could have survived the LGM, migrating downwards on the eastern slopes of the Andes, then settling down along its base in Argentina. However, populations could have survived there only in a narrow strip between the frigid glacial ice sheet and the steppe (Pastorino & Gallo 2002). Populations could also have migrated northward to partly glaciated or unglaciated regions of the Andes. Another possible refugium might have been in the very south of the continent, in Tierra del Fuego, which was not glaciated in its lower parts (the Pleistocene sea level was lower than today's; Hulton *et al.* 2002).

Several studies have revealed that populations in colonized areas differ in genetic characteristics from those of

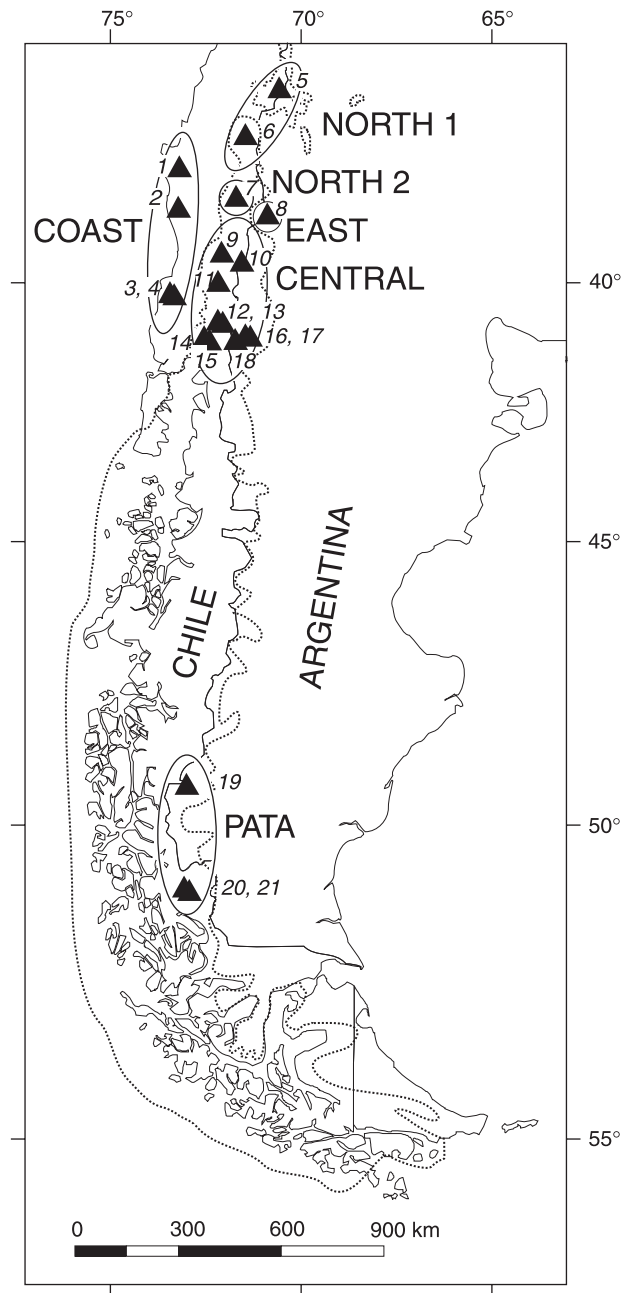


Fig. 1 Southern Chile and Argentina showing glacial extent during the Last Glacial Maximum (LGM, dotted line; after Hollin & Schilling 1981; Hulton *et al.* 2002) and locations of populations of *Hypochaeris palustris* sampled (see Table 1).

their respective source populations. Most studies characterized source populations by higher levels of allelic richness (e.g. Hewitt 1996, 2000; Amsellem *et al.* 2000; Comps *et al.* 2001; Widmer & Lexer 2001; Chauvet *et al.* 2004; Olsen *et al.* 2004). Colonized areas are expected to harbour only a subset of their source gene pools (Hewitt 1996, 2000; Soltis *et al.* 1997; Taberlet *et al.* 1998) and genetic diversity often parallels this pattern (Shapcott 1998; Premoli *et al.* 2002;

Rendell & Ennos 2002; Olsen *et al.* 2004). In some studies, however, populations of colonized areas are found to be equally or even more genetically variable than those of refugia, probably because of amalgamation of immigrants of several different refugial areas (Comps *et al.* 2001; Petit *et al.* 2003; Olsen *et al.* 2004; Schoenswetter *et al.* 2004).

Here, we report a phylogeographical study of *H. palustris* based on AFLP (amplified fragment length polymorphism) data covering large portions of its total distributional area. We include accessions from the coastal Cordillera, the central, northern, eastern, and southern (Patagonian) ranges of the Andes of southern South America, and focus on the following questions:

Did populations from the coastal Cordillera serve as a source for postglacial recolonization of the southern Andes? The coastal Cordillera has never been glaciated, hence might have served as a source for recolonization of the Andean region. This would imply genetic similarity between populations from the coastal Cordillera and from the main Andean Cordillera, and a depletion of genetic diversity of the Andean populations as compared with populations from the coastal Cordillera.

Do populations from the previously glaciated central Andean range originate from one or more source areas? Apart from refugial populations in the coastal Cordillera, populations could have survived in the N, E, and S of the present distributional area of *H. palustris*. These populations might have served as additional source populations apart from populations from the coastal Cordillera.

What was the direction of gene flow within the Andes after the glacial retreat? Gene flow is expected to have occurred from refugia at the unglaciated edges to the centre of the Andean Cordillera. This would imply a depletion of genetic variation from the edges to the centre.

Materials and methods

During radiation of *Hypochaeris* in South America, a group of taxa has evolved at higher elevations along the southern Andean chain: pioneer *Hypochaeris tenuifolia* on volcanic peaks; and *Hypochaeris acaulis* and *Hypochaeris palustris* in moist seeps and near rivulets (Tremetsberger *et al.* 2003a,b). *H. palustris* is a distinct species, approximately 10–30 cm tall, with one or several yellow, ligulate flower heads.

The present distribution of the (facultatively) autogamous *H. palustris* is on the South American continent in Chile and Argentina from c. 36° S to the southern tip of the continent (Tierra del Fuego; 56° S) and on the Falkland Islands (Islas Malvinas; as *H. arenaria*; Cabrera 1963). It is confined to oceanic climate (i.e. the western side of the continent). Barriers within the total distributional area are

provided by the longitudinal valley (Valle Longitudinal) between the coastal and Andean Cordilleras in the northern part of its range and by the Patagonian icefield in the central part of its range.

Sampling

Leaf material of *H. palustris* was collected and dried in silica gel from the following populations (Fig. 1, Table 1): four populations (1–4) from the coastal Cordillera, Chile (Regiones VIII, IX and X); and 17 populations from the Andean Cordillera, of which three populations (5, 6, 7) were from the northern part of the distributional area, Chile (Regiones VII, VIII, and IX); wherein one population (8) was from the eastern side of the Andes, Chile (Región IX); 10 populations (9–18) were from the central range, Chile (Regiones IX and X) and Argentina (Río Negro); and three populations (19–21) were from the southern part of the distributional area, Chile (Región XII) and Argentina (Santa Cruz). Distance between samples was variable (Fig. 1). Vouchers of each population are deposited to WU, most of them with duplicates at CONC or LP. Populations 1–4 are from areas not glaciated during the LGM. Population 8 is from an area probably not glaciated, whereas populations 5 and 6 are from areas partially glaciated, N of the closed ice shield (cf. Figure 1). Table 1 shows the number of individuals per population.

DNA isolation and AFLP fingerprinting

Total genomic DNA was extracted from similar amounts of dried tissue following a CTAB protocol (Doyle & Doyle 1987) with modifications and quantified photometrically (UV 160 A Spectrophotometer, Shimadzu). The AFLP procedure was carried out according to Tremetsberger *et al.* (2003a). Because *H. palustris* and *H. tenuifolia* are closely related (Stuessy *et al.* in press), the same primer combinations used for the study of *H. tenuifolia* (Tremetsberger *et al.* 2003a) were also applied to *H. palustris*. These are: *EcoRI*(Fam)-ACT/*MseI*-CAG, *EcoRI*(Joe)-ACG/*MseI*-CTC, and *EcoRI*(Ned)-AGC/*MseI*-CAG. AFLP fragments were scored in GENOGRAPHER (version 1.6.0, © Montana State University 1998; <http://hordeum.oscs.montana.edu/genographer>) and exported as presence/absence matrix.

Data analysis

To test correspondence of data gathered by each of three primer combinations, Jaccard similarity matrices on all 206 individuals analysed were constructed for each primer combination separately using R PACKAGE (version 4.0; Casgrain & Legendre 2000). The similarity matrices were used to compute pairwise correlations among them by a Mantel test (standardized Mantel statistic *r*; Casgrain &

Table 1 Regional acronyms, population numbers, collectors and population accession numbers, number of investigated individuals, locations, percentage of different AFLP phenotypes, percentage of polymorphic fragments, Shannon diversity (H_{Sh}), and number of private fragments (fixed private fragments in parentheses) of *Hypochaeris palustris*. Diversity indices in the last three columns are calculated from the standardized data set (eight randomly selected individuals per population). CMB, C. M. Baeza; RH, R. Hössinger; GK, G. Kottirsch; PS, P. Schönswetter; TFS, T. F. Stuessy; KT, K. Tremetsberger; AT, A. Tribsch; EU, E. Urtubey

Regional acronym	Pop. no.	Collectors and number	N_{ind}	Location	Phenotypes (%)	Polymorphism (%)	H_{Sh}	$N_{private\ fragments}$
COAST	1	KT & RH 49	9	Chile, Región VIII, Parque Nacional Nahuelbuta:	100	13.9	5.1	4 (0)
	2	KT & RH 57	6	Laguna Las Totoras Chile, Región IX, drained remnant of a bog W of Villa Araucaria	100			
	3	KT & RH 102	9	Chile, Región X, Cordillera Pelada, Monumento Natural Alerce Costero:	100	13.2	4.5	0 (0)
	4	KT & RH 103	8	Turbera Lañinagual Chile, Región X, Cordillera Pelada, Monumento Natural Alerce Costero:	100	20.7	7.6	4 (1)
NORTH 1	5	TFS & CMB 15572	2	El Mirador Chile, Región VII, Laguna del Maule	100			
	6	TFS, CMB & GK 15566	10	Chile, Región VIII, Termas de Chillán: Valle de las Nieblas	100	8.6	3.7	5 (2)
NORTH 2	7	TFS & CMB 15581	12	Chile, Región IX, along road between Malalcahuello and village Lonquimay	17	0.7	0.3	0 (0)
EAST	8	TFS & CMB 15588	8	Chile, Región IX, Paso Pino Hachado	63	2.6	1.1	1 (1)
CENTRAL	9	TFS & CMB 15606	9	Chile, Región IX, Volcán Villarrica	100	17.2	6.5	2 (0)
	10	TFS & CMB 15824	13	Chile, Región IX, Volcán Lanín: near Laguna Huinfuicá	92	9.3	3.5	0 (0)
	11	TFS & CMB 15825	16	Chile, Región IX, Volcán Choshuenco	13	4.0	1.4	0 (0)
	12	TFS & CMB 15629	14	Chile, Región X, Volcán Casablanca: Crater Rayhuen	50	3.3	0.8	0 (0)
	13	TFS & CMB 15630	12	Chile, Región X, Volcán Casablanca: Cerro Mirador	100	12.6	4.7	0 (0)
	14	TFS & CMB 15829	11	Chile, Región IX, Volcán Osorno	64	7.9	3.8	0 (0)
	15	TFS & CMB 15830	15	Chile, Región IX, Volcán Osorno	47	4.6	1.5	0 (0)
	16	TFS, EU & KT 18029	4	Argentina, Prov. Río Negro, Cerro López	75			
17	TFS, EU & KT 18030	13	Argentina, Prov. Río Negro, Cerro López	62	7.3	2.9	0 (0)	
18	TFS, EU & KT 18048	10	Argentina, Prov. Río Negro, Cerro Tronador	100	11.9	4.7	0 (0)	

Table 1 Continued

Regional acronym	Pop. no.	Collectors and number	N_{ind}	Location	Phenotypes (%)	Polymorphism (%)	H_{Sh}	$N_{private}$ fragments
PATA	19	PS & AT 5642	9	Argentina, Prov. Santa Cruz, Parque Nacional Los Glaciares	78	4.6	1.9	2 (0)
	20	PS & AT 5639	9	Chile, Región XII, Parque Nacional Torres del Paine: Refugio Pehoe	67	6.0	2.1	2 (0)
	21	PS & AT 5648	7	Chile, Región XII, Parque Nacional Torres del Paine: between Valle Ascencio and Mirador Torres del Paine	86			

Legendre 2000). One-tailed test statistic probabilities were obtained through 9999 permutations (Casgrain & Legendre 2000).

Within-population genetic diversity was assessed for eight individuals per population, chosen by random generator (for those populations with at least eight individuals), using the percentage of polymorphic fragments, and Shannon diversity, $H_{Sh} = -\sum(p_i * \ln(p_i))$, where p_i is the frequency of the i th fragment in the respective population based on all AFLP fragments recorded (i.e. Shannon diversity was calculated for each putative locus and then summed without averaging by the number of loci) (Legendre & Legendre 1998). The number of AFLP phenotypes and the number of polymorphic fragments were assessed using ARLEQUIN (version 1.1; Schneider *et al.* 1997). The number of polymorphic fragments was divided by 151 (total number of fragments in *H. palustris*) to obtain the percentage of polymorphic fragments. The numbers of private and fixed private fragments were estimated for populations (also from eight randomly chosen individuals per population) as well as for regions (including populations with at least eight individuals).

To test the hypothesis of the populations from the coastal Cordillera being the source of the Andean populations, the number of fragments shared between pooled AFLP profiles (i.e. the combination of presences of AFLP markers found in the single individuals) of the coastal Cordillera populations (COAST), central (CENTRAL), northern (NORTH, comprising NORTH 1 and NORTH 2), eastern (EAST), and southern, Patagonian (PATA) Andes was estimated (see Table 1 for population affiliations). Populations were grouped a priori according to their geography. Furthermore, a Bayesian clustering approach implemented in the program STRUCTURE (Pritchard *et al.* 2000) was used as a guide for

detailed final grouping of the populations (results not shown). Correlation among geographical and genetical (Jaccard) distances was obtained by a Mantel test by calculating 9999 permutations using R PACKAGE (version 4.0; Casgrain & Legendre 2000). A phenogram representing genetic distances among populations was created by importing population-pairwise F_{ST} values generated by ARLEQUIN (version 1.1; Schneider *et al.* 1997) into SPLITSTREE [version 3.2 (<http://bibiserv.techfak.uni-bielefeld.de/splits/>)]. Robustness of nodes was tested by 1000 bootstrap replicates in a neighbour-joining analysis with Nei-Li distances (using all individuals; PAUP* version 4.0b10; Swofford 2002). Genetic differentiation within and among populations (groups) was assessed by analysis of molecular variance (AMOVA) using ARLEQUIN (version 1.2; Schneider *et al.* 1997) with 10% allowance for missing data with three hierarchical levels defined: among regions, among populations within regions, and among individuals within populations. The significance of differentiation was tested with 1023 permutations, where P denotes the probability of observing a random value as large or larger as the observed value.

Results

Analysis of AFLP fragments of all three primer combinations of all individuals and populations of *Hypochaeris palustris* yielded a total of 151 unambiguously scorable DNA fragments, of which 126 (83%) were polymorphic. A total of 206 individuals investigated represented 147 different AFLP phenotypes. The same phenotypes occurred mainly within populations (see Table 1), with the only exception of one phenotype shared by one individual of populations 20 and 21 each (both: Torres del Paine). The primer combinations *EcoRI*(Fam)-ACT/*MseI*-CAG yielded 54 fragments,

EcoRI(Ned)-AGC/*MseI*-CAG 50 fragments, and *EcoRI*(Joe)-ACG/*MseI*-CTC 47 fragments. A correlation test performed on each pairwise combination of three Jaccard similarity matrices, obtained from analysis of each primer combination separately, yielded high values: Mantel's r were 0.923 among *EcoRI*(Fam)-ACT/*MseI*-CAG and *EcoRI*(Ned)-AGC/*MseI*-CAG, 0.940 among *EcoRI*(Fam)-ACT/*MseI*-CAG and *EcoRI*(Joe)-ACG/*MseI*-CTC, and 0.931 among *EcoRI*(Ned)-AGC/*MseI*-CAG and *EcoRI*(Joe)-ACG/*MseI*-CTC with all values being significant (1-tailed $P < 0.0001$) after 9999 permutations.

Large-scale genetic relationships

The Mantel test revealed almost no correlation among geographical and genetical distances, when all 206 individuals from all populations were analysed together (Mantel's $r = 0.097$; $P < 0.0317$). A high correlation was found when only individuals from COASTAL populations (without Andean populations; a total of 32 individuals) were analysed together (Mantel's $r = 0.701$; $P < 0.0001$). When all individuals from all Andean populations were analysed together (without populations from the coastal Cordillera; a total of 174 individuals), a weaker correlation among geographical and genetical distances was found (Mantel's $r = 0.480$; $P < 0.0001$). The correlation was even weaker when only individuals from CENTRAL populations (a total of 117 individuals) were analysed together (Mantel's $r = 0.210$; $P < 0.0001$).

The comparison of the pooled AFLP profiles of all individuals of each region (Table 2) revealed that most fragments were shared by CENTRAL and PATA (35; without counting fragments occurring in all regions), followed by CENTRAL and NORTH 1 (30), CENTRAL and EAST (27), and CENTRAL and NORTH 2 (26). Least fragments were shared by COAST and NORTH 2 (2), COAST and EAST (6), COAST and NORTH 1 (8), and COAST and PATA (10). Twenty one fragments were shared by five regions, but were absent in a sixth region. Of these, 18 fragments were shared by NORTH 1, NORTH 2, EAST, CENTRAL, and PATA, but did not occur in COAST.

These pooled AFLP profiles are reflected in highly supported groupings in AMOVA. AMOVA carried out at two hierarchical levels apportioned 78% of variance among populations ($P < 0.000$) and 22% among individuals within populations. The largest variance (76%) was revealed comparing COAST with all Andean regions NORTH 1-NORTH 2-EAST-CENTRAL-PATA combined ($P < 0.001$). We also investigated the other pairwise comparisons among regions. Comparing groupwise each Andean region with every other revealed the largest variances among NORTH 1 and NORTH 2-EAST-CENTRAL-PATA (38%; $P < 0.005$) and among PATA and NORTH 1-NORTH 2-EAST-CENTRAL (38%; $P < 0.001$). Variance between NORTH 2 and NORTH 1-EAST-CENTRAL-PATA was 30% ($P < 0.126$) and variance between EAST and NORTH 1-NORTH 2-CENTRAL-PATA was 13% ($P < 0.463$). Variances among pairwise comparisons of regions were 72% between COAST and NORTH 1, 75% between COAST and NORTH 2, 72% between COAST and EAST, 80% between COAST and CENTRAL, and 81% between COAST and PATA, respectively (Table 2). Variances between NORTH 1 and NORTH 2, EAST, CENTRAL, and PATA were 35%, 37%, 47%, and 71%, respectively. Variances between NORTH 2 and EAST, CENTRAL, and PATA were 94%, 45%, and 76%, respectively. Variances among pairwise comparisons of regions EAST and CENTRAL, and EAST and PATA were 29% and 70%, respectively. Variance between CENTRAL and PATA was 43% (Table 2). AMOVA apportioned 45% variance among populations of COAST ($P < 0.000$), 50% among populations of NORTH 1 ($P < 0.013$), 41% among populations of CENTRAL ($P < 0.000$), and 40% among populations of PATA ($P < 0.000$).

Phenetic analysis resulted in a phenogram with the largest divide between populations from COAST and all Andean populations (100% bootstrap percentage, BP; Fig. 2). Within COAST, the two more northern populations 1 and 2 formed a group (60% BP), as did the two more southern populations 3 and 4 (less than 50% BP). CENTRAL populations diverged in a star-like pattern. The populations of NORTH were nested within the CENTRAL samples. The same applied to the eastern population 8 and to the populations of PATA, the latter of which grouped together (90% BP).

Table 2 Variance (AMOVA; significance in parentheses; above diagonal) and numbers of shared fragments (excluding fragments occurring in all regions; below diagonal) between regions in *Hypochaeris palustris* based on analysis of 151 AFLP fragments

	COAST	NORTH 1	NORTH 2	EAST	CENTRAL	PATA
COAST		72 ($P < 0.058$)	75 ($P < 0.189$)	72 ($P < 0.193$)	80 ($P < 0.001$)	81 ($P < 0.033$)
NORTH 1	8		35 ($P < 0.332$)	37 ($P < 0.320$)	47 ($P < 0.020$)	71 ($P < 0.104$)
NORTH 2	2	24		94 ($P < 0.000$)	45 ($P < 0.086$)	76 ($P < 0.262$)
EAST	6	23	19		29 ($P < 0.176$)	70 ($P < 0.276$)
CENTRAL	17	30	26	27		43 ($P < 0.001$)
PATA	10	24	22	23	35	

Table 3 Mean percentage of different phenotypes (SD in parentheses), mean percentage of polymorphic fragments (SD in parentheses), Shannon Diversity Indices (H_{Sh} , SD in parentheses), and number of private fragments (fixed private fragments in parentheses) compared among regions of *Hypochaeris palustris*. Values in the last three columns are calculated for the standardized data set of 17 populations with eight randomly selected individuals per population

Region	Percentage of different phenotypes	Percentage of polymorphism	H_{Sh}	$N_{\text{private fragments (fixed)}}$
COAST	100 (0)	15.9 (4.1)	5.7 (1.6)	25 (5)
NORTH 1	100 (-)	8.6 (-)	3.7 (-)	5 (2)
NORTH 2	17 (-)	0.7 (-)	0.3 (-)	0 (0)
EAST	63 (-)	2.6 (-)	1.1 (-)	1 (1)
CENTRAL	70 (29)	8.7 (4.6)	3.3 (1.9)	5 (0)
PATA	77 (10)	5.3 (1.0)	2.0 (0.1)	7 (3)

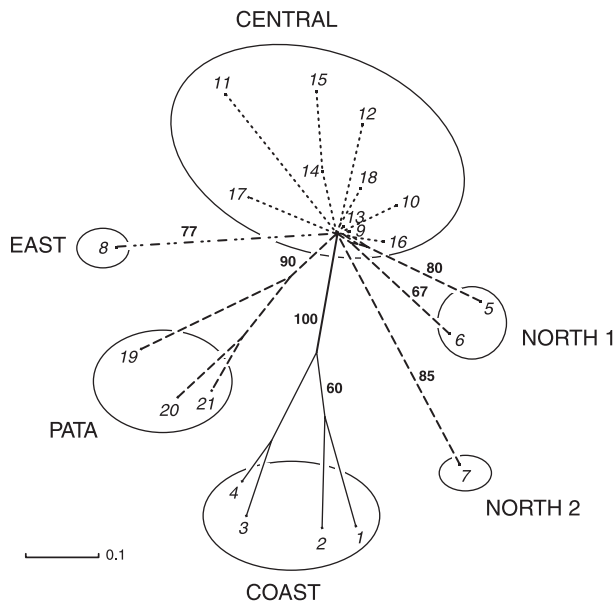


Fig. 2 Unrooted phenogram of AFLP data from populations of *Hypochaeris palustris* obtained from SplitsTree analysis of population-pairwise F_{ST} values. Numbers in bold indicate bootstrap percentages (1000 replicates).

Within PATA populations, the more northern population 19 was separated from the two more southern populations and was closer to the CENTRAL populations.

Regionally structured levels of genetic diversity

Populations of COAST were the most diverse and distinct (Table 3; Fig. 3). In populations of COAST, each individual had a unique multilocus phenotype. The average polymorphism in the populations from COAST was 15.9% (SD = 4.1). The populations from COAST had a mean Shannon diversity of 5.7 (SD = 1.6), exhibiting the highest number of private fragments (Table 3; Fig. 3). Thus, 25 private fragments (five fixed) were found in the populations from COAST (Table 3).

In NORTH 1, each individual had a unique multilocus phenotype as well (Table 3; Fig. 3). Population 6 had an average polymorphism of 8.6%, and a mean Shannon diversity of 3.7. Five (two fixed) private fragments were found in NORTH 1 (Table 3).

In populations of CENTRAL, 70% (SD = 29) of individuals had different phenotypes (Table 3; Fig. 3). The average polymorphism in the populations from CENTRAL was 8.7% (SD = 4.6) and the mean Shannon diversity was 3.3 (SD = 1.9). Five (none fixed) private fragments were found in the CENTRAL populations (Table 3).

In PATA, 77% (SD = 10) of individuals had different phenotypes (Table 3; Fig. 3). 5.3% (SD = 1.0) of fragments were found to be polymorphic on average. PATA populations had a mean Shannon diversity of 2.0 (SD = 0.1). Seven (three fixed) private fragments were found in PATA (Table 3).

In EAST, 63% of individuals had different phenotypes (Table 3; Fig. 3). The average polymorphism in the population 8 was 2.6%. Population 8 had a mean Shannon diversity of 1.1 (Table 3). One fixed private fragment was found in EAST (Table 3).

In population 7 of NORTH 2, 17% of individuals had a unique multilocus phenotype (Table 3; Fig. 3). Population 7 had an average polymorphism of 0.7%, and a mean Shannon diversity of 0.3. No private fragment was found in this population (Table 3).

Discussion

Our paper suggests that (1) populations from the coastal Cordillera have not served as source for postglacial recolonization and have been separated from Andean populations during the LGM, and that (2) the central Andean ranges have been recolonized after the LGM from source populations from several nearby refugia (probably in the N and E of the present range). This is supported by several lines of evidence.

First, distance analysis (Fig. 2) as well as AMOVA between regions (Table 2) reveal that the largest genetic divide in

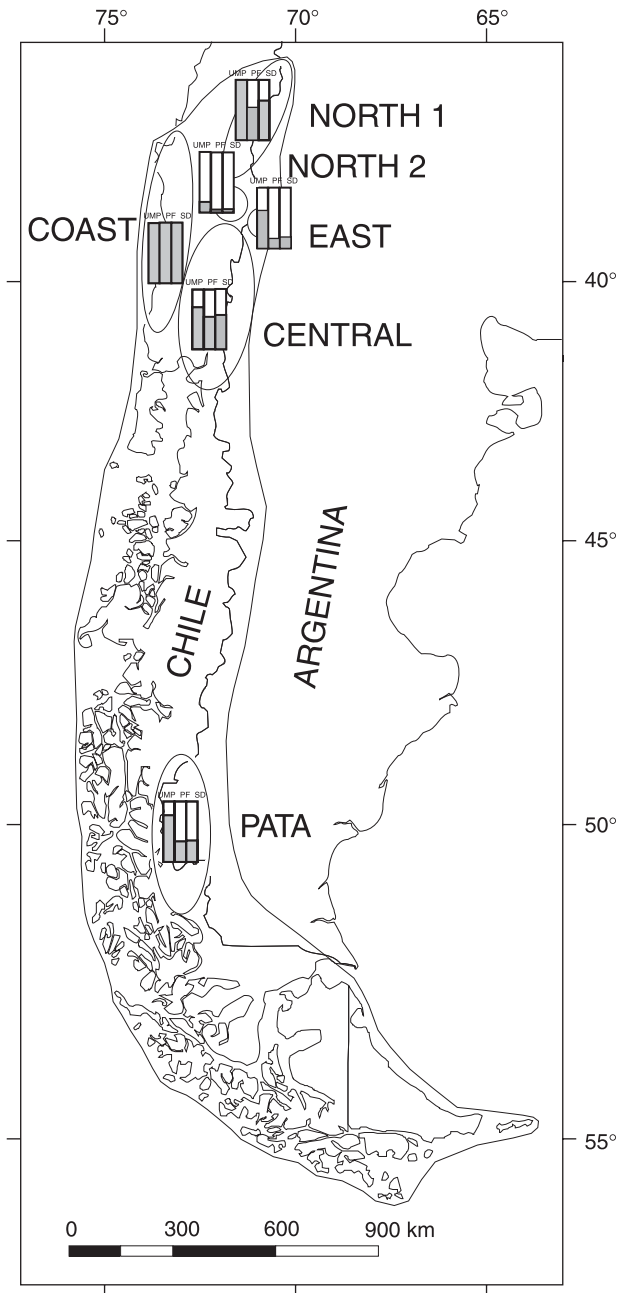


Fig. 3 Total distribution area of *Hypochaeris palustris* and population diversity levels of regions. UMP = unique multilocus phenotypes, PF = polymorphic fragments, and SD = Shannon diversity.

Hypochaeris palustris is between populations from the coastal Cordillera and those from the Andes, the first of which are genetically well differentiated (100% BP; Fig. 2). This conclusion is further illustrated by comparison of pooled AFLP profiles of each region (COAST, CENTRAL, NORTH, EAST, and PATA; Table 2), which reveals that the coastal Cordillera shares least fragments with the Andean regions. In this latter area, CENTRAL shares most

fragments with PATA, followed by NORTH 1 and EAST. This suggests that source populations for these three regions may have colonized other parts of the central Andes. Indications for routes of migration of source populations out of possibly northern and eastern refugia provided by shared fragments between regions are further suggested by distance analysis (Fig. 2). The postglacial spread of *H. palustris* into the newly available central ranges of the Andes was probably rapid, as there is scant genetic divergence among populations. The central Andean populations spread star-like from a common centre in the distance analysis (Fig. 2), and there is low correlation of geographical and genetic distances in the central Andean area (Mantel's $r = 0.406$).

Second, populations from the coastal Cordillera harbour most private fragments (Table 3; Fig. 3), which could indicate that they are the oldest populations. Survival of populations on the coastal Cordillera during the LGM might have enabled them to accumulate more private fragments than populations from any other regions. Premoli *et al.* (2000) found a similar pattern in *Fitzroya cupressoides*, investigating isozyme variation of populations sampled over the entire modern range of this endemic conifer in temperate South America. Their study indicated that present populations of *F. cupressoides* are the result of spreading from at least two glacial refugia located in coastal Chile (but further south than the relict populations of *H. palustris*) and on the flanks of the Andes in southern Argentina.

Third, the rate of polymorphism in the populations from the coastal Cordillera is highest compared with all other regions (Table 3; Fig. 3). Glacial refugia often harbour higher levels of genetic diversity than those that have been colonized after the retreat of glaciers (Hewitt 1996). Accordingly, Shannon diversity, which accounts for both number of fragments and their frequency, was also highest in populations of the coastal Cordillera (Fig. 3). This was also the case for the number of private fragments.

The relationship of Patagonia is clearly with the central Andean range, but it is also set apart from it. The Patagonian area itself is well supported in the distance analysis (90% BP; Fig. 2). Because populations were not available for analysis from the intermediate range of the southern Andes (between CENTRAL and PATA), nor further south from Tierra del Fuego, our data cannot say more about relationships of these populations. The differentiation between CENTRAL and PATA could be because of isolation by distance, or the accumulation of mutations and bottleneck/drift events on the way down to Patagonia. Another hypothesis might be recolonization of the Patagonian Andes by populations from refugia further south or southeast in Patagonia. Evidence that such refugia could have existed is provided by both empirical reconstruction (Hollin & Schilling 1981) and modelling (Hulton *et al.* 2002) of the LGM in southern South America. Palaeoclimatological data show that the eastern as well as the very southernmost

parts of southern Patagonia were partly ice-free during the LGM (Hollin & Schilling 1981; Hulton *et al.* 2002; Fig. 1). The major part of Patagonia appears to have been a bogland (Auer 1958; Simpson-Vuilleumier 1971) dissected by outwash streams and lakes from glacial melt waters, which presumably isolated some peripheral populations and caused differentiation. This is now observable by the numerous plant taxa that occur throughout the Patagonian steppe (Simpson-Vuilleumier 1971). Additional population sampling can test these hypotheses.

H. palustris is facultatively autogamous. We therefore expected it to have less within-population genetic diversity than an allogamous species, but rather than a more strictly autogamous species. The allogamous *Hypochaeris tenuifolia* has 63%–100% different AFLP phenotypes in its populations and 11%–72% polymorphic fragments (Tremetsberger *et al.* 2003a). The more strictly autogamous *Hypochaeris acaulis* has 11%–69% different AFLP phenotypes and 1%–13% polymorphic fragments (Tremetsberger *et al.* 2003b). *H. palustris* has an intermediate position with 13%–100% different AFLP phenotypes and 1%–21% polymorphic fragments. The large range of variation among populations in the three species might suggest that pronounced differences in the breeding system may exist among local populations. A similar result was also obtained in the western Mediterranean *Hypochaeris salzmanniana* (Tremetsberger *et al.* 2004), which shows local differences in its compatibility system.

Acknowledgements

We thank: Fonds zur Förderung der Wissenschaftlichen Forschung (FWF grant P13055 BIO) for financial support; Departamento de Botánica, Universidad de Concepción, for providing work space in Chile and for the permission to consult herbarium material (CONC); Corporación Nacional Forestal (CONAF) for the permission to collect samples in Chilean national parks; G. Kadlec for technical assistance; all collectors of plant material; two anonymous reviewers for their valuable comments on the manuscript.

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